Response of Zooplankton Community of Lake Winnipeg to Environmental Changes

by

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ABSTRACT

Lake Winnipeg has been subject to intense eutrophication and invasive species such as Rainbow Smelt and *Eubosmina coregoni* for the last 40 years. This study demonstrated significant increases in total phosphorus, total nitrogen, chlorophyll-*a*, overall zooplankton abundance, and specifically Cladocera, between 1969 and the first decade of the 21st century. There were specific basin differences in the long-term changes of the Cladocera community, with the species *Chydorus sphaericus* and *Ceriodaphnia quadrangula* negatively affected by high levels of phosphorus, nitrogen and chlorophyll-*a*. Moreover, long-term change in the Cladocera community composition and abundance throughout the years (1969-2011) during summer was correlated with intensifying eutrophication. Additionally, weak diel vertical migration in the zooplankton community was observed for the first time in Lake Winnipeg.

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CHAPTER 1: INTRODUCTION

Zooplankton play an important role in the ecosystem of oceans and freshwater systems. A very dynamic and diverse group, they are a link between primary producers and organisms in the upper trophic levels (e.g. fish). Being a key part in the trophic web in aquatic ecosystems, the density and species richness of zooplankton are affected by the environment through physical and chemical characteristics of the water body, the phytoplankton community that plays a role as a food source (bottom-up effect), and the species of the fish community (top-down effect), particularly planktivorous fish (Sommer and Lampert, 2007). Both effects play a crucial role in determining the composition of the zooplankton community and are not mutually exclusive (Carpenter *et al.*, 1985). On this basis, zooplankton has been shown to be great indicators of water quality and overall trophic condition of a lake (Gannon and Stemberger, 1978).

Eutrophication is a natural phenomenon that reflects gradually increasing productivity of a lake with concurrent increase in nutrient loading, typically resulting in an increase in primary producers, especially phytoplankton, over long periods of time. However, anthropogenic eutrophication is a serious and widespread concern in lakes worldwide. The main cause of anthropogenic eutrophication is the accelerated increase in nutrients (both phosphorus and nitrogen) derived from human sources that lead to a proliferation of algae, especially cyanobacteria, high oxygen consumption in the sediments, and fish kills as a consequence of low oxygen concentrations during the winter (Schindler *et al.*, 2008; Sommer and Lampert, 2007). Changes in all trophic levels have been documented in experimentally manipulated lakes (Schindler *et al.*, 2008), as well as in large lakes around the world, e.g. the Laurentian Great Lakes (Nicholls *et al.*, 2001), Lake Champlain, USA (Mihuc *et al.*, 2012), Lake Balaton,

Hungary (Devai and Moldovan, 1983; Padisak and Reynolds, 1998), and Lake Taihu, China (Mao *et al.*, 2012, Guijun *et al.*, 2012, Sun *et al.*, 2012). The rate of eutrophication of Lake Winnipeg has accelerated over the last two decades, primarily as a consequence of increased loading and concentration of phosphorus (McCullough *et al.*, 2012; Schindler *et al.*, 2012). This has resulted in the proliferation of nuisance blooms of heterocystous cyanobacteria, correlated with increased livestock production and use of fertilizers in the watershed (Bunting *et al.*, 2011) and increased frequency and intensity of spring floods in the Red River watershed (McCullough *et al.*, 2012).

This research aimed to study the long-term changes (1969 – 2011) within the zooplankton community (with a focus on Cladocera) in response to eutrophication. This is a rich data set that has not been analyzed before. This study is crucial to understand the dynamics within a trophic food web in Lake Winnipeg and has been relatively unexplored in large shallow lakes. Furthermore, we studied diel vertical migration (DVM) of the zooplankton community in Lake Winnipeg. DVM is a trade-off between minimizing visual predation and maximizing food consumption (Ringelberg, 2010). We hypothesise that the crustacean zooplankton community would increase in density in response to eutrophication, as higher primary production becomes a potential food source for the crustacean zooplankton community (Patalas and Salki, 1992; McCauley and Kalff, 1981; Hanson and Peters, 1984).

Zooplankton

The main crustacean groups, Cladocera, commonly known as “water fleas”, and Copepoda (copepods), constitute the dominant and most important components within the zooplankton (Wetzel, 2001; Sommer *et al.*, 2003). In general, Cladocera (Class Branchiopoda)

range from 0.2 to 6 mm in size (with the exception of the predatory cladocerans, e.g. *Leptodora kindti* which reaches 14 - 20 mm). They are mainly filter-feeders, ingesting detrital material and phytoplankton, bacteria and protozoa. Cladocera have a certain degree of selection for their food, sorting edible phytoplankton by size through filtration and mouthpart size, as well as some chemical cues, e.g. *Bosmina* has a preference for algae rather than other small particles (DeMott, 1986). Cladocera are considered to be the main competitors with other small-sized phytoplankton-feeding protozoa and predatory protozoa as well (Sommer and Sommer, 2006). Importantly, *Daphnia,* one of the most commonly occurring genera of Cladocera, has been identified as a model organism to use in assessing effects of multiple stressors in freshwater ecosystems (Altshuler *et al.*, 2011).

Predatory crustaceans (such as *L.kindti*, *Bythotrephes longimanus,* and *Cercopagis pengoi*) feed on other zooplankton, such as other cladocerans and rotifers. They reside in littoral, pelagic and benthic zones and are found in most continental fresh water bodies (Wetzel, 2001; Forro *et al.*, 2008; Sommer and Sommer, 2006). *B. longimanus* has also successfully invaded the Laurentian Great Lakes (Demelo and Hebert, 1994) and Lake of the Woods, Ontario (Suchy *et al.*, 2010), and has been reported from gut contents of fish caught in Traverse Bay near the outlet of the Winnipeg River in Lake Winnipeg (A. Olynyk, pers.comm.) .

Copepods vary in length from 1 to 4 mm and can be found in many freshwater habitats (2,814 species are estimated to exist); their greatest diversity is found within the marine environment (Boxshall and Defaye, 2008). Within the five orders that constitute the freshwater copepods, two are considered to be the main constituents of the zooplankton, the Calanoida and Cyclopoida (Wetzel, 2001). Copepods rely on chemical cues for feeding, so they can avoid algae that are toxic or chemically unsuitable (Sommer *et al.*, 2003). Previous research showed that they

prefer larger food items rather than small phytoplankton; however, they can still thrive when large food items are not available (Sommer and Sommer, 2006). Depending on the species or stage of development, copepods will feed on detritus or small algae. However, adult copepods (especially cyclopoid copepods) are predators and can feed on other components from the zooplankton, including protozoa, other copepods (calanoid copepods) and Cladocera (Wetzel, 2001; Boxshall and Defaye, 2008; Feuchtmayr *et al.*, 2004). In fact, calanoids and *Daphnia* can be considered to occupy the same trophic level, sharing the same food source (Sommer *et al.*, 2003). However, mature cyclopoid copepods and cladocerans feed on different food resources and are positioned in different trophic levels (Feuchtmayr *et al.*, 2004).

Cladocera are capable of both asexual and sexual reproduction. They grow through several morphologically similar juvenile stages called instars, finally moulting to become mature adult females. When reproducing asexually, they undergo parthenogenesis, where mature females produce broods of clonal female offspring from eggs that develop in their brood chambers. After completing development, the free-swimming neonates (all females) are released from the brood chamber, and the adult female moults and releases another brood of eggs into the brood chamber (Gilbert and Williamson, 1983). However, sexual reproduction ensues after a number of asexual generations have occurred or when stimulated by a particular combination of environmental cues, e.g. decline in photoperiod, temperature and increased crowding that trigger mature females to produce eggs that undergo diapause (Gyllström and Hansson, 2004). Most cladocerans produce diapausing eggs that must be fertilized by males and those eggs are enveloped by a protective structure known as an ephippium. Other cladocerans shed their diapausing eggs with no ephippium being produced (e.g. *Diaphanosoma*, *Leptodora*, *Sida*, *Holopedium*, *Polyphemus*). Males are produced seasonally from parthenogenetically produced

eggs in response to environmental stimuli. They typically undergo several juvenile instars prior to ecdysis to the mature male instar which produces haploid sperm. Sexual reproduction is frequently synchronized with the end of peak density of the population, typically occurring late in the open water season in large temperate zone lakes.

Copepods reproduce via sexual reproduction exclusively. Life history stages after hatching from eggs include 6 naupliar stages, followed by 5 copepodid instars. The final moult of a fifth instar copepodid culminates in a mature adult female or male instar.

Calanoid copepods reproduce sexually and are capable of producing resting eggs. This is a strategy to escape from strong predation pressure, and the eggs hatch when predation pressure is lower (Einsle, 1993 in Santer, 1998). Furthermore, increasing day length and temperature are common stimuli that trigger resting egg production, be it in a permanent or temporary pond (Hairston and Kearns, 1995 in Santer, 1998; Hairston *et al.*, 1990 in Santer, 1998). Some calanoids such as *Eudiaptopmus graciloides*, in their adult stage, enter diapause (known as motile diapause), having stored lipids in their body and staying stationary in the pelagic zone (Fenova, 1979 in Santer, 1998).

Cyclopoid copepods do not produce diapausing eggs. They rely on dormant stages to overcome unsuitable conditions; generally, it entails a pause in their development between the fourth and fifth copepodid instars (Kiefer, 1978 in Santer, 1998). However, second and third copepodid instars, as well as mature adult females and fertilized females have been found to diapause (Naess and Nilssen, 1991 in Santer, 1998). Fryer and Smyly (1954 in Santer, 1998) established that individuals in diapause can stay in the pelagic zone or sink to the bottom sediments (active and passive diapause, respectively). Diapause can be accompanied by

encystment, especially for species living in temporary ponds (Rzoska, 1961 in Santer, 1998). Diapause can happen during the winter or summer depending on the species (Burgis, 1971 in Santer, 1998). Moreover, in a single species one can find variation in onset and termination of diapause as well, depending on the habitat (Santer and Lampert, 1995 in Santer 1998). Within a population, some individuals may enter diapause while others maintain normal activity (Nilssen, 1977 in Santer, 1998).

Lake Winnipeg

Lake Winnipeg is the tenth largest freshwater body in the world by surface area and an important resource for the province of Manitoba. Approximately 23,000 residents live in 30 communities along the lake, 11 of which are First Nations (Lake Winnipeg Stewardship Board 2006). A fishing industry has existed on Lake Winnipeg since the end of the 19th century and currently the lake supports the largest freshwater commercial fishery in western Canada. Recreational and bait fishing are also active contributors to the economy on the lake. Furthermore, the lake is used as a source of drinking water with direct withdrawals of untreated water from the lake as well as from wells, tributaries or local aquifers, mainly to small communities along the eastern side. Tourism and eco-tourism thrive on Lake Winnipeg, around Grand Beach, the city of Gimli, through its beaches, festivals, water sporting events and all-season resorts. Unfortunately, the frequency and intensity of algal blooms in the lake have increased concurrent with the increases in phosphorus and nitrogen loading.

Lake Winnipeg can be subdivided into three main regions, a North and South Basin, separated by the Narrows (Patalas and Salki, 1992). The land surrounding the west and south region of the lake is underlain by sedimentary rock and that surrounding the east of the lake is

underlain by Precambrian Shield bedrock. To add more complexity, the lake receives its water inflow and nutrient input through a number of rivers. The main water inflow into the South Basin comes from the Winnipeg River (from the Precambrian Shield). However, the main nutrient input comes from the Red River, which carries the nutrients from the agricultural regions to the south. In the North Basin, the main water inflow comes from the N. Saskatchewan River that does not carry as high nutrient load as the Red River in the South Basin (Patalas and Salki, 1992). In addition, there are many smaller rivers entering the North Basin around its margins. The mean depth of the North Basin is approximately 13 m; and the South Basin averages less than 10 m (Lévesque and Page, 2011). The greatest depth is located in the Narrows, with a depth of 60 meters north of Black Island (Lévesque and Page, 2011). As a consequence of its shallow mean depth and large fetch, the lake displays a high degree of wind mixing and a general absence of thermal stratification during the open water period; therefore, it is classified as polymictic.

The first limnological survey on Lake Winnipeg (Bajkov, 1934) examined water chemistry and biological characteristics of the lake. This study was followed in 1969 with an extensive survey of the morphology, hydrology, physical and chemical characteristics of the lake, conducted by scientists at the Freshwater Institute, Winnipeg, MB (Fisheries Research Board of Canada, currently Fisheries and Oceans Canada), with results summarized in Brunskill *et al.* (1980). The crustacean zooplankton community, also included in the survey, was described with respect to its spatial heterogeneity in distribution (both species occurrence and densities) by Patalas and Salki (1992). Only in the late 1990s and first decade of the 21st century, have intensive surveys been conducted under the auspices of the Lake Winnipeg Research Consortium (LWRC), utilizing the M.V. *Namao*. Paleolimnological studies have provided a timeline for evaluation of long-term changes in the lake (Bunting *et al.*, 2011). A synthesis of conditions in

Lake Winnipeg was published as a State of Lake Winnipeg: 1999-2007, Environment Canada and Manitoba Water Stewardship Report (Lévesque and Page, 2011). Unfortunately, during the 1970s and 1980s there were no whole lake surveys conducted which leaves a gap in information on the increase of nutrients in Lake Winnipeg; however, phosphorus concentration, algal biomass, and the frequency and extent of cyanobacteria blooms have increased (McCullough *et al.*, 2012). All of these are considered to be proxies for eutrophication.

Lake Winnipeg has been subjected to a number of stressors over the past several decades, including increased nutrient loading (eutrophication), toxic chemicals, invasive species and climate change (Gewurtz *et al.*, 2006; Schindler *et al.*, 2012). The Laurentian Great Lakes, particularly Lake Erie, Lake Ontario and Lake Michigan, and other large, shallow lakes of the world have been influenced by this same array of stressors; therefore, these lakes provide a suitable comparison against which to assess historical patterns of change in response to multiple environmental stressors (Munawar *et al.*, 2010; Schindler, 2006). Laurentian Great Lakes and other Shallow Lakes

Many large, shallow lakes of the world, e.g. Lake Balaton, Hungary (Honti *et al.*, 2007; Tatrai *et al.*, 2008), Lake Taihu, China (Guijun *et al.*, 2012), are experiencing increased nutrient loading and accelerated eutrophication similar to that occurring in Lake Winnipeg, currently in a similar eutrophic state to that of Lake Erie in the 1960s and 1970s (Sweeney, 1993). Recovery from one stressor will likely be influenced by onset of effects of new stressors impacting a lake at a subsequent time and will present a unique lake-specific pattern of response. Nevertheless, comparison of lakes that have experienced similar stressors over time may permit predictions of consequences of environmental stressors in newly impacted lakes.

Makarewicz (1993) compared zooplankton community composition in Lake Erie from 1939 to 1987, encompassing the pre-eutrophication period, through increased nutrient loading in the 1960s and 1970s, into the era of phosphorus control implementation in the 1980s. Crustacean zooplankton abundance in 1939 was similar to that reported in 1983-87 in the western basin of Lake Erie; however, Cladocera density was significantly lower and Copepoda density higher in 1983-87 compared to observed maxima in 1961 and 1967 (Makarewicz, 1993), prior to passage of the Great Lakes Water Quality Agreement (GLWQA) in 1972 which led to reduction in phosphorus loading to the lake from municipal sources (Conroy *et al.*, 2005). When studying zooplankton community response to eutrophication, Gannon and Stemberger (1978) developed an index, calanoid/cyclopoid plus cladoceran ratio, which has proven useful. The underlying assumption of their index is that calanoid copepods are better adapted to oligotrophic conditions, whereas cladocerans and cyclopoid copepods thrive in more eutrophic lakes. Hence, the index would have a higher value in oligotrophic conditions and conversely a lower value in eutrophic waters. This pattern is reflected across basins of Lake Erie and over the decades of his study, as well as among the Laurentian Great Lakes of differing trophic status (Makarewicz *et al.* 1992).

Barbiero *et al.* (2001) gathered information about the zooplankton community in the Laurentian Great Lakes from 1998 data. Lake Erie's zooplankton population varied greatly in density amongst the sampling stations, reflecting limnological differences among basins. Temperature and chlorophyll-*a* accounted for most variability in the zooplankton community, as had been found by Patalas (1969) for Lake Ontario (in Barbiero *et al.*, 2001). Lake Erie had the highest species richness and variability within its three basins, compared to the other Great Lakes. Barbiero *et al.* (2001) mention that Lake Erie has seen an improvement, by reduction of eutrophic state and the re-emergence of calanoid copepods. However, the glacial relict calanoid

Limnocalanus macrurus, that was extensively present throughout Lake Erie in the past, was confined to only the western basin and in reduced numbers.

Currently Lake Ontario is considered to be a healthy, oligotrophic lake (Munawar *et al.*, 2010), with no excess of nutrient loading and with a low phytoplankton biomass and acceptable water clarity, except for the impacted Bay of Quinte region (Minns *et al.*, 1986a,b). Lake Ontario has suffered the invasion of predatory cladocerans (*Bythotrephes longimanus,* and *Cercopagis pengoi*) during the late 1980s and early 1990s (Munawar *et al*., 2010). Stewart *et al.* (2010) compared zooplankton communities in Lake Ontario between 1987 -1991 to 2001 – 2005, showing there was a pronounced decrease in cyclopoid copepods concurrent with an increase in invasive cladocerans which, nevertheless, remained only a small proportion of the overall zooplankton community; this shift in proportional composition may be a consequence of the predation pressure of alewives and planktivorous fish on these invasive cladocerans. Furthermore, a reduction in primary production and the effects of invasive dreissenid mussels may also have contributed to the decline of zooplankton production. Because cyclopoid copepods are the dominant group, this observed decline within Lake Ontario after the invasion might disrupt the energy flow and trophic transfer in a substantial manner (Stewart *et al*., 2010). Lake Ontario may be more resistant to alterations in its food web dynamics, perhaps due to the heterogeneity of zooplankton composition which has been found to reduce the impact of invaders and increase the likelihood of coexistence of native and invading species (Stewart *et al.*, 2010). Sommer *et al.* (2003) inferred a similar increased resistance in conjunction with elevated functional diversity in the trophic food web.

In Lake Michigan, shallow and near shore sites had high levels of nutrients and this was reflected in the phytoplankton and zooplankton communities (Carrick *et al*., 2001)

Cyanobacteria *Anabaena cyanae* and *A. flos-aquae* were considered to be indicators of moderate eutrophication and the zooplankton consisted in *Mesocyclops edax* and *Daphnia retrocurva* and *Ceriodaphnia* sp. (Carrick *et al*., 2001). These assemblages were observed despite an overall reduction of TP loading to the lake (Carrick *et al*., 2001).

Research regarding the zooplankton composition of Lake Balaton (Zankai and Ponyi, 1986) showed that during intense cyanobacterial blooms in the summer between 1981 – 83 (*Anabaenopsis*) filter-feeding species of calanoid copepods and Cladocera decreased, with the zooplankton community constituting mainly cyclopoid copepods, reflecting the effects of cyanobacterial blooms in the grazing rate (Zankai and Ponyi, 1986).

In contrast, Mihuc *et al.* (2012) studied the zooplankton community of Lake Champlain from 1992 – 2010, showing that water quality variables such as chlorophyll-*a*, total nitrogen, and total phosphorus were not the drivers of change observed in the zooplankton community during this time period. The introduction of alewife was found to be a possible cause for the reduction in size of daphniid species in the lake (Mihuc *et al*., 2010).

Thus, a study examining the zooplankton community of this lake and how it has changed through time in response to environmental changes related to eutrophication is urgently needed. Comparison with zooplankton communities in other large, shallow lakes exposed to a similar suite of environmental stressors, such as the Laurentian Great Lakes, especially Lake Erie and other shallow lakes of the world, e.g. Lake Champlain, USA, Lake Balaton, Hungary, and Lake Taihu, China, may permit prediction of the trajectories that the zooplankton community of Lake Winnipeg may take in the future in response to novel invaders and climate change.

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CHAPTER 2: VERTICAL DISTRIBUTION OF ZOOPLANKTON IN LAKE WINNIPEG

INTRODUCTION

Zooplankton, aquatic organisms whose position in the water column is affected by current velocities greater than their swimming capabilities, have their temporal and spatial distributions determined by biological and physical processes (Huber *et al.*, 2011; Kiørboe, 2011). Such processes include: wind-induced circulation, swarming behaviour, diel vertical migration, horizontal migrations, and the horizontal and vertical patters of variation in temperature, food concentration and predation (Kiørboe, 2011). Although there is some evidence of wind-induced currents affecting vertical distribution of zooplankton through up- or downwelling (Huber *et al.*, 2011; Kiørboe, 2011), the majority of currents generated by wind action affect the horizontal distribution of plankton, particularly towards the surface, in contrast to middle and deeper layers where internal currents become more relevant (Huber *et al.*, 2011). In fact, Blukacz *et al.* (2009; 2010) have shown that wind force is the main driver for the downwind accumulation and spatial overlap of zooplankton and phytoplankton, particularly for the 2 meter surface layer. Recent research has shown that active vertical transport, in this case diel vertical migration, is more relevant than passive transport in a temporal scale of days for zooplankton distribution. Horizontal transport dominates at longer temporal scales (Huber *et al.*, 2011).

Diel vertical migration (DVM) is the movement of zooplankton within the water column in response to light, temperature, food levels and predation pressure (Lampert *et al.*, 2003; Ringelberg, 1999; 2010). DVM is an important component in the behaviour of zooplankton (Dodson *et al.*, 1997) that allows plankton to adjust to environmental cues and facilitate their

survival, which emphasizes the adaptive significance of this behaviour (Ringelberg, 1999). In thermally stratified temperate zone lakes, zooplankton usually reside in deep waters during daytime and rise toward the surface during the night. This is a complex process as it involves a compromise between benefits accrued from evasion of predation by visual predators and increased food availability on the one hand, and energetic costs involved for such a migration on the other hand (Dodson, 1990).

"Reverse" migrations, when zooplankton move upward during the daytime, have been related to unusual daytime distributions of planktivorous fish within the hypolimnion. For example, in Lake Rudolf, Africa, *Tropodiaptomus banforanus* migrates to the surface in response to these fish distribution patterns (Leibold, 1991; Dini and Carpenter, 1992; Ringelberg, 2010). This emphasizes the role of fish presence on diel vertical migration of zooplankton (Dini and Carpenter, 1992). Reverse migrations have been observed even when planktivorous fish are not present, which allows them to access shallower depths with better quality and quantity food during daytime, but calls into question why migration would be advantageous at all under these conditions. Typically, such reverse migrations have been associated with non-visually hunting invertebrate predators, such as *Chaoborus* and *Leptodora*, which may themselves avoid fish predation through DVM (Alajarvi and Horppila, 2004). Complex interactions and migration responses result when zooplankton co-exist with both invertebrate and vertebrate planktivores; invertebrate planktivores may also perform DVM to avoid predatory fish, and zooplankton in turn will try to evade these invertebrate predators and execute reverse migration (Ringelberg, 2010).

Factors that initiate DVM can be classified as abiotic and biotic. The abiotic factors or cues are light intensity, and UV radiation and temperature; the primary biological factor

considered is predator presence through environmental signals, known as kairomones, released by both planktivorous fish and invertebrate predators (Ringelberg, 2010). The relative importance of these factors remains the subject of an ongoing debate. The factors that stimulate DVM can be categorized also into proximate and ultimate factors. Proximate factors relate to how zooplankton are stimulated to initiate DVM, manifested in physiological and behavioural mechanisms (Ringelberg, 2010; Ringelberg and Van Gool, 2003), whereas ultimate factors involve cues that relate to why DVM occurs, i.e. the adaptive significance of the behaviour and its magnitude (Ringelberg 2010; Ringelberg and Van Gool, 2003). Physiological studies conclude that light is the factor that triggers migration, whereas ecological research relates the movement more frequently to predator evasion (Ringelberg, 2010).

The rate of change in light intensity that occurs near dusk and dawn is a direct stimulus (proximate factor) triggering DVM. In essence, light alone, and the response of zooplankton to differences in its intensity, known as phototaxis, has been identified as the most important mechanism initiating DVM (Ringelberg, 1999; 2010). Other factors such as food, fish predation, and temperature affect the magnitude (the range of depth) over which DVM occurs, and are generally considered to be ultimate factors (Ringelberg, 2010). Before sunrise, zooplankton, sensitive to the rate of change in light intensity, typically initiate downward migration. A migration caused by relative changes in light intensity is usually considered to be a strategy to avoid visually-feeding predators during daytime (Ringelberg, 2010). Consequently, the rapid descent by zooplankton occurs at very low light intensities before visual predation by fish becomes crucial. In the evening, the opposite process occurs, leading to a slow upward swimming before sunset, which thereafter accelerates (Ringelberg, 2010). This pattern of upward

and downward migration is accelerated if fish kairomones are more concentrated in the surface waters (Ringelberg, 2010).

Temperature can influence DVM when thermal (and density) stratification occurs, observed commonly in summer in north temperate, dimictic, clear water lakes (Ringelberg, 2010). The depth of the thermocline varies depending upon the physical characteristics of light incidence, water transparency, and temperature profiles in a lake (Ringelberg, 2010). DVM of zooplankton might be slowed during the descent as passing through a denser thermocline requires more expenditure of energy (Ringelberg, 2010). The converse would occur during the ascent, transitioning through a gradient to decreasing density.

Fish presence increases the magnitude of downward movement of zooplankton that naturally occurs due to the changes in light before sunrise and sunset, confirming via many experiments that fish predation is a conditional factor in initiating DVM in zooplankton. This might be due to a chemical cue, i.e. fish kairomones in water (Ringelberg, 2010), and possibly a minimum concentration of kairomones that will trigger a DVM response, depending on the sensitivity of the zooplankton species. Other evidence suggests chemicals liberated by injured individuals (prey) or a mechanical cue involving the swimming movement of planktivorous fish can also be responsible for these responses in DVM (Ringelberg, 2010; Cohen and Forward, 2009). Von Elert and Pohnert (2000) found that *Daphnia* sp. respond with DVM to free dissolved kairomones in the environment from either piscivorous and planktivorous fish. This was inferred as an evolved adaptive response by the zooplankton as they cannot differentiate planktivorous fish from piscivores, thus evading all fish populations directly to ensure survival (Von Elert and Pohnert, 2000). Furthermore, it has been shown that kairomones also affect life

histories of both Cladocera and Copepoda by stimulating a faster maturation rate and smaller adult individuals (Chakri *et al*., 2010; Gutierrez *et al*., 2010).

Vanderploeg *et al*. (2009a; 2009b) observed a 'hypolimnion refuge' in the central basin of Lake Erie where zooplankton sought refuge from fish predation. Swimming towards a hypoxic hypolimnion may be advantageous for zooplankton because of the reduced presence of planktivorous fish, lower bioenergetic demands, and the presence of benthos that serve as an alternative food source to cold-water dwelling fishes such as rainbow smelt and yellow perch (Vanderploeg *et al*., 2009a). *Daphnia pulicaria* have been found concentrated in the upper portion of the hypolimnion (just below the thermocline with a dissolved oxygen concentration of 1 mg O_2 L^{-1}) when planktivorous fish were present (Larsson and Lampert, 2011). Although this was a viable survival strategy in comparison to a fully oxygenated hypolimnion, a trade-off between predation evasion and survival in hypoxic conditions must exist (Larsson and Lampert, 2011). Despite the advantages of avoiding predation by occupying a hypolimnetic refuge in daytime, rate of reproduction and resting egg production would be expected to be affected by low DO levels in the hypolimnion (Vanderploeg *et al.*, 2009b).

There have been few studies of DVM of zooplankton in shallow, turbid, polymictic lakes where mechanisms involved with DVM, and proximate and conditional cues have not yet been fully studied. Such a lake is generally well mixed throughout the entire water column during the open water season, thermal stratification is rare or absent, oxygen is well distributed throughout the entire water column and light is rapidly attenuated as a consequence of high turbidity (Baranyai *et al*., 2011). Therefore, only the obvious day/night light cue and kairomones released by predators remain as potential triggers for DVM in turbid lakes.

In turbid systems, the *reactive distance* of planktivorous fish, or the distance needed to recognize zooplankton, is reduced, allowing zooplankton to seek refuge safely in shallower waters (Williamson *et al.*, 2011). However, there is some evidence of visual predation by planktivorous fish even in lakes with high turbidity as size-selectivity was observed in turbid reservoirs from Oklahoma and Texas (Schulze, 2011), in sites classified as shallow, unstratified, well-mixed water bodies. In these sites, as usually happens in turbid environments, there was no littoral macrophyte cover that could account for a horizontal migration evasion strategy as an alternative explanation, and it appears that visual foraging by planktivorous fish can only be excluded in areas where Secchi transparency is less than 0.2 m (Schulze, 2011). In systems with turbidity that exceeds 20 NTU, cyclopoids and the invertebrate predator *L. kindti* take advantage of the high turbidity and do not suffer negative effects on abundance as they escape visual predation by planktivorous fish (Liljendahl-Nurminen *et al*., 2008).

In Lake Balaton (Hungary), the largest shallow lake in Europe, turbulence acts as a proximate factor for zooplankton distribution, aside from light condition and predation (Baranyai and Lazlo, 2010; Baranyai *et al*., 2011). During calm days (no wind), DVM was still observed for Cladocera and copepods, with migration towards the mid or deeper depth during daytime (Baranyai *et al*., 2011). During wind active days, however, DVM was interrupted (Baranyai and Lazlo, 2010) and most zooplankters were still situated close to the surface during the day, i.e. no DVM was observed.

In Lake Hiidenvesi with an average depth of 3 m, DVM and reverse DVM were observed, the latter associated with possible predation by invertebrates (Alajarvi and Horppila, 2004). Furthermore, Alajarvi and Horppila (2004) recognized the importance of horizontal migration by

zooplankton when avoiding fish predation, as DVM might not be a good avoidance strategy in waterbodies with such shallow depths.

The objectives of this study are to: 1) examine the vertical distribution of zooplankton in Lake Winnipeg, which is a turbid and unstratified lake through most of the open water period; 2) compare vertical distribution of the zooplankton between day and night; and 3) assess the existence of DVM in Lake Winnipeg, crucial to the dynamics of the trophic food web.

METHODS

Study Site

Lake Winnipeg is the tenth largest freshwater body in the world based on surface area (approximately 24,500 square kilometres (km)) and has a length of 436 km. It has the largest watershed to lake surface area ratio (39:1) of any large lake in the world (Lake Winnipeg Stewardship Board, 2005). Lake Winnipeg can be differentiated into two basins connected by the Narrows, a channel of 2.5 km wide on average (Lake Winnipeg Stewardship Board, 2005). The North Basin is the larger of the two basins, being 111 km wide at the widest point and with a mean depth of 13.3 meters (m). The South Basin is approximately 40 km wide with an average depth of 9.3 m (Lake Winnipeg Stewardship Board, 2005; Sheppard *et al.*, 2011). Because of its shallow depth and large fetch, it is rarely stratified which leads to a well-mixed water column with high turbidity during the open water season (Sheppard *et al.*, 2011). Lake Winnipeg receives most of its water inflow from Winnipeg River, N. Saskatchewan River, and Red River and their tributaries.

Field Collection and Sampling Methods

To examine diurnal and nocturnal distribution patterns of planktivorous fish and zooplankton, day/night sampling cruises were undertaken during July and August of 2011 by Fisheries Branch of Manitoba Conservation and Water Stewardship, on board the M. V. *Namao*.

Three random starting points for the fish trawls were established by the Fisheries Branch of Manitoba Conservation and Water Stewardship in the North and South Basins (Figure 2.2 and 2.3) (Site 1, 2 and 3). For this research, Site 1 of North Basin was not included as problems with the closing net hindered sampling at that site.

A. Environmental variables and Zooplankton sampling

At each site in the North and South Basin, the following sequence was followed:

1. The water depth of the site was determined using sonar on the M. V. *Namao*. With that information, we estimated the depth used for the integrated vertical zooplankton sample and established how the water column would be partitioned for the stratified zooplankton samples. For the latter, three approximately equal depths were assigned to represent the surface, middle, and deep layers (i.e. North Basin with 15 meters depth: 0 – 5 m, 5 – 10 m, 10 – 14 m; South Basin with 10 meters depth: 0 – 3 m, 3- 6 m, 6 – 9 meters). Sampling times were approximately 12 hours apart to study the position of zooplankton after sunrise and after sunset, when zooplankton may normally be found in contrasting positions within the vertical water column (Farrell and Hodgson, 2012). North Basin sampling occurred at Site 1 on July 25th (11:10 PM) and July 26th (10:20 AM); Site 2 on July 26th for nighttime and daytime data (1:56 AM and 1:15 PM respectively); Site 3 on July 26th for daytime and nighttime data (3:27 PM and 11:15 PM respectively). South Basin sampling occurred at Site 1 on August 2nd (11:30 PM) and August 3rd (10:15 AM);

Site 2 on August 2nd (10:10 AM) and August 3rd (2:10 AM); Site 3 on August 3rd (11:15 PM) and August 4th (10:10 AM).

2. At the sampling site, the Rosette (SeaBird®) was deployed to take profiles of light irradiance (photosynthetically active radiation; PAR), temperature (°C), dissolved oxygen (mg/L), conductivity (µS/cm), chlorophyll fluorescence (µg Chl/L) and turbidity (NTU) at 0.1 m intervals. Mean values for each depth stratum were calculated by averaging the values recorded in the same layers established for the zooplankton stratified sampling (surface, mid, deep).
3. Stratified zooplankton sampling was done using a closing net (Downing and Rigler, 1984) equipped with two filtering nets each of 1 m in length, and 73 µm mesh size. The area of each net opening was 0.018 m^2. Deep, mid, and surface samples were taken in that order. An integrated zooplankton haul followed, from 1 meter above the bottom sediments to the surface with the use of a single net, 1 m long, with 73 µm mesh and net opening of 0.049 m^2.
4. All zooplankton samples (from stratified and integrated hauls) were made up to a standard volume of 125 ml and preserved in 10% formalin in glass sample bottles.
5. North Basin was sampled first and the sampling points and schedule for the North Basin and South Basin zooplankton sampling are displayed in the maps Figures 2.2 and 2.3.

B. Fish Sampling (Lumb *et al.*, 2012)

Fish were collected in daylight and nighttime hours with a 3-meter square beam trawl, 10.8 meters in length. Mesh size was graduated with a size of 76.2 mm at the mouth of the trawl and 19.1 mm at the end of the fish trawl. The towing speed was 3.9 km/h and the duration was

on average 30 minutes. Surface trawls corresponded to a depth of approximately 2.5 meters; midwater trawls were from approximately 2.7 m to 5.3 m; and deep trawls were from approximately 9.8 m to 12.4 m.

Laboratory methods

Based on methods described in Sheppard *et al.* (2011), the 125 mL sample was thoroughly mixed, and then a subsample of 5 ml was taken with a wide-bore syringe and placed in a square gridded petri dish. At least 200 individuals were counted within each subsample. With the use of a dissecting microscope at 40× magnification, Cladocera were identified to species level (Pennak, 1989; Balcer *et al.,* 1984). Copepods were identified as cyclopoids, calanoids, and nauplius larvae and counted. Identifications of individuals in a representative sample were verified by Mr. Alex Salki, DFO biologist (retired), Winnipeg, Manitoba.

The density of each species and classified group was calculated from the total count of all individuals in the 5 mL aliquot, then dividing by the total 5 mL, and multiplying by the total volume of the sample (125 mL). This gave an estimate of the total number of individuals within the sample that had been filtered from the stratum of the water column. The resulting value was divided by the volume of water that was filtered through the closing net, calculated by multiplying depth interval where the stratified sample was taken by the combined area of both mouths of the closing net (0.018 m^2 x 2), resulting in the density of individuals in each stratum of the water column (individuals/L). Percentage values were calculated for each classified group in each stratum of the entire water column. For the integrated vertical sampling, the calculations were the same as a 5 mL volume was used to identify and count the taxa in a 125 mL sample and

the depth of the water column filtered was used to determine the filtered volume. The mouth opening of the net was 0.049 m^2.

Data Analysis

The environmental variables, averaged within depth strata, were analyzed in a two-way ANOVA without replication (Microsoft Excel Analysis Tool-Pak) to determine if any of these variables differed between day and night, and with depth.

Densities of each taxon were averaged across all three depth strata (n=3), giving an overall estimate of density within the water column which was compared with the density estimate determined from the integrated sample. These estimates of zooplankton density at each site were compared using a paired t-test for North Basin and South Basin separately (Microsoft Excel Analysis Tool-Pak).

Average values from the stratified sampling at each sites (daytime and nighttime separately) for the major crustacean zooplankton groups and Cladocera species were calculated to explore patterns between day and night. The values from which the averages were calculated are shown in the Appendix (Table A2.1).

Correlation analysis (Data Desk 6.1; Data Description Inc.) was done between the averaged environmental variables and average densities for Cladocera, copepods, and nauplii and each individual Cladocera species for each three depth strata (at day and night, n=3), within each basin averaged across sites (North Basin with Site 2 and 3; South Basin with Site 1, 2 and 3).

Mean Residence Depth (MRD) was calculated according to Baranyai *et al*. (2011). MRD is the depth where the mean density of a given taxon resides within the water column. MRD was

calculated for Cladocera, calanoid and cyclopoid copepods, for day and night distributions, and for individual species of Cladocera.

$$MRD = \frac{\sum(N_i \times d_i)}{\sum N_i}$$

N_i is the density calculated from the stratified samples of an identified group or species, from depth *i* corresponding to a specific stratum. The d_i is the abundance (*d*) of an identified group species of a given depth interval (*i*), in this case, surface, mid and deep stratum at each sampled site.

Fish biomass data were obtained for each stratified trawl at all sites and in the North Basin and South Basin (Chelsey Lumb, Fisheries Branch of Manitoba Conservation and Water Stewardship), and volumetric density (biomass density, g/m^3) was calculated according to Lumb *et al.* (2012). Mean residence depth (MRD) of planktivorous fish was calculated from these data according to methods in Baranyai *et al*. (2011).

The correlation between density of planktivorous fish and zooplankton with depth (n=3) within the vertical column was examined at each measured site with Data Desk 6.1 (DataDescription Inc.).

RESULTS

Environmental variables

Mean values for each environmental variable obtained from the multiple measures done by the Rosette, are shown for each depth stratum at each sampling site during day and night in the North Basin (Table 2.1) and in the South Basin (Table 2.2). Generally, there was little variation with depth or between day and night in any environmental variable as would be

expected in a well-mixed water body. PAR declined exponentially with depth at all sites during daytime, indicative of a highly turbid water body. However, temperature and conductivity showed significant variation with depth (p-value lower than 0.05) at both sites in the North Basin with higher temperatures and conductivities in the surface layer during both day and night (Table 2.3). In the South Basin at one site (Site 2) only, dissolved oxygen (mg/L) was significantly lower in surface waters during both day and night, and conductivity (µS/cm) was significantly higher at night than during the day (Table 2.4). Wind conditions were calm during sampling in the North and South Basin. However, turbulence that is relevant for DVM was not measured.

Zooplankton Distribution Patterns

Densities of major crustacean zooplankton taxa varied with depth strata in both the North and South Basins (Table 2.5). In the North Basin, at site 2 during daytime, Cladocera, calanoids and nauplii presented higher density at the surface with cyclopoids having a higher density in the middle layer. During daytime in site 3, Cladocera, cyclopods and nauplii had higher concentration in the middle layers, whereas calanoids presented a much higher abundance in the deep layer. At night time, all major crustacean zooplankton groups presented higher abundances in the middle layer for site 2, and the surface layer for site 3. For the South Basin, all major crustacean zooplankton groups had higher abundances in the surface and middle layers at all three sites, during both daytime and night time.

To determine the reliability of zooplankton sampling done in Lake Winnipeg, a comparison of the calculated densities from the integrated and stratified sampling was done. Comparing the integrated and the calculated average values from the stratified sampling, Cladocera densities were higher during the day than at night at both sites in the North Basin,

whereas in the South Basin the opposite pattern of higher Cladocera densities at night at all sites was observed (Table 2.6). Calanoid densities were generally higher during the day than at night with a few exceptions. Cyclopoid and naupliar densities showed no clear pattern between day and night in either basin (Table 2.6).

Although site-specific differences in densities of zooplankton determined from integrated and stratified hauls existed at some sites during day and night (Table 2.7), differences were found with Cladocera being significantly higher in the integrated sampling in the North Basin and nauplii higher in the South Basin.

Patterns of distribution of zooplankton with depth during day and night were examined using mean residence depth (MRD) (Table 2.9, Figure 2.4), to determine if the depth at which the mean density of the zooplankton group occurred in the water column differed between day and night, thereby providing evidence for vertical movement diurnally.

In the North Basin, MRD of cladocerans and calanoids was more than a meter shallower during the day than at night at site 2, providing evidence of downward migration at night. In contrast, at site 3, cladocerans and calanoids were more than a meter shallower during the night, showing evidence of diel vertical migration for both taxa. There was no consistent pattern for cyclopoids and nauplii at the two sites.

In the South Basin, Cladocera migrated upwards at night at Site 1 and 2, while in Site 3 reverse migration was observed; calanoids moved downwards at night at all site. Cyclopoid copepods moved upwards at night in Site 2 and 3, and showed reverse migration in Site 1 (Table 2.9, Figure 2.4)

Cladocera were examined in more detail to determine if certain species demonstrated differences in MRD between night and day (Figure 2.4). In the North Basin and South Basin,

there were no clear patterns between sites. However within each site, most species reflected the pattern observed by the MRD of the overall Cladocera group during the daytime and nighttime.

Correlation analysis across all sites done for each basin and time of sampling did not show consistent patterns between environmental variables and the abundance of the major crustacean zooplankton groups or individual Cladocera species (Table 2.10).

Fish Data Comparison

In the North Basin, at both sites, maximum fish density occurred in deep water during the day but at mid-water depths at night (Table 2.4). In North Basin at site 2, Cladocera densities were highest near the surface during the day and moving to the middle layer towards at night (Table 2.4). Other zooplankton taxa showed a similar pattern to Cladocera, concentrated in the mid-water layer during the night (Table 2.4), precisely where fish were also concentrated (high positive correlation, Table 2.11). At site 3, however, Cladocera were concentrated at mid-water depths during the day and moved upward to shallower water during the night (Table 2.10). This was reflected in the correlation analysis, as almost all groups and Cladocera species showed a high negative correlation with fish density and position within the water column (Table 2.11). Most planktivorous fish in the North Basin collected in the trawls were rainbow smelt (Lumb *et al.*, 2012).

In South Basin, highest fish densities occur at mid-water depths at night, but distributions are variable during the day (Table 2.4). There is no consistent pattern of distribution of any zooplankton taxon with that of fish (Table 2.4, Table 2.11). Most planktivorous fish in the South Basin in the trawls were emerald shiners and cisco (Lumb *et al.*, 2012).

DISCUSSION

Cladocera and calanoid copepods generally showed evidence of low amplitude diel vertical migration in the North Basin of Lake Winnipeg at one site, whereas cyclopoid copepods and nauplii did not; all Cladocera species tended to show DVM. In overall, there were no consistent patterns within and between all identified groups. This could be a relfection of the well-mixed characteristic of Lake Winnipeg and other large shallow lakes. All zooplankton taxa migrated to shallower water than the water depth at which planktivorous fish were concentrated. However, at the other site, despite similar patterns of planktivorous fish distributions during day and night, evidence supported "reverse" migration for most taxa, which would have resulted in increased exposure to fish predators at night. In the South Basin, there were no clear consistent patterns among sites, perhaps reflective of the well-mixed and more turbid conditions. Regardless, at 2 of the 3 sites, zooplankton were concentrated at shallower depths than planktivorous fish at night.

There has been very little research that deals with diel vertical migration in shallow, well-mixed lakes. In terms of the environmental cues deemed the most relevant in triggering the diel vertical migration, specifically temperature and fluorescence (associated with chlorophyll-a and algal food availability) (Ringelberg, 2010), none presented a statistical or biological variation for depth or day/night difference. This might implicate a biotic influence, i.e. visual predation by planktivorous fish that triggers or determines the magnitude in which DVM occurs in Lake Winnipeg. Schulze (2011) showed that visual fish predation is an important element in food web dynamics even in turbid lakes (with NTU values as high as 20). Lake Winnipeg has turbidity values substantially lower than this (e.g. average values from samplings sites of NTU of 6 during the day and 6.4 at night in the North Basin, and 10.7 for South Basin for both day and night),

suggesting that planktivore predation may be a stimulus. This was shown in the correlation analysis result of Site 3 of North Basin.

Turbulence (not quantified) has been shown to be an important factor affecting vertical distribution of zooplankton, especially in shallow, well-mixed lakes (Baranyai and Lazlo, 2010); Cladocera (e.g. *Daphnia* and *Bosmina* species) were the first of the crustacean zooplankton community constituents to be unable to maintain their migratory behaviour, followed by calanoid and cyclopoid copepods (Baranyai and Lazlo, 2010). Another factor related to turbulence is the stress induced by the movement of suspended particles and physical damage to zooplankton (Lazlo *et al.*, 2011). Such information is crucial for explaining the patterns of vertical migration in lakes such as Lake Winnipeg, and must be included in further research.

Lumb *et al.* (2012) indicated that fish biomass is highest during the summer season, where the sampling was done. The community of fish is quite different depending on the basin. Planktivorous fish community is dominated by the non-native species Rainbow Smelt in the North Basin, and Cisco and Emerald Shiner in the South Basin (Lumb *et al.*, 2012) and their feeding strategies are different. Rainbow Smelt typically feed in deeper water during the day and move upwards at night, a pattern reflected in the distribution of planktivorous fish sampled in the North Basin (Stewart and Watkinson, 2004). In contrast, Emerald Shiner are more predominantly visual surface-feeders, especially in daytime, a pattern also observed in the South Basin (Stewart and Watkinson, 2004). This may influence the vulnerability of zooplankton to specific fish species.

However, it should be noted that the fish trawls were not done at the same depth as the stratified zooplankton sampling. Gaps in the vertical water column exist where trawls were not taken, particularly between 5.3 (mid-water trawl depth) and 9.4 m (deep water trawl depth)

(Lumb *et al.*, 2012). Consequently, using these data to determine MDR of fish and correlating with MRD zooplankton data might not reflect a complete picture of fish distribution and visual predation pressure on zooplankton. This would be accentuated in the North Basin were turbidity is lower than in the South Basin.

Many studies have shown the effect of visual predation by planktivorous fish on copepods and Cladocera community, identified as a trigger that modifies the magnitude (ultimate factor) of DVM executed in response to light cues (proximate factor) (Gool and Ringelberg, 1998, 2010; Folt and Burns; 1999; Brewer *et al.*, 1999).

Reverse diel vertical migration is frequently associated with the presence of invertebrate predators, such as *Bythotrephes longimanus* or *L. kindti* (Alajarvi and Horppila, 2004; Farrell and Hodgson, 2012). *L.kindti* has been shown to be present in the lake in integrated samples (Patalas and Salki, 1992) but was not found in the stratified sampling in 2011. This might be due to inadequate sampling as Chang and Hanazato (2004) showed that filtering water samples with 40 µm mesh was adequate for DVM studies of *L. kindti*. *B. longimanus* has not been found yet in zooplankton sampling in Lake Winnipeg. As *L. kindti* was not found in the samples used for the DVM study, it is unclear if they drove the observed DVM patterns.

Unfortunately, there have not been many studies to examine DVM of copepods in turbid well-mixed shallow lakes. However, in temperate stratified lakes (Farrell and Hodgson, 2012), calanoid and cyclopoid copepods have been seen to respond more intensely than Cladocera to invertebrate predators through reverse DVM. Cladocera, cyclopoid copepods and copepod nauplii in general, on the other hand, have been shown to be more sensitive to vertebrate predation, responding with DVM (Farrell and Hodgson, 2012). This hints at the importance of prey size for visually feeding vertebrates that rely on more visible larger prey, while smaller

species are vulnerable to invertebrate predation (Farrell and Hodgson, 2012), resulting in a zooplankton community of smaller individuals when vertebrate predation is present. The understanding of DVM becomes more complex as this phenomenon varies according to the life history stage and size of a given species affecting timing of phototaxis and transit distances; different populations are adapted to the presence of different group of predators and environmental conditions (Neill, 1992).

Furthermore, abiotic factors relevant to DVM such as temperature and fluorescence (chlorophyll a) in monomictic temperate deep lakes (Doulka and Kehayias, 2011) cannot be expected to have the same importance in well-mixed unstratified lakes such as Lake Winnipeg. Other environmental factors must be taken into account in these environments, such as turbulence, that has been shown previously to be an important factor (Baranyai and Lazlo, 2010).

Despite the evidence of weak diel vertical migration by the crustacean zooplankton in the North Basin of Lake Winnipeg, it must be noted that a more exhaustive sampling program is needed to track the movement of this community. In previous studies, zooplankton have been sampled over a 24- to 48-hour period at every 1 meter and at every 4 to 6 hours, at a given sampling site (Alajarvi and Horppila, 2004; Doulka and Kehayias, 2011; Armengol and Miracle, 2000). Furthermore, the paired t-test has shown only few statistically significant variations between stratified and integrated zooplankton sampling, within daytime and nighttime data for each basin. Livings *et al.* (2010) suggested column samplers to be more precise than Wisconsin nets in determining zooplankton density, as they sample at a constant depth, particularly at homogenous shallow lakes. Wisconsin net results vary possibly due to operator errors and mesh escapement. Furthermore, column samples are more effective in capturing smaller species in contrast to large plankton nets shown to capture large, rare or active species. However, a more

robust comparison is necessary as to include, variations in a spatial and seasonal scale between the two sampling methods (Downing and Rigler, 1984; Livings *et al.*, 2010). However, this is the first indication that diel vertical migration in Lake Winnipeg does occur. This is crucial information that can lead to a better understanding on how food web dynamics works within the context of this lake and how that is affecting the food web itself. Further studies must be done regarding DVM in Lake Winnipeg. This will indicate food accessibility and survivability not only for the zooplankton community, but also for the upper trophic levels (planktivorous fish) as these organisms are their main food source.

Table 2.1 Environmental variables for day and night sampling at North Basin sites. TEMP=Temperature; DO=Dissolved Oxygen; FLUOR=Flourescence; Chla=Chlorophyll-a; COND=Conductivity; TURB=Turbidity; PAR=Photoactive Radiation

SITE 2	DEPTH (m)	TEMP (°C)	DO (mg/L)	DO (%)	FLUOR/Chla (µg/L)	COND (µS/cm)	TURB [NTU]	PAR
Day	0 - 5	17.1	9.4	97.3	1.7	254.7	6.3	342.8
	5 - 10	16.5	8.6	87.7	1.4	250.7	6.4	2.9
	10 - 15	16.1	8.1	82.2	1.5	248.8	7.2	0.0
Night	0 - 5	17.1	9.2	95.3	1.6	254.3	6.4	0.0
	5 - 10	16.6	9.0	92.1	1.7	251.9	6.6	0.0
	10 - 15	16.3	8.4	85.8	1.2	249.7	6.5	0.0
SITE 3	DEPTH (m)	TEMP (°C)	DO (mg/L)	DO (%)	FLUOR/Chla (µg/L)	COND (µS/cm)	TURB [NTU]	PAR
Day	0 - 5	17.7	9.5	99.7	1.4	262.6	5.0	322.4
	5 - 10	16.5	8.9	90.7	1.3	254.5	5.3	4.8
	10 - 15	16.0	8.5	86.1	1.0	253.9	5.9	0.0
Night	0 - 5	17.3	9.1	95.2	1.3	264.0	5.3	0.0
	5 - 10	16.7	9.0	92.2	1.5	254.4	5.5	0.0
	10 - 15	15.8	7.9	79.7	1.5	251.3	8.4	0.0

Table 2.2 Environmental variables for day and night, sampling at South Basin sites

SITE 1	DEPTH (m)	TEMP (°C)	DO (mg/L)	DO (%)	FLUOR/Chla (µg/L)	COND (µS/cm)	TURB [NTU]	PAR
Day	0 - 3	22.1	7.4	85.2	1.0	504.4	7.8	149.6
	3 - 6	20.0	7.0	80.4	0.9	453.4	6.8	1.8
	6 - 10	22.1	7.6	87.2	0.9	510.8	7.9	0.0
Night	0 - 3	22.4	7.1	82.3	1.1	486.6	8.7	0.0
	3 - 6	22.4	7.8	90.6	1.2	491.3	8.6	0.0
	6 - 10	22.1	7.7	88.3	1.0	501.3	7.8	0.0
SITE 2	DEPTH (m)	TEMP (°C)	DO (mg/L)	DO (%)	FLUOR/Chla (µg/L)	COND (µS/cm)	TURB [NTU]	PAR
Day	0 - 3	22.4	7.3	84.7	1.0	374.4	14.9	61.0
	3 - 6	22.3	7.7	88.1	1.0	373.7	15.6	0.2
	6 - 10	22.3	7.7	88.4	1.0	373.8	16.1	0.0
Night	0 - 3	22.3	7.3	83.6	1.0	404.5	13.7	0.0
	3 - 6	22.3	7.8	89.4	1.0	400.5	14.2	0.0
	6 - 10	22.2	7.7	88.5	0.9	391.3	15.5	0.0
SITE 3	DEPTH (m)	TEMP (°C)	DO (mg/L)	DO (%)	FLUOR/Chla (µg/L)	COND (µS/cm)	TURB [NTU]	PAR
Day	0 - 3	22.6	7.8	90.7	1.1	475.8	8.6	321.6
	3 - 6	22.3	7.7	88.3	0.9	474.0	9.1	17.7
	6 - 10	22.3	7.7	88.4	0.8	474.5	9.1	0.0
Night	0 - 3	22.3	7.7	88.5	1.2	475.4	8.9	0.0
	3 - 6	22.3	7.8	89.9	1.2	475.7	9.2	0.0
	6 - 10	22.3	7.8	90.3	1.2	476.0	9.5	0.0

Table 2.3 Two-way ANOVA for Temperature and Conductivity at site 2 and 3 (North Basin). Only significant results shown

Temperature (Site 2)	α = 0.05					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Depth	0.859783	2	0.429892	45.89596	*0.021324*	19
Day / Night	0.012159	1	0.012159	1.298169	0.372622	18.51282
Error	0.018733	2	0.009367			
Total	0.890676	5				

Conductivity (Site 2)	α = 0.05					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Depth	27.70621	2	13.8531	36.39361	*0.026743*	19
Day / Night	0.454032	1	0.454032	1.192792	0.388781	18.51282
Error	0.761293	2	0.380647			
Total	28.92153	5				

Conductivty (Site 3)	α = 0.05					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	131.3725	2	65.68624	32.36991	*0.029967*	19
Columns	0.277605	1	0.277605	0.136803	0.746974	18.51282
Error	4.058475	2	2.029237			
Total	135.7086	5				

Temperature (Site 3)	α = 0.05					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Depth	2.587751	2	1.293875	25.50496	*0.037729*	19
Day / Night	0.020055	1	0.020055	0.39532	0.593751	18.51282
Error	0.101461	2	0.05073			
Total	2.709266	5				

Table 2.4 Two-Way ANOVA for Dissolved Oxygen and Conductivity at site 2 (South Basin). Only significant results shown

Dissolved Oxygen (Site 2)	α = 0.05					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Depth	0.212039	2	0.10602	20.4597	*0.046599*	19
Day / Night	0.000336	1	0.000336	0.064836	0.822799	18.51282
Error	0.010364	2	0.005182			
Total	0.222739	5				

Conductivity (Site 2)	α = 0.05					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Depth	49.46134	2	24.73067	1.15367	0.464324	19
Day / Night	925.7844	1	925.7844	43.18725	*0.022381*	18.51282
Error	42.87305	2	21.43652			
Total	1018.119	5				

Table 2.5 Volumetric densities of fish (g/m3) and major crustacean zooplankton community (ind/L) calculated from data obtained from Lumb *et al.* (2012) and zooplankton stratified sampling – Summer 2011. SURF=Surface layer; MID= Middle layer; DEEP= Deep layer; CLAD= Cladocera; CAL= Calanoid copepod; CYC= Cyclopoid copepod; NAP= Nauplii; RETRO= *D. retrocurva*; MEND= *D. mendotae*; REMIS= *D. longiremis*; ROSTRIS= *B. longirostris*; CORE= *E. coregoni*; DIA= *Diaphanosoma sp.*

BASIN-SITE	DEPTH	FISH	CLAD	CAL	CYC	NAP	RETRO	MEND	REMIS	ROSTRIS	CORE	DIA
NB - S2 Day	SURF	0.3	8.5	1.7	0.9	0.9	0.2	0.1	0	0.2	7.8	0.2
	MID	0.0	0.4	0.4	2.1	0.6	0.0	0	0	0.3	0.1	0
	DEEP	0.7	1.7	0.7	0.9	0	0.8	0	0	0.8	0.1	0.0
NB - S2 Night	SURF	0.3	0.6	9.6	5.7	1.1	0	0	0	0.1	0.4	0.0
	MID	3.7	4.9	19.6	11.5	6.2	1.3	0.3	0.1	1.4	1.8	0.0
	DEEP	2.6	0.4	0.9	0.7	0.2	0	0.0	0	0.3	0.1	0
NB - S3 Day	SURF	0.6	18.3	16.4	27.7	23.7	5.2	1.1	0.3	7.9	3.7	0
	MID	0.2	83.9	14.9	28.6	29.4	23.6	6.1	0.4	39.9	11.9	2.0
	DEEP	1.7	12.7	29.4	3.4	0	2.2	0.4	0.2	7.5	2.4	0
NB - S3 Night	SURF	0.0	9.2	13.9	11.1	11.3	4.0	1.1	0.1	0.6	3.2	0.1
	MID	2.5	7.4	7.2	10.4	11.3	2.3	0.3	0	2.3	2.4	0.1
	DEEP	1.7	2.6	2.1	3.3	1.9	0.6	1.1	0	0.2	0.6	0.1

BASIN-SITE	DEPTH	FISH	CLAD	CAL	CYC	NAP	RETRO	MEND	DIA
SB - S1 Day	SURF	0.2	0.2	26.7	18.2	18.5	0	0	0
	MID	0.7	0.8	33.3	41.4	45.2	0	0.4	0
	DEEP	1.1	0.9	9.4	10.0	14.1	0	0.8	0
SB - S1 Night	SURF	0.1	7.4	0	2.5	0	0.7	6.0	0.5
	MID	6.9	3.2	18.8	16.6	0.6	0.0	3.1	0
	DEEP	3.2	0.8	2.2	1.9	0.3	0.1	0.7	0
SB - S2 Day	SURF	13.2	0.6	9.1	2.5	0.9	0	0.5	0
	MID	2.7	2.4	17.0	6.6	0.9	0	2.4	0
	DEEP	0.7	0.4	13.9	6.0	5.0	0.1	0.2	0
SB - S2 Night	SURF	2.6	0.0	1.6	0.3	0	0	0	0
	MID	4.5	3.5	20.4	11.9	6.3	0	3.4	0
	DEEP	1.2	0.3	3.8	1.0	0.7	0	0.2	0
SB - S3 Day	SURF	1.7	0.4	11.3	5.7	5.0	0	2	0.8
	MID	3.2	0.0	12.6	3.5	3.8	0	2	0.1
	DEEP	0.1	0.2	9.4	10.4	8.5	0	0	0.0
SB - S3 Night	SURF	0.6	2.6	32.0	43.3	9.7	0	0	0.0
	MID	7.9	2.1	14.1	11.6	15.4	0	0	0.0
	DEEP	4.3	0.4	30.8	9.7	2.5	0	0	0.1

Table 2.6 Density (ind/L) of major crustacean zooplankton groups between integrated sampling and stratified sampling (average between layers)

North Basin		Int Day	Int Night	Strat Day	Strat Night
SITE 2	*Cladocera*	38.3	24	3.5	2.0
	Calanoid	26.5	9.9	0.9	10.3
	Cyclopoid	1.8	10.2	1.3	6.0
	Nauplii	3.9	1.1	0.5	2.5
SITE 3	*Cladocera*	46.0	26.5	38.3	6.4
	Calanoid	16.3	21.8	20.2	7.7
	Cyclopoid	27.4	7.7	19.9	8.3
	Nauplii	5.2	23.4	17.7	8.2
South Basin		Int Day	Int Night	Strat Day	Strat Night
SITE 1	*Cladocera*	1.1	2.5	0.6	4
	Calanoid	24.3	19.0	23.1	7.0
	Cyclopoid	18.8	18.4	23.2	7.0
	Nauplii	26.1	22.2	25.9	0.3
SITE 2	*Cladocera*	0.7	2.2	1	1.3
	Calanoid	38.5	23.6	13.3	8.6
	Cyclopoid	20.2	12.7	5.0	4.4
	Nauplii	28.3	19.7	2.3	2.3
SITE 3	*Cladocera*	0.6	2.1	0.2	1.7
	Calanoid	26.5	18.8	11.1	25.6
	Cyclopoid	18.4	13.6	6.5	21.5
	Nauplii	29.9	29	5.8	9.2

Table 2.7 P-values of comparison between integrated and stratified sampling (T-Test) for each major crustacean zooplankton taxa.

	NB		SB	
	Day	Night	Day	Night
Cladocera	0.18	0.03*	0.66	0.99
Calanoid	0.60	0.52	0.18	0.43
Cyclopoid	0.46	0.58	0.34	0.58
Nauplii	0.67	0.56	0.18	0.004*

* indicates $p < 0.05$

Table 2.8 Average density across depth strata of major crustacean zooplankton groups and of Cladocera species (ind/L) from day / night stratified sampling

		NB				SB					
		SITE 2		SITE 3		SITE 1		SITE 2		SITE 3	
		DAY	NIGHT	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT
Groups	**Cladocera**	3.5	2.0	38.3	6.4	0.6	3.8	1.1	1.3	0.2	1.7
	Calanoid	0.9	10.3	20.2	7.7	23.1	7.0	13.3	8.6	11.1	25.6
	Cyclopoid	1.3	6.0	19.9	8.3	23.2	7.0	5.0	4.4	6.5	21.5
	Nauplii	0.5	2.5	17.7	8.2	25.9	0.3	2.3	2.3	5.8	9.2
Cladocera	***D. retrocurva***	0.35	0.42	10.35	2.33	0.00	0.28	0.03	0.00	0.00	0.00
	D. mendotae	0.04	0.09	2.54	0.85	0.39	3.26	1.00	1.20	1.38	0.16
	D. longiremis	0.00	0.02	0.31	0.02	0.00	0.00	0.00	0.00	0.00	0.00
	B. longirostris	0.43	0.61	18.43	1.03	0.00	0.00	0.00	0.00	0.00	0.00
	E. coregoni	2.65	0.78	6.00	2.07	0.00	0.00	0.00	0.00	0.00	0.00
	Diaphanosoma sp.	0.08	0.01	0.66	0.12	0.26	0.22	0.07	0.07	0.31	0.04

Table 2.9 MRD values of Zooplankton groups from the stratified day/night sampling. D=day, N=night.

MRD North Basin						
Site		Fish Depth	Cladocera Depth	Calanoid Depth	Cyclopoid Depth	Nauplii Depth
2	D	9.2	4.5	5.8	7.7	4.9
	N	7.6	7.7	6.5	6.5	7.4
3	D	9.5	7.6	8.1	6.0	5.8
	N	7.8	6.1	5.3	6.2	5.9

MRD South Basin						
Site		Fish Depth	Cladocera Depth	Calanoid Depth	Cyclopoid Depth	Nauplii Depth
1	D	8.6	6.2	4.1	4.5	4.7
	N	7.0	3.3	5.2	4.8	7.1
2	D	2.4	4.8	5.0	5.3	6.4
	N	4.8	5.2	5.1	5.1	5.2
3	D	3.7	4.4	4.8	5.7	5.6
	N	7.1	3.7	5.0	3.4	4.2

Table 2.10 Correlation coefficients of mean values of environmental variables and zooplankton taxa within each depth stratum in each basin (averaged across sites) and between day and night

Pearson Product-Moment Correlation - North Basin (Daytime)

	CLADOCERA	CALANOID	CYCLOPOID	NAUPLII	*D. retrocurva*	*D. mendotae*	*D. longiremis*	*B. longirostris*	*E. coregoni*	*Diaphanosoma sp.*
TEMP (°C)	0.152	-0.002	0.455	0.475	0.005	0.003	0.22	-0.06	0.264	-0.081
DO (mg/L)	0.281	0.162	0.47	0.491	0.154	0.163	0.335	0.117	0.535	0.113
FLUOR/Chla (µg/L)	-0.62	-0.814	-0.163	-0.11	-0.242	-0.22	-0.515	-0.332	0.117	-0.111
COND (µS/cm)	0.608	0.553	0.643	0.64	0.211	0.194	0.618	0.195	0.333	0.037
TURB [NTU]	-0.859	-0.719	-0.835	-0.821	-0.587	-0.576	-0.875	-0.594	-0.547	-0.429

Pearson Product-Moment Correlation - North Basin (Nighttime)

	CLADOCERA	CALANOID	CYCLOPOID	NAUPLII	*D. retrocurva*	*D. mendotae*	*D. longiremis*	*B. longirostris*	*E. coregoni*	*Diaphanosoma sp.*
TEMP (°C)	0.532	0.597	0.595	0.509	0.538	-0.126	0.452	0.122	0.559	-0.067
DO (mg/L)	0.503	0.712	0.702	0.516	0.417	-0.345	0.432	0.374	0.526	-0.215
FLUOR/Chla (µg/L)	-0.015	0.543	0.437	0.017	-0.184	-0.141	0.139	0.302	0.012	-0.196
COND (µS/cm)	0.767	0.441	0.598	0.701	0.866	0.522	0.56	0.04	0.788	0.494
TURB [NTU]	-0.669	-0.414	-0.587	-0.692	-0.632	0.229	-0.352	-0.475	-0.663	-0.05

Pearson Product-Moment Correlation - South Basin (Daytime)

	CLADOCERA	CALANOID	CYCLOPOID	NAUPLII	*D. retrocurva*	*D. mendotae*	*Diaphanosoma sp.*
TEMP (°C)	0.027	-0.783	-0.947	-0.95	0.123	0.068	-0.94
DO (mg/L)	0.142	-0.712	-0.784	-0.788	0.224	0.146	-0.887
FLUOR/Chla (µg/L)	0.339	-0.001	-0.266	-0.287	0.189	0.215	-0.282
COND (µS/cm)	-0.356	0.16	0.262	0.347	-0.478	-0.457	0.144
TURB [NTU]	0.345	-0.4	-0.544	-0.616	0.546	0.464	-0.419

Pearson Product-Moment Correlation - South Basin (Nighttime)

	CLADOCERA	CALANOID	CYCLOPOID	NAUPLII	*D. retrocurva*	*D. mendotae*	*Diaphanosoma sp.*
TEMP (°C)	0.284	0.16	0.466	-0.051	0.355	0.62	0.417
DO (mg/L)	0.456	0.801	0.548	0.622	-0.721	-0.123	-0.284
FLUOR/Chla (µg/L)	0.294	0.446	0.709	0.385	0.1	0.382	0.46
COND (µS/cm)	0.107	0.085	0.412	0.152	0.343	0.382	0.269
TURB [NTU]	-0.131	-0.072	-0.409	-0.172	-0.342	-0.38	-0.297

Table 2.11 Correlation analysis between zooplankton density (ind/L) at each site and depth with fish volumetric density (g/m^3) for North Basin and South Basin

		NORTH BASIN				SOUTH BASIN					
		S2 - D	**S2 - N**	**S3 - D**	**S3 - N**	**S1 - D**	**S1 - N**	**S2 - D**	**S2 - N**	**S3 - D**	**S3 -N**
Groups	**Cladocera**	0.069	0.721	-0.754	-0.452	0.95	-0.594	-0.263	0.876	-0.645	-0.215
	Calanoid	0.16	0.355	0.986	-0.727	-0.654	0.934	-0.853	0.856	0.995	-0.889
	Cyclopoid	-0.822	0.362	-0.974	-0.288	-0.188	0.873	-0.958	0.882	-0.981	-0.848
	Nauplii	-0.66	0.65	-0.997	-0.203	-0.067	0.999	-0.623	0.857	-0.969	0.424
Cladocera species	***D. retrocurva***	0.976	0.749	-0.793	-0.664	NA	-0.914	-0.623	NA	NA	NA
	D. mendotae	-0.082	0.749	-0.786	-0.746	0.989	-0.511	-0.247	0.886	0.929	-0.985
	D. longiremis	NA	0.749	-0.856	-0.95	NA	NA	NA	NA	NA	NA
	B. longirostris	0.789	0.804	-0.715	0.59	NA	NA	NA	NA	NA	NA
	E. coregoni	-0.078	0.617	-0.787	-0.484	NA	NA	NA	NA	NA	NA
	***Diaphanosoma* sp.**	0.069	0.749	-0.706	-0.203	-0.092	-0.705	-0.922	-0.775	0.144	0.015

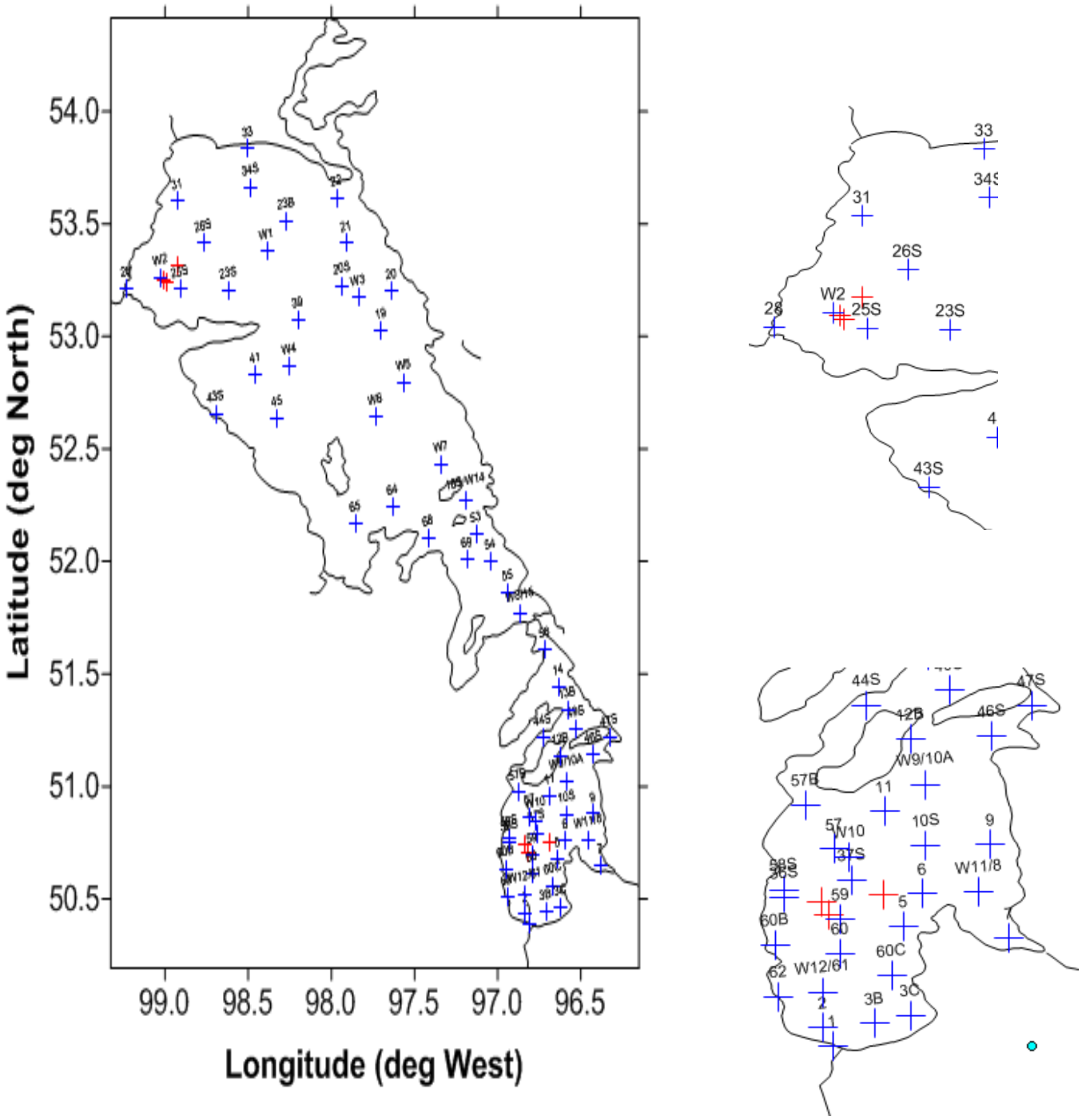


Figure 2.1 Map of Lake Winnipeg with all the sampling stations marked in blue. Stations were stratified zooplankton sampling was done marked in red.

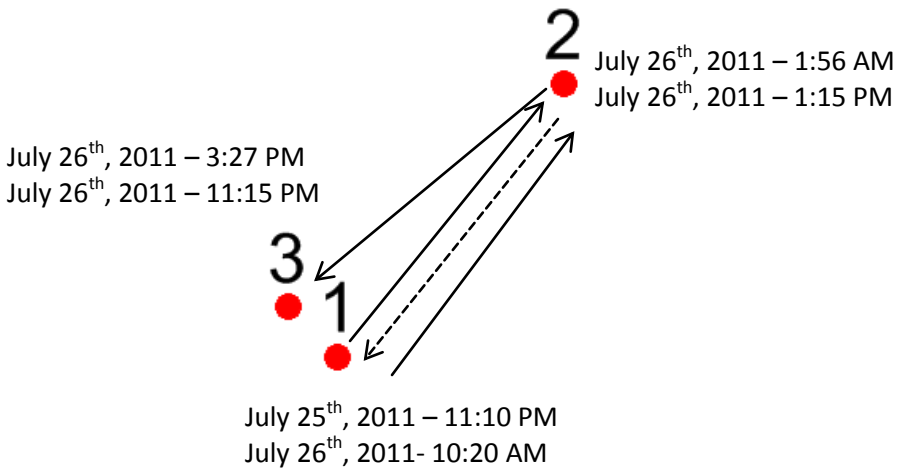


Figure 2.2 North Basin - MV *Namao* travel for stratified sampling.

a= Night sampling between July 25th – 26th

Dash line= boat trip to return to point 1

b= Day sampling on July 26th

c= Day sampling on July 26th, and anchored at Point 3 for Night sampling.

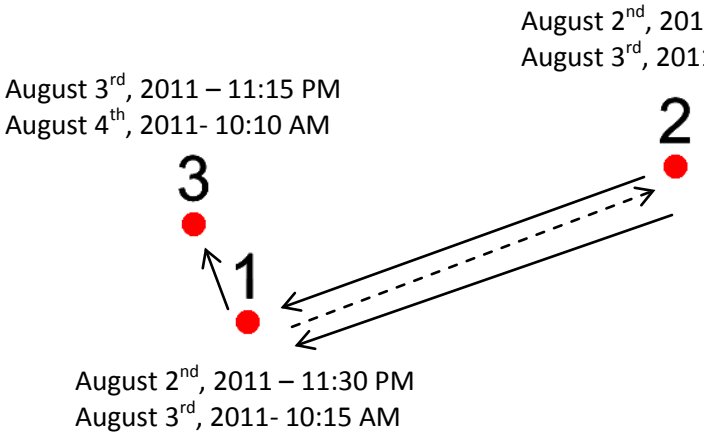


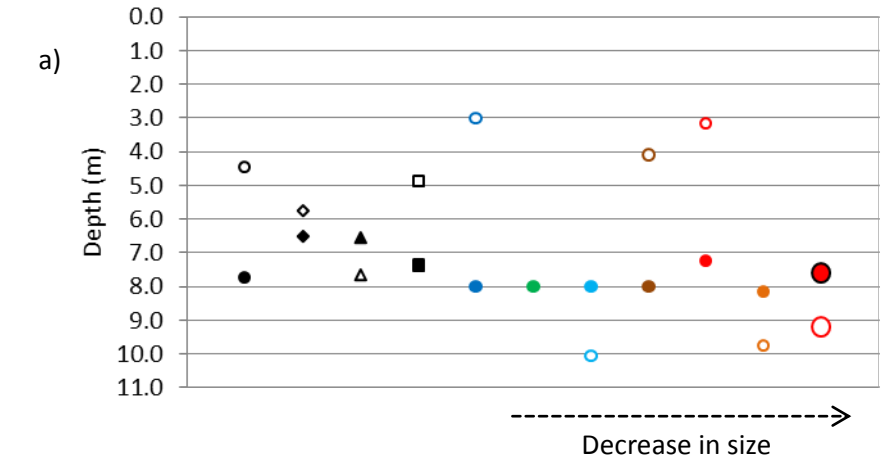
Figure 2.3 South Basin - MV *Namao* travel for stratified sampling.

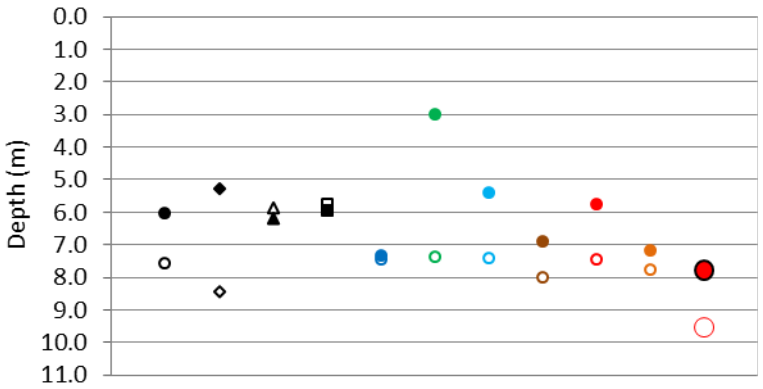
a= Day sampling at Point 2 and Night sampling at Point 1 (August 2nd)

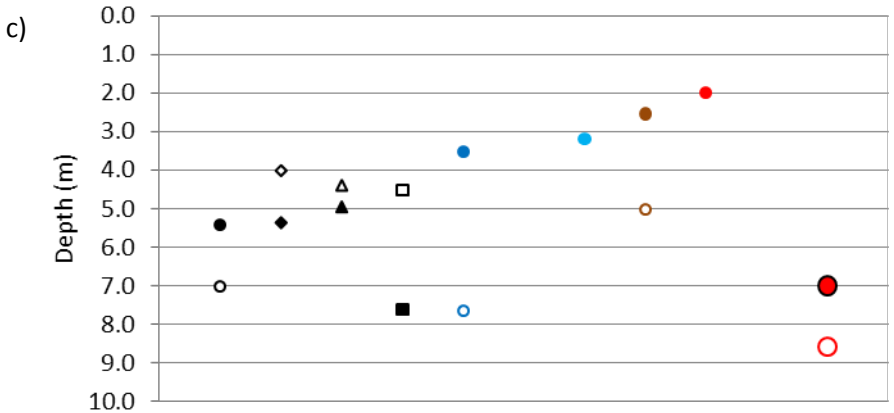
Dash line= boat trip to return to point 2

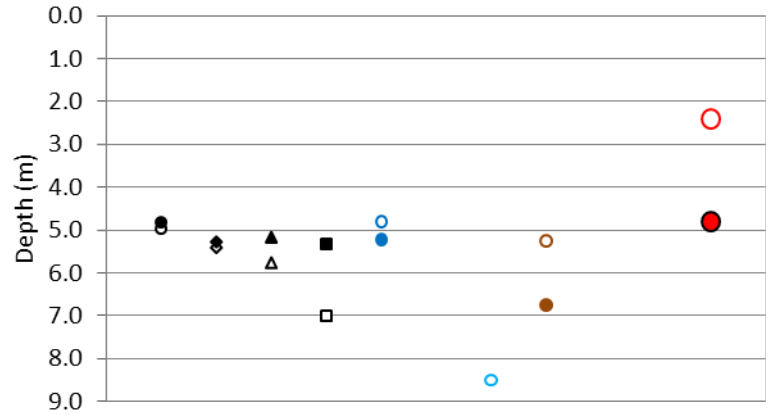
b=Night sampling at Point 2 and Day sampling at Point 1 (August 2nd)

c= Travel to Point 3 for day and night sample









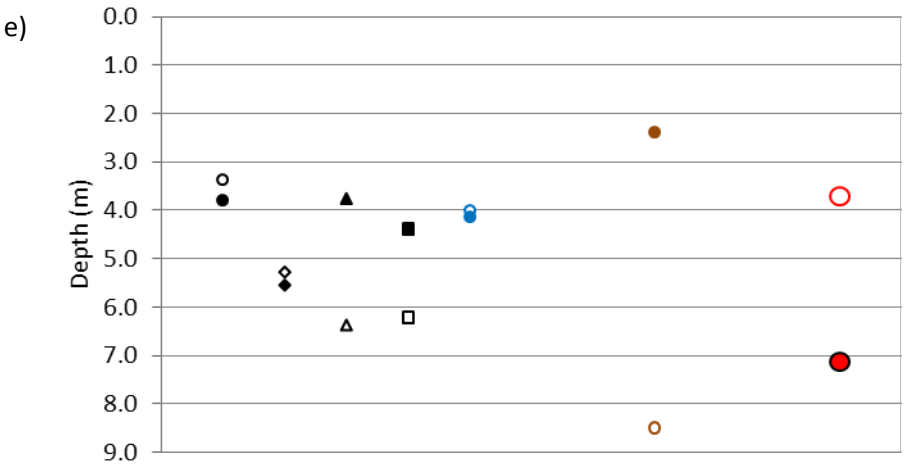


Figure2. 4 Mean Residence Depth for day and night sampling. Empty symbols correspond to daytime data. Filled symbols represent nighttime data. Cladocera (circle); Calanoid (diamond); Cyclopoid (Triangle); Nauplii (square).

Daphnia mendotae *(*blue); ***Daphnia longiremis*** (green); ***Daphnia retrocurva*** (light blue); ***Diaphanosoma sp.***(brown); ***Eubosmina coregoni*** (red); ***Bosmina longirostris*** (orange) – ordered in decreasing size; **FISH (Red)**

a) Point 2 NB; b) Point 3 NB; c) Point 1 SB; d) Point 2 SB; e) Point 3 SB

CHAPTER 2: APPENDIX

Densities of all identified Cladocera species also varied with depth strata in North and South Basin (Table A2.1). In North Basin, all species had higher abundance in the surface and middle layers for both daytime and nighttime at all sites. For South Basin, there were fewer species present in the samples. *Daphnia retrocurva* had higher abundance in the surface during the day but in deep layers at night. *Daphnia mendotae* had higher abundance in the middle and deep layers at all sites during daytime. At night at all sites, *D. mendotae* was present at higher concentrations in the surface and middle layers. *Diaphanosoma sp.* was more abundant in the surface and middle layers during daytime and higher at the surface and middle layers during the night.

Table A2.1 Abundance (ind/L) of Cladocera species from day / night stratified sampling

		NB				SB					
SPECIES	DEPTH	SITE 2		SITE 3		SITE 1		SITE 2		SITE 3	
		DAY	NIGHT	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT	DAY	NIGHT
D. retrocurva	SURF	0.21	0	5.23	4.03	0	0.71	0	0	0	0
	MID	0.04	1.27	23.63	2.33	0	0	0	0	0	0
	DEEP	0.80	0	2.20	0.62	0	0.12	0.09	0	0	0
D. mendotae	SURF	0.11	0	1.13	1.13	0	6.01	0.47	0.00	1.77	0.35
	MID	0	0.28	6.08	0.28	0.35	3.07	2.36	3.42	2.00	0.00
	DEEP	0	0	0.39	1.13	0.83	0.71	0.18	0.18	0.35	0.12
D. longiremis	SURF	0	0	0.28	0.07	0	0	0	0	0	0
	MID	0	0.07	0.42	0	0	0	0	0	0	0
	DEEP	0	0	0.24	0	0	0	0	0	0	0
B. longirostris	SURF	0.18	0.14	7.92	0.64	0	0	0	0	0	0
	MID	0.32	1.41	39.90	2.26	0	0	0	0	0	0
	DEEP	0.80	0.27	7.47	0.18	0	0	0	0	0	0
E. coregoni	SURF	7.76	0.42	3.68	3.18	0	0	0	0	0	0
	MID	0.07	1.84	11.88	2.41	0	0	0	0	0	0
	DEEP	0.11	0.09	2.44	0.62	0	0	0	0	0	0
Diaphanosoma sp.	SURF	0.22	0	0	0.14	0.18	0.53	0	0.12	0.83	0
	MID	0	0.04	1.98	0.14	0.47	0.12	0.12	0.00	0.12	0
	DEEP	0.04	0	0	0.09	0.12	0	0.09	0.09	0	0.12

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CHAPTER 3: LONG-TERM CHANGES IN THE ZOOPLANKTON COMMUNITY OF LAKE WINNIPEG

INTRODUCTION

Eutrophication is one of the most significant environmental problems for aquatic ecosystems. This process involves the excessive input of nutrients, such as phosphorus and nitrogen from external sources (e.g. municipal waste water, agriculture, factory farms, large-scale land uses) (Carpenter *et al*., 1998; Schindler, 2006, Schindler *et al.*, 2012, Bunting *et al*., 2011). Surplus concentrations of these nutrients may lead to changes in the phytoplankton community, one of which is an increasing predominance of often toxic buoyant cyanobacteria that are generally inedible by the zooplankton community (Carpenter 2003; Carpenter and Brock, 2006; Rondel *et al*., 2007; Ghadouani *et al.*, 2003).

Lake Winnipeg is a eutrophic lake and has experienced algal blooms (including cyanobacteria) since at least 1990 (North/South Consultants Inc., 2006). The lake receives its water inflow and nutrient input predominantly via a number of rivers. The main water inflow into the South Basin comes from the Winnipeg River (from the Precambrian Shield). However, the majority of nutrients come from the Red River, which carries nutrients from the agricultural regions in the south. A report from North/South Consultants Inc. (2006), showed a long-term historical pattern of escalating nutrient loading (phosphorus and nitrogen) to the lake, and McCullough *et al*. (2012) indicated that over a 30-year period, this abrupt rise of nutrient loading occurred during the 1990s caused by increased runoff and flooding from the main source of phosphorus to Lake Winnipeg, the Red River. The main water inflow into the North Basin comes

from the N. Saskatchewan River, which does not carry as high nutrient load as its South Basin counterpart, the Red River (Patalas and Salki, 1992), despite draining a much larger watershed.

Lake Winnipeg has been affected by invasive species, specifically Rainbow Smelt (*Osmerux mordax),* which was first detected in Lake Winnipeg in 1990 (Sheppard *et al*., 2011), and has altered the food web structure and energetic pathways in the Laurentian Great Lakes (Johnson *et al*., 2004; Roth *et al*., 2010). In Lake Winnipeg, Sheppard *et al*. (2011) found that Rainbow Smelt selectively consumed larger species of zooplankton, mainly *Daphnia* spp., as a consequence of their high visibility and slow swimming speeds in comparison with copepods. Therefore, invasion of a size-selective planktivore would be expected to have a substantial impact on the composition and overall abundance of the zooplankton community of the lake. Another invasive species is the cladoceran, *Eubosmina coregoni,* that has successfully established within the North Basin of Lake Winnipeg (Suchy *et al.*, 2010), and the invasion of which coincided with the introduction of Rainbow Smelt within the lake. Furthermore, Suchy *et al*. (2010) observed a decrease of *Bomina longirostris* density, another bosminid, in the same region of Lake Winnipeg.

The study of eutrophication and invasive species in Lake Winnipeg has been sporadic throughout the last 40 years. The first limnological survey was conducted in 1928-29 (Bajkov, 1934) and was followed only in 1969, when the Canadian Government Ship, M.V. *Bradbury*, conducted an extensive open water and ice cover season survey for both environmental and biological data under the direction of scientists of the Freshwater Institute (then Fisheries Research Board of Canada, FWI, Winnipeg, MB) (McCullough *et al*, 2012; North/South Consultants Inc., 2006). With the data from the 1969 survey, Patalas and Salki (1992) published the first detailed description of spatial and seasonal variation in the crustacean zooplankton

community of Lake Winnipeg. In the last years of the 1990s, a satellite image survey documented algal blooms extending over large areas of the North Basin of the lake that attracted public and government attention and concern (Liu *et al*., 2007; North/South Consultants Inc., 2006). Lake Winnipeg Research Consortium Inc. (LWRC) was formed in 1998 to facilitate and co-ordinate research on Lake Winnipeg between different provincial and federal agencies and numerous lake-based and non-profit organizations. Since 2002, the LWRC has conducted 3 open water season surveys in all years (except 2005) to gather information on physical, chemical and biological aspects of the lake (McCullough *et al.*, 2012; North/South Consultants Inc., 2006). According to these recent studies, phosphorus concentration, algal biomass, and the frequency and extent of cyanobacterial blooms have strongly increased since 1969. Between the 1970s and 1990s no complete lake surveys were conducted; therefore, there is no information available from which to infer when and how these changes occurred (McCullough *et al*., 2012). During 1969, cyanobacteria constituted only 56% of the total heterogeneous phytoplankton community (Kling *et al.*, 2011,). However, since that time, cyanobacteria have increased to comprise 90% of the total phytoplankton biomass, especially seen as an increase in the phytoplankton and total net plankton during the mid-summer (Kling *et al.,* 2011). This increase was correlated with an increase in total phosphorus (TP) (McCullough *et al*., 2012). Furthermore, South Basin is more turbid than the North Basin (Patalas and Salki, 1992) which might have an impact of visual predation by planktivorous fish.

The critical need for a detailed study regarding the zooplankton community of this lake and how it has changed through time cannot be understated. More importantly, taking into consideration other large, shallow lakes such as Lake Champlain, Lake Balaton, and many of the Laurentian Great Lakes (Stewart *et al.*, 2010; Johannsson *et al*., 2000; Barbiero *et al.,* 2008;

Kane *et al*., 2004; Miller *et al*., 2010), we might predict the directions that Lake Winnipeg will take in regards to changes in its zooplankton community, and effects on commercial fisheries and water quality, if nutrient loading continues unabated.

The first question to be addressed in this study was the effect on the overall crustacean zooplankton community structure of two major environmental stressors (nutrient loading and invasive species) in Lake Winnipeg. We selected 1969 and 2002 for detailed comparison as comprehensive datasets from all open water seasons for crustacean zooplankton were available; 1969 represents the state of the lake prior to intensification of nutrient loading (McCullough *et al*., 2012) and invasion of several non-indigenous species, whereas 2002 reflects conditions during the most recent decade of eutrophication as well as post-invasion of aquatic taxa. The extensive analysis of the crustacean zooplankton community in 1969 (e.g. abundance, spatial and seasonal distribution, life history) (Patalas and Salki, 1992) is relevant as a baseline for comparison with all subsequent surveys executed in the lake.

The second question addressed was a more detailed examination of long-term changes that occurred in the Cladocera community, specifically during the summer season of 1969, 1994, 1999, 2002-06, and 2011. Patterns of change were investigated intensively in summer as this is a period with a higher air and water temperature, consequently higher metabolic activity and cyanobacterial bloom formation (Sommer and Lampert, 2007; Wetzel, 2001). Cladocera are the most species-rich, well-studied zooplankton organisms in modern ecological and paleolimnological studies; hence, extensive comparisons are possible with responses of Cladocera in other large lakes subject to similar environmental stressors (Jansen *et al.*, 2011; Altshuler *et al.,* 2011; Sweetman and Finney, 2003; Eggermont and Martens, 2011; Rumes *et al.*, 2011; Seda and Petrusek, 2011).

We hypothesise that the crustacean zooplankton community would increase in density due to eutrophication, as increased nutrients could lead to higher primary production and potentially more food for the crustacean zooplankton community with consequently an overall higher density (McCauley and Kalff, 1981; Hanson and Peters, 1984; Patalas and Salki, 1992). However, both composition and relative abundance of the phytoplankton (primary producers) would be expected to change but in an unknown fashion with eutrophication of the lake, thus affecting the quality and quantity of the diet available for crustacean zooplankton. Whereas an increase in nutrient loading in general may be expected to lead to higher primary producer biomass, the consequences of possible changes in N:P ratio and predominance of cyanobacteria which may be inedible and/or toxic, may offset the effects of increased nutrient loading to the lake (Haney, 1987; Ferrao-Filho, 2003; Bednarska *et al*., 2011, Kling *et al*., 2011). Rainbow Smelt and *E. coregoni* were first reported in the North Basin of Lake Winnipeg in 1990 (Sheppard *et al.*, 2012; Suchy *et al.*, 2010), and over the subsequent 20 years have become dominant constituents of the planktivorous fish and zooplankton communities, respectively (Sheppard *et al.*, 2011). Hence, the structure of the food web in that basin, and predation pressure on the zooplankton community, may have altered the structure and composition of the crustacean zooplankton community relative to what existed in 1969. However, almost all previous studies of the consequences of invasion of Rainbow Smelt on zooplankton communities have been on dimictic, kettle lakes (Christie *et al.*, 1972; Evans and Loftus, 1987; Mercado-Silva *et al*., 2006; Vander Zanden and Olden, 2008), in sharp contrast with limnological conditions of Lake Winnipeg; hence, only tenuous predictions with respect to consequences of increased predation pressure on larger-bodied Cladocera species and other zooplankton in Lake Winnipeg are feasible.

The objectives of this research are: (1) to contrast seasonal (spring, summer, and fall) and spatial (North Basin vs. South Basin) patterns within the major crustacean zooplankton groups (Cladocera, calanoid copepods, cyclopoid copepods and copepod nauplii) before (1969) and after (2002) intensive eutrophication of Lake Winnipeg and invasion of Rainbow Smelt; and (2) to examine long-term patterns of change in Cladocera in response to eutrophication as an environmental stressor, during the summer only in 1969, 1994, 1999, 2002, 2003, 2004, 2006 and 2011.

METHODS

Study Site

Lake Winnipeg is the 10th largest lake in the world by surface area, covering approximately 3.7% of the surface area of Manitoba. It can be separated into three main regions, a North and South Basin, separated by the Narrows (Patalas and Salki, 1992; North/South Consultants Inc., 2006). The land surrounding the west and south region of the lake is underlain by sedimentary rock and that surrounding the east of the lake is comprised of Precambrian Shield. To add more complexity, the lake receives its water inflow and nutrient input via a number of rivers (summarized in McCullough *et al.,* 2012). For the South Basin the main water inflow comes from the Winnipeg River (draining from the Precambrian Shield). Lake Winnipeg supports a large commercial fishery, subsistence fishery, recreation and livelihood to lakeshore populations such as Gimli, and many First Nation communities (North/South Consultants Inc., 2006).

Field Sampling (Figure 1)

In all years, field collections were done at the same predetermined stations in Lake Winnipeg. Stations are located in all regions of the lake, including the North Basin, Narrows, and South Basin (Figure 1).

In 1969, regular field surveys were conducted aboard M.V. *Bradbury* at approximately biweekly intervals throughout the open water season. In all other years, field collections were done 3 times annually during the open water season, representing spring, summer, and fall periods using the M. V. *Namao*, a scientific vessel supported by the Lake Winnipeg Research Consortium. Collection of water samples during 1969 cruises is described in Patalas and Salki (1992).

Zooplankton samples in all years of the study were collected using consistent methods, i.e. an integrated zooplankton haul, from 1 meter above the bottom sediments to the surface with the use of a single net, 1 m long, with 73 μm mesh and net opening of 0.049 m^2. Samples were made up to a standard volume of 125 mL and preserved with 10% formalin (Sheppard *et al.*, 2011).

Laboratory analyses

a) Environmental variables

The water samples collected in 1969, and 2002 – 2006 were analyzed by the FWI water chemistry laboratory under the supervision of Mr. Michael Stainton (DFO, Winnipeg, MB) for many chemical and physical properties., Total phosphorus (TP), total nitrogen (TN), and chlorophyll-*a* (CHLA) data for the relevant years were provided by Mr. M. Stainton (also

available on the Environment Canada Data Portal; http://lwbi.cc.umanitoba.ca). TP, TN, and CHLA data for summer 2011 was provided by Ms. Elaine Page (Manitoba Conservation and Water Stewardship, Winnipeg, MB).

b) Zooplankton

Zooplankton collected in 1969, 1994, 1999, 2002-2006 were identified and enumerated by Mr. Alex Salki (formerly DFO and Salki Consultants, Inc., Winnipeg, MB). Datasets of the zooplankton community density for all seasons in 1969 and 2002 were provided by Mr. Alex Salki (Salki Consultants, Inc., Winnipeg, MB; also available on the Environment Canada Data Portal, http://lwbi.cc.umanitoba.ca). Cladocera community density (individuals per litre) during the summer season for 1969, 1994, 1999, 2002 – 2006 were also provided by Mr. Alex Salki (Salki Consultants, Inc., Winnipeg, MB). At each station, an integrated vertical haul was sampled using a Wisconsin net of 73-µm mesh size from approximately a meter above the bottom (Patalas and Salki, 1992). In 2011, I collected zooplankton samples on board the M.V *Namao,* during the summer season from the same pre-established stations in Lake Winnipeg. Data for summer of the year 2005 were unavailable as it was an unusually wet year and the summer scientific survey could not be executed.

For 2011 samples, according to methods described in Sheppard *et al.* (2011), the 125 mL sample was thoroughly mixed, and a subsample of 5 ml was taken with a wide-bore syringe and placed in a square gridded petri dish. With the use of a dissecting microscope at 40× magnification, Cladocera were identified to species (Pennak, 1989; Balcer *et al.*, 1984). To ensure consistency, identifications of representative specimens from 2011 samples were verified by Mr. Alex Salki (Salki Consultants, Winnipeg, MB). Copepods were identified as calanoid

copepods, cyclopoid copepods, and nauplii larvae. A minimum of 200 individuals was counted in each subsample. Within the Cladocera community common in Lake Winnipeg, *Daphnia galeata mendotae*, previously identified by Patalas and Salki (1992), was identified in this research as *Daphnia mendotae*, as a consequence of taxonomic revision of the species (Taylor *et al.*, 1996). Furthermore, the species previously identified as *Diaphanosoma leuchtenbergianum* is valid for the European taxon, but the North American taxon is currently identified as *Diaphanosoma birgei* (Kořinek, 1981).

Data analyses

Because sampling intervals in 1969 (6 surveys) and subsequent surveys (3 surveys annually) were different, environmental variables and the density of the zooplankton community from 1969 surveys that were most representative for each season were pooled (e.g. spring: June 4-12, summer: July 9-16, July 24 – August 1, fall: September 2 – 10, October 3-13, October 27-31).

In publications arising from the 1969 surveys (e.g., Patalas and Salki, 1992), North Basin (NB), the Narrows (Nar), and South Basin (SB) were treated separately. In this study, data collected from stations in the North Basin and the Narrows were pooled (see analyses below), whereas those data representing the South Basin remained separate.

Environmental variables

a. 1969 vs 2002, 3 seasons, Total Nitrogen (TN), Total Phosphorus (TP) and Chlorophyll-*a* (CHLA).

To determine if there were significant differences in TP, TN, and CHLA between the basins (NB, Nar, SB) and years in each season independently, a two-way ANOVA was used

(with basin and year as factors). Tukey's HSD Test was used as a post-hoc test to determine where significant differences occurred (Dytham, 2011).

b. Summer Season (1969, 1994, 2002 – 2006, 2011)

The values of TP, TN and CHLA for the summer season of 1969, 2002 – 2006 and 2011 were examined individually via a one-way ANOVA to determine if a temporal pattern existed across years in North Basin (including Narrows) and South Basin separately (Dytham, 2011). Tukey's HSD Test was subsequently used as a post-hoc test to identify significant differences within each variable among years (Dytham, 2011).

Zooplankton community

a. 1969 vs 2002, 3 seasons, Cladocera, Cyclopoida, Calanoida, Calanoid nauplii, Cyclopoid nauplii

In 1969, the average density (individuals per litre) for the major crustacean zooplankton groups (cladocerans, calanoid, cyclopoid copepods and nauplii) of the two sampling periods corresponding to each of three seasons (spring, summer, and fall) was calculated with standard error. With 2002 data, the density (individual per litre) average of the overall major crustacean zooplankton groups from each season was calculated with their corresponding standard error.

A two-way ANOVA (with basin and year as factors) was done with all major crustacean zooplankton groups to detect differences between 1969 and 2002 in each basin in each season independently (Dytham, 2011). Tukey's HSD Test was used as a post-hoc test (Dytham, 2011). In this analysis, calanoid copepods and cyclopoid copepods included calanoid and cyclopoid nauplii, respectively.

In addition, Canonical Correspondence Analysis (CCA) (Borcard *et al.*, 2011), was used to compare the differences between the basins of 1969 and 2002 according to the major crustacean zooplankton groups and TP, TN and CHLA. CCA is a multivariate analysis technique, where the unimodal response of species (response variables) to environmental variables (predictors) is studied (Jongman *et al.*, 1995). This analysis is a general linear model (GLM) analogous to bivariate correlation (Pearson *r*), in this case, between two variable data sets (Sherry and Henson, 2005).The resulting ordination diagram shows variation in species composition and also the relationships between the species and environmental variable (Jongman *et al.*, 1995). Sites that are positioned close to a point of a species are likely to have a high abundance of that particular species. Site points close together represent sites that have similar species composition and abundance. Sites positioned apart from each other differ in species abundance or species composition (Jongman *et al.*, 1995). The environmental variables are shown as vectors. The longer the environmental vector, the higher the correlation between the environmental variable and the correlation axes, and the greater its influence on the overall pattern of species composition (ter Braak, 1988). Monte Carlo permutation tests were used to calculate the statistical significance between the environmental variable set and the species composition (Borcard *et al.*, 2011).

In this analysis, calanoid and cyclopoid copepod densities included the corresponding densities of nauplii. The three major zooplankton groups and the environmental variables studied for each year and basin were separated by season and tested for normality as required by the multivariate test using Shapiro-Wilk Test and Quantile-Quantile Plots (Borcard *et al.*, 2011; Mead, 1990). The data required transformation and was then $\log_{10}$-transformed to fit normality (Quinn and Keough, 2002) and tested once more through Shapiro-Wilk Test and Quantile-

Quantile plots. Mean values were then calculated from the $\log_{10}$-transformed data(log[x+1]) and used in the CCA in each season, comparing biological and environmental for all basins of the years 1969 and 2002.

b. Changes in Cladocera Community– Summer Season

One-way ANOVA and post-hoc test (Tukey HSD Test) were used to analyze the variation of densities of species of Cladocera in Lake Winnipeg for 1969, 1994, 1999, 2002 – 2006 and 2011 (Dytham, 2011).

CCA was used to determine the association of TP, TN, and CHLA on the Cladocera community (Dytham, 2011; Borcard *et al.*, 2011) of Lake Winnipeg with available information of 1969, 2002 – 2006 and 2011 during the summer season where algal blooms occur with higher frequency with R software 2.13.2 (R Development Core Team). Comparative environmental data for 1994 and 1999 were not available so these data were not included in the CCA.

Cladocera densities during the summer season of 1969, 1994, 1999, 2002, 2003, 2004, 2006, and 2011 were tested for normality using a Shapiro-Wilk test (Dytham, 2011). Density data required transformation and a $\log_{10}$-transformation was applied (Mead, 1990; Dytham, 2011). Shapiro-Wilk Test and Quantile-Quantile Plots (Dytham, 2011) were used to confirm normal distribution after $\log_{10}$-transformation. When using logarithms to transform data, the base is considered to be inconsequential although base-10 logs are more familiar and commonly used (Quinn and Keough, 2002). From these $\log_{10}$-transformed data, mean values from all stations in the North Basin (including the Narrows) and South Basin were determined for each Cladocera species.

Environmental variables were tested for normality using Shapiro-Wilk Test (Dytham, 2011) for the same years as mentioned above, and were also $\log_{10}$-transformed; success of the transformation was confirmed with Shapiro-Wilk Test and Quantile-Quantile Plots (Dytham, 2011). From the $\log_{10}$-transformed data, average values representing each year for the summer season were calculated.

The calculated mean values for the biological and environmental $\log_{10}$-transformed data were then applied to the CCA analysis. Correlation coefficients were obtained from the CCA analysis.

RESULTS

Number of species of crustacean zooplankton reported in 1969 was 26 and in 2002 was 23 (Table 3.1). Cladocera showed a reduction in species richness. *Daphnia ambigua* and *D. shloedleri* were not present in 2002. Calanoid copepods showed a reduction in species richness in 2002, where *Epischura nevadensis*, *Diaptomus leptopus* and *Diaptomus clavipes* were no longer reported. *Limnocalanus macrurus*, *Epischura lacustris*, *Diaptomus ashlandi*, *D. oregonensis*, *D. siciloides*, *D. sicilis* and *D. minutus* were present in both years. Cyclopoid copepods showed no changes in species richness between years; *Cyclops bicuspidatus thomasi (*now *Diacyclops thomasi*), *Cyclops vernalis*, *Mesocyclops edax*, *M. albidus*, and *Eucyclops agilis* were present in both 1969 and 2002.

Cladocera community mean density showed considerable variation among years during summer season (Figure 3.2 and Figure 3.3), and an overall increase in TP and TN levels in both basins (Figure 3.2 and Figure 3.3).

Environmental analysis

a) Comparison of Pre-eutrophication (1969) and Post-eutrophication (2002) Conditions in Lake Winnipeg

TP and TN concentrations (Table 3.2) were significantly higher in 2002 than in 1969 in all seasons in each basin of the lake (2-way ANOVA, Table 3.3). Concentrations in the North Basin (NB) and Narrows (Nar) were significantly lower than in the South Basin (SB) in spring and summer of 2002 but TP and TN were higher in NB and Nar than in SB in fall of 2002 (Tukey HSD Test, Table 3.3).

Chlorophyll-*a* concentrations (Table 3.2) were significantly higher in all basins in summer and fall in 2002 than in 1969 (Table 3.3); spring values were higher in 2002 than in 1969 but the difference was not significant (2-way ANOVA, Table 3.3). In 1969, no significant seasonal variation in CHLA was detected in any basin, whereas in 2002, concentrations were lower in NB than in SB in in all seasons, although the difference is not significant in fall (Tukey HSD Test, Table 3.3).

b) Changes in TN, TP and CHLA – Summer Season (1969, 2002 – 2006, 2011)

TP (Table 3.4) was significantly higher in the South Basin than in the North Basin (and Narrows) in all years except 2004 (one-way ANOVA, Table 3.5; Tukey HSD Test, Table 3.6). TN was also significantly higher in the South Basin than in the North Basin (and Narrows) in all years except 2003 and 2004 (one-way ANOVA, Table 3.5; Tukey HSD Test, Table 3.6). CHLA was significantly higher in the South Basin than in the North Basin (and Narrows) in 1969 only

(one-way ANOVA, Table 3.5; Tukey HSD Test, Table 3.6). The Narrows and North Basin did not differ significantly in TP and TN concentrations across all years (Table 3.6).

In all basins, there were significant differences across years in TP and TN (Table 3.7); CHLA varied significantly across years in SB only. Within the North Basin, TP and TN were significantly higher between 2002 and 2011 than in 1969 (one-way ANOVA, Table 3.7; Tukey HSD Test, Table 3.8). For subsequent analyses, the data from stations in the Narrows were pooled with those in the North Basin as there were no significant differences in TP or TN between these two basins in any year (Table 3.6). In the South Basin, all values of TP and TN were significantly higher from 2002 to 2011 than in 1969. CHLA varied considerably among years; values were elevated above 1969 values in all years except 2011; however, they were significantly higher only in 2003 (one-way ANOVA, Table 3.7; Tukey HSD Test, Table 3.8).

Zooplankton Community responses to Environmental Stressors

a) Comparison of Pre-eutrophication (1969) and Post-eutrophication (2002)

Cladocera density (Table 3.9) was significantly higher in 2002 than in 1969 in all seasons and all basins (with one exception, i.e. in SB in summer and in NB in fall, cladoceran density was lower in 2002) (two-way ANOVA, Table 3.10). In 1969, there was no substantial variation in cladoceran density among basins within a season; summer densities were general higher than in spring or fall in a given basin (Table 3.9).In 2002, there was considerable variation among basins and among stations in each season (Table 3.9). Densities in the Narrows and NB were generally higher in spring and summer, whereas SB densities were elevated in fall. Calanoid copepod density (Table 3.9) was significantly higher in all basins in 2002 than in 1969 in spring and summer (Two-way ANOVA, Table 3.10). There was no consistent pattern in calanoid

density among basins seasonally or in either 1969 or 2002. Cyclopoid copepod density (Table 3.9) was significant higher in 2002 than in 1969 only in spring (Two-way ANOVA, Table 3.10). In summer, North Basin density was higher than in the South Basin and Narrow Basin (Tukey HSD Test, Table 3.10), and in both years, density in the North Basin was higher than South Basin. In Fall, North Basin density of 1969 was higher than Narrow Basin and South Basin densities (Tukey HSD Test, Table 3.10).

Canonical correspondence analyses (Figure 3.4 – 3.6) showed distinct seasonal differences in response of zooplankton taxa to environmental conditions that existed in 1969 in contrast with 2002. In spring (Figure 3.4), 89% variation was accounted for in CCA1 axis. CCA axes 1 and 2 comprised of 90 % of the total variation. CCA axis 1 was strongly positively correlated with TP and TN ($r = 0.91$ and $r = 0.82$ respectively) and to a lesser degree with CHLA($r = 0.11$). Zooplankton communities representing each basin within a given year were tightly clustered but points representing 1969 were widely separated from those of 2002; those of 1969 presented negative site scores with CCA1, whereas those of 2002 had positive high scores with CCA1. In 1969, cyclopoid copepods were predominant components of the zooplankton community, especially in NB and SB (Figure 3.4, Table 3.9). In 2002, cladocerans were abundant in all basins, particularly in the Narrows; calanoids were also prevalent in all basins (Figure 3.4, Table 3.9).

In summer (Figure 3.5), 51% of variation was accounted for in CCA1 and both CCA1 and CCA2 axes variation was 57%. TP had a negative correlation with CCA2 axis of $r=-0.97$ and CHLA and TN a correlation of $r = -0.88$. Zooplankton communities represented in all basins in 1969 were clearly partitioned from those in 2002 along CCA2. There was more separation among basins within each year along CCA1 than on CCA2. Cladocera, and to a lesser extent

calanoid copepods, were associated with conditions of higher TP and TN present in 2002. Cyclopoid copepods were associated with conditions present in 1969. In 1969, the zooplankton community in the North Basin was predominantly composed of cyclopoids, whereas that found in the Narrows and South Basin was overwhelmingly calanoid copepods (Figure 3.5, Table 3.9). In contrast, the zooplankton community in 2002 was predominantly cladocerans in NB but calanoids in SB (Figure 3.5, Table 3.9).

In fall (Figure 3.6), CCA1 accounted for 63% of variation and both CCA1 and CCA2 axes accounted for 71% of variation. Environmental variables were positively correlated with CCA1, with TP presenting a correlation of r=0.62, TN a correlation of r=0.58 and CHLA with r=0.28. In regards with CCA2 axis, they were negatively correlated with r=-0.45 for TP, r=-0.52 for TN and r=-0.56 for CHLA. In 1969, zooplankton communities representative of all basins were tightly clustered, with calanoid copepods being the predominant component of the community (Figure 3.6, Table 3.9). In contrast, the basins in fall of 2002 were quite dissimilar. Cladocera and cyclopoid copepods were associated with conditions present in 2002, and calanoid copepods with the environment of 1969.

According to Monte Carlo permutation tests, all models comparing 1969 versus 2002 in the three seasons were found to be not significant ($p > 0.05$). .

b) Changes in Cladocera Community and the influence of eutrophication (1969, 1994, 1999, 2002 – 2006, 2011)

CCA results show that for North Basin (Figure 3.7), CCA2 axis summarized the variation between 1969 (pre-eutrophication) and the subsequent years corresponding to the first decade of 2000 (post-eutrophication). TP and TN were highly correlated with CCA2 axis (r=0.69 and

r=0.57 respectively). CHLA was slightly correlated with CCA2 axis, with r=0.45. . Furthermore, CCA2 axis summarizes the variation within the Cladocera community, grouping all species (positive score along CCA2 axis) to be correlated to eutrophication conditions, with a negative score along the CCA2 axis (i.e. between the first decade of 2002 and 1969). CCA1 axis summarized the variation along the first decade of 2000 within the Cladocera community. Although these share a common eutrophic state, 2002, and 2006 were associated with higher density of *Daphnia longiremis* (positive species score along CCA2 axis). Higher density of *Eubosmina coregoni* characterized the year 2011 from the other years of the first decade of 2000 (positive score along CCA1 axis). The density of other Cladocera species was associated with the year 2003 and also 1969. According to Monte Carlo permutation test, this result was not statistically significant. Data analyses with rare species (*Daphnia pulex*, Ceriodaphnia *quadrangula* and *Chydorus sphaericus)* removed and with rare species present (results not shown) did not differ.

South Basin (Figure 3.8), also showed high levels of nutrient loading around the first decade of 2000 in contrast to low levels of TP and TN in 1969, this difference accentuated along CCA1 axis with both variables having a high correlation with this axis (both with r=0.89). In the case of CHLA, had a correlation of r=0.47 with CCA1 and r=-0.14 with CCA2 axes, reflecting differences not only between the first decade of 2000 and 1969, but also within the 2002 – 2011 period. CCA1 shows the variation between 1969 and the 2002 – 2011 period within the Cladocera community, presenting all species (positive scores along CCA1 axis) except *B. longirostris* and to a lesser extent *D. retrocurva* (negative scores along CCA1 axis), associated with eutrophication conditions. In contrast to North Basin, CCA2 axis showed differences within the first decade of 2000 due to biological differences The years 2002, and 2011 reflect an

association with high densities and *Diaphanosoma birgei*.The year 2006 appears to be associated with *Daphnia mendotae*. . A Monte Carlo permutation test showed that this result was not statistically significant.

In North Basin, TP, TN and CHLA values were closely correlated indicated by the angle and position of the vectors between them. In contrast, CHLA in the South Basin was not so tightly correlated with TP and TN.

The correlation coefficients for NB and SB reflected the distribution of sites and species within the ordination diagram presented in the CCA analyses (Table 3.11 & 3.12). The low correlation of CHLA with TP and TN in the South Basin might be due to the differences in turbidity present between North and South Basin (Manitoba Water Stewardship, 2011).

DISCUSSION

Changes in the zooplankton community occurred in parallel with the substantial changes in nutrient levels in the lake between pre-eutrophication (1969) and post-eutrophication (2002), Within the Cladocera community, *D. ambigua* and *D. schoedleri*, were no longer present in 2002. The calanoid community was also affected, as *Epischura nevadensis*, *D. minutus* and *D. clavipes* were no longer observed in 2002. In contrast, the cyclopoid copepod community did not present changes in their species composition. This might be a reflection of calanoid copepods and Cladocera being more adapted to oligotrophic conditions than cyclopoid copepods (Gannon and Stemberger, 1978; Hsieh *et al.*, 2011).

Daphnia longiremis and *Eubosmina coregoni* appeared to be mainly restricted to the North Basin and highly associated with eutrophication conditions according to multiavariate

analysis. *E. coregoni*, an invasive species, has remained largely confined to the North Basin and appears unable to spread successfully through the South Basin (Suchy *et al*., 2010). In the South Basin, *D. mendotae* is the only species that showed a positive correlation with the environmental variables considered in this study. There is a definite contrast between both regions of the lake, and this might be due to the differences in turbidity present, South Basin being the most turbid with an average NTU = 10. This might hinder visual predation by planktivorous fish. However, the higher abundance of *D. longiremis*, a large bodied Cladocera in North Basin in the early years of 2000, does not support this hypothesis. As in North Basin, the turbidity is lowest and visual predation would not be hindered.

In Lake Winnipeg, the density of large species such as *D. retrocurva, D. mendotae* have remained low in comparison to *E. coregoni* and *B. longirostris*. Previous research done by Sheppard *et al.* (2011) has indicated the positive selectivity by Rainbow Smelt on larger zooplankton species such as *Daphnia* in Lake Winnipeg, as they are more conspicuous to visual predation. Fish predation might be a strong factor to explain a pattern of the Cladocera population due to the nature of the turbidity of this basin; again this hypothesis must be explored in future multivariate long term analysis in Lake Winnipeg. In fact, Schulze (2011) showed that even in turbid environments, fish predation can be a main driver in shaping zooplankton community in a top-bottom manner. Visual predation by planktivorous fish has been observed in turbid environments (Schulze, 2011) and Rainbow Smelt have been shown to selectively feed on large-bodied Cladocera (Sheppard *et al*., 2011). The multivariate analysis showed that the density of the large *D. mendotae* in the South Basin where turbidity is highest, increased in later year. However, to confirm this hypothesis, fish abundance data must be included in future long-term studies of the zooplankton community (i.e. Cladocera) of Lake Winnipeg. Barbiero *et al.*

(2001) showed overall increases in abundance of *Daphnia*, *Bosmina* and *Eubosmina* were observed during summer of 1998 in Lake Erie. This was observed in the North Basin of Lake Winnipeg. Also Barbiero *et al.* (2001), showed that *Bosmina longirostris* and *L. kindti* were dominant in the western basin of Lake Erie, which seems to indicate that these species can thrive in eutrophic conditions.

Daphnia pulex, Ceriodaphnia *quadrangula* and *Chydorus sphaericus* were rare and sporadic species within the data set and this must be taken into account when interpreting the multivariate analysis. Arnott *et al.* (1999) indicated that much of a detected species turnover is due to sampling error. Because a large system such as a lake presents high turnover rates where in a short-term study the zooplankton diversity changes every year, and one third of the total species pool was able to be identified and counted, much of the patterns displayed might not be explained (Arnott *et al.*,1999)Vijverberg and Boersma (1997) have shown that *C. sphaericus* as well as other "small-bodied" Cladocera responded negatively to intense eutrophication (increase in chlorophyll-*a*), whereas larger Cladocera, such as *D. mendotae* thrived in response to chlorophyll-*a* increase. *D. pulex* has been shown to be an effective control agent of phytoplankton biomass in large within lake enclosures (Vanni, 1984) and this might be the cause of its increase in the South Basin during summer of 2004, coupled with high levels of chlorophyll-*a*. Holm *et al.* (1983) demonstrated the filtering capabilities of *D. pulex* on small colonies or single filaments of the cyanobacteria *Aphanizomenon flos-aquae,* with its effectiveness increased in relation to the body size. In contrast, previous research (Guijun *et al.*, 2012) has shown that *Ceriodaphnia* can survive during periods of extensive cyanobacterial

blooms. *Ceriodaphnia* can develop a tolerance against toxic *Microcystis* strains (Guo and Xie, 2005).

Lake Winnipeg experienced significant increases in TP, TN and CHLA concentrations between 1969 and 2002 in all seasons, but there was substantial variation within 1969. This corroborates the pattern observed by others (McCullough *et al.,* 2012) of increased nutrient loading, especially in years with elevated flows in the Red River over the last two decades, leading to substantial eutrophication of the entire lake. Although nutrient (phosphorus and nitrogen) conditions are adequate for the growth of algae and cyanobacterial blooms throughout the lake, algae growth and blooms are most extensive in the North Basin during late summer and fall. In contrast, in the South Basin, high turbidity translates to a reduced light penetration through the water column, limiting growth for these organisms despite the elevated nutrient concentrations (Manitoba Water Stewardship, 2011). The higher levels of TP and TN in the North and Narrow Basins than in the South Basin, during autumn is probably due to the flow of these nutrients from the Red River that are highest in spring as they travel north (Brunskill *et al.,* 1980).

Chlorophyll-*a* is used as a proxy of algal and cyanobacterial blooms (overall algal biomass) (Boyer *et al.*, 2009; Sondergaard *et al.*, 2011). However, care must be taken when considering CHLA as a proxy, as Fitzpatrick *et al.*, (2007), in Lake Erie, showed that an increase in cyanobacterial blooms was coupled with a decrease in CHLA values. Furthermore, Sondergaard *et al*. (2011) found that although CHLA presented high correlations with the overall phytoplankton community, there were less clear relationships with this predictor and cyanobacteria.

Average values for the biological and environmental data were used to focus on temporal variation for each basin (North Basin and the Narrows pooled together and South Basin). However, intra-basin variation was found in Lake Winnipeg (Patalas and Salki, 1992) and should be explored in future long term eutrophication studies on the lake as the lake's well-mixed characteristics due to wind action might contribute much to horizontal patchiness (Huber *et al.*, 2011).

Lake Champlain has suffered the effects of eutrophication, with an increased level of phosphorus from 1979 – 2009, reductions in nitrogen levels and an increased dominance of cyanobacteria in the phytoplankton community since the 1970s (Smeltzer *et al.*, 2012; Levine *et al.*, 2012). In a survey done in Lake Erie during 1996 to 2002, Conroy *et al*. (2005) concluded that an increase in zooplankton and phytoplankton biomass was found, noticeably within the eutrophied western basin of the lake during spring and summer and cyanobacterial blooms had increased particularly in the summer.

The gap within the data set between the years 1969 and 2002 is an issue, as the differences between both years might be due to normal, climate-related and historical differences. Furthermore, there is a severe lack of long term studies in zooplankton community in shallow polymictic lakes such as Lake Winnipeg.

The role of invasive species must be considered when studying temporal changes in the zooplankton community. In Lake Erie, the presence of invasive zebra mussels as well, facilitated the predominance of cyanobacterial blooms as they are filter feeders selective to phytoplankton (Conroy *et al.*, 2005; Fitzpatrick *et al.*, 2007) In a recent research of Lake Champlain (Mihuc *et al.*, 2012), they concluded that the influence of alewife predation affected the constitution of the

community, as individuals of smaller size increased in density and larger bodied zooplankton diminished. In Lake Winnipeg, more than 80% of the phytoplankton biomass has been shown to be constituted mainly by three genera of cyanobacteria, *Aphanizomenon*, *Anabaena*, and *Microcystis* (Manitoba Water Stewardship, 2011). The cyanobacteria are considered to be of low nutrient quality to the zooplankton because they lack essential polyunsaturated lipids needed for zooplankton growth (Ferrao-Filho, 2007) and are quite inedible as well due to their size and shape, and depending on the species, toxic (Fitzpatrick *et al.*, 2007). Although, eutrophication can lead to the higher algal growth, the cyanobacterial blooms that arise in response to this phenomenon are quite detrimental for zooplankton. *D.pulex* has been shown to be unable to feed on colonies of *Anabaena flos-aquae* as their size made them unfit for their filtering apparatus (Holm *et al.*, 1983). The cyanobacteria *Microcystis aeruginosa* has shown to decrease growth of both male and female individuals of *Daphnia magna*, affecting more severely the reproduction rate of the latter (Lurling and Beekman, 2006). *Daphnia galeata* was proven to be unable to feed on *Planktothrix* (other cyanobacteria) microcystin-producing and microcystin-free strains (Pires *et al.*, 2007). Besides physical hindrance *Microcystis aeruginosa* has a lethal effect on zooplankton, such as *Daphnia galeata*, due to their natural producing toxin microcystin (Rohrlack, 1999).

We conclude that there has been discernible evidence of eutrophication in Lake Winnipeg, considerably affecting the zooplankton community despite the data gap throughout the years. Further research is recommended to take into account within basin spatial variation that was documented previously (Patalas and Salki, 1992) to determine these effects in a much more detail.

Table 3.1 List of species of the major crustacean zooplankton groups present in the years 1969 and 2002 in Lake Winnipeg

	1969	2002
CALANOID COPEPODS	**n=10**	**n=6**
Limnocalanus macrurus	+	+
Epischura lacustris	+	+
E. nevadensis	+	-
Diaptomus ashlandi	+	+
D. oregonensis	+	+
D. siciloides	+	-
D. sicilis	+	+
D. minutus	+	+
D. leptopus	+	-
D. clavipes	+	-
CYCLOPOID COPEPODS	**n=5**	**n=5**
Dyaciclops thomasi	+	+
C. vernalis	+	+
Mesocyclops edax	+	+
Macrocyclops albidus	+	+
Eucyclps agilis	+	+
CLADOCERA	**n=13**	**n=11**
Daphnia retrocurva	+	+
D. mendotae	+	+
D. longiremis	+	+
D. parvula	+	+
D. ambigua	+	-
D. schoedleri	+	-
Ceriodaphnia quadrangula	+	+
Bosmina longirostris	+	+
Chydorus sphaericus	+	+
Holopedium gibberum	+	+
Diaphanosoma birgei	+	+
Leptodora kindti	+	+
Alona guttata	+	+

Table 3.2 Total Nitrogen (TN), Total Phosphorus (TP) and Chlorophyll-*a* (CHLA) levels (mean ± SE) in North Basin (NB), Narrow Basin (Nar) and SB (South Basin) in 1969 (Pre-Eutrophication) and 2002 (Post-Eutrophication).

SPRING

		n	TN (µg/L)	TP (µg/L)	CHLA (µg/L)
NB	**1969**	12	33.6 ± 2.1	1.1 ± 0.1	4.5 ± 0.8
	2002	30	487.2 ± 89	31.5 ± 5.8	4.9 ± 0.9
NRB	**1969**	4	32 ± 10.4	0.64 ± 0.21	3.4 ± 1.2
	2002	4	561.8 ± 280.9	32.6 ± 5.9	4.7 ± 0.9
SB	**1969**	7	50.9 ± 8.3	3.1 ± 0.4	11.2 ± 3.1
	2002	34	701.2 ± 120.3	68.2 ± 11.7	11.1 ± 1.9

SUMMER

		n	TN (µg/L)	TP (µg/L)	CHLA (µg/L)
NB	**1969**	18	29.8 ± 7	1.1 ± 0.3	1.3 ± 0.3
	2002	28	495.9 ± 93.7	31.5 ± 5.9	6 ± 1.1
NRB	**1969**	4	35.2 ± 17.6	1.9 ± 0.9	0.9 ± 0.4
	2002	4	701 ± 350.5	69 ± 34.5	14.9 ± 7.5
SB	**1969**	24	49.2 ± 10	4.4 ± 0.9	6.4 ± 1.3
	2002	32	927 ± 113.3	137.9 ± 16.8	10.5 ± 1.3

FALL

		n	TN (µg/L)	TP (µg/L)	CHLA (µg/L)
NB	**1969**	31	25.8 ± 4.5	1.2 ± 0.2	4 ± 0.8
	2002	27	647.1 ± 124.5	86.6 ± 16.7	6.4 ± 1.2
NRB	**1969**	4	33.1 ± 11.7	1.9 ± 0.7	2.3 ± 0.6
	2002	4	742.5 ± 371.2	120.8 ± 60.4	2.4 ± 1.2
SB	**1969**	15	43.4 ± 11.2	3.1 ± 0.8	4.7 ± 1.2
	2002	14	388.1 ± 62.1	79 ± 10.6	14.9 ± 2

Table 3.3 Two-Way ANOVA Analysis comparing Total Phosphorus (TP), Total Nitrogen (TN) and Chlorophyll-*a* (CHLA) values between basins and 1969 (pre-eutrophication) and 2002 (post-eutrophication) across seasons and Tukey's HSD Test (post hoc analysis)

TN Two-Way ANOVA (p-values)

	Spring	Summer	Fall
YEAR	*	*	*
BASIN	*	*	*
BASIN:YEAR	*	*	*

TP Two-Way ANOVA (p-values)

	Spring	Summer	Fall
YEAR	*	*	*
BASIN	*	*	*
BASIN:YEAR	*	*	*

CHLA Two-Way ANOVA (p-values)

	Spring	Summer	Fall
YEAR		*	*
BASIN	*	*	
YEAR:BASIN			

TN Tukey HSD Test

	Spring	Summer	Fall
YEAR	*	*	*
BASIN			
NRB-NB			
SB-NB	*	*	*
SB-NRB		*	*
BASIN:YEAR			
NRB:Y1-NB:Y1			
SB:Y1-NB:Y1			
SB:Y1-NRB:Y1			
NRB:Y2-NB:Y2			
SB:Y2-NB:Y2	*	*	*
SB:Y2-NRB:Y2			
NB:Y2-NB:Y1	*	*	*
NRB:Y2-NRB:Y1	*	*	*
SB:Y2-SB:Y1	*	*	*

* indicates p-value < 0.05

TP Tukey HSD Test

	Spring	Summer	Fall
YEAR	*	*	*
BASIN			
NRB-NB			
SB-NB	*	*	*
SB-NRB	*	*	*
BASIN:YEAR			
NRB:Y1-NB:Y1			
SB:Y1-NB:Y1			
SB:Y1-NRB:Y1			
NRB:Y2-NB:Y2			
SB:Y2-NB:Y2	*	*	*
SB:Y2-NRB:Y2			*
NB:Y2-NB:Y1	*		*
NRB:Y2-NRB:Y1	*		*
SB:Y2-SB:Y1	*	*	*

* indicates p-value < 0.05

CHLA Tukey HSD Test

	Spring	Summer	Fall
YEAR		*	*
BASIN			
NRB-NB			
SB-NB	*	*	
SB-NRB			
BASIN:YEAR			
NRB:Y1-NB:Y1			
SB:Y1-NB:Y1			
SB:Y1-NRB:Y1			
NRB:Y2-NB:Y2			
SB:Y2-NB:Y2	*		
SB:Y2-NRB:Y2			
NB:Y2-NB:Y1			
NRB:Y2-NRB:Y1			
SB:Y2-SB:Y1		*	*

* indicates p-value < 0.05

Table 3.4 Total Phosphorus (TP), Total Nitrogen (TN) and Chlorophyll-*a* (CHLA) values (mean ± SE) for North Basin (NB), Narrow Basin (Nar) and South Basin (SB) of Lake Winnipeg through years - Summer Season Only

		n	TN (µg/L) ± SE	n	TP (µg/L) ± SE	n	CHLA (µg/L) ± SE
1969	NB	18	29.8 ± 7	20	1 ± 0.25	18	1.3 ± 0.3
	NRB	4	35.2 ± 17.6	4	1.9 ± 0.9	4	0.85 ± 0.42
	SB	24	49.2 ± 10	13	4.4 ± 0.9	24	6.4 ± 1.3
2002	NB	28	495.9 ± 93.7	28	31.5 ± 6	28	6 ± 1.1
	NRB	4	701 ± 350.5	4	69 ± 34.5	4	14.9 ± 7.5
	SB	32	927 ± 113.2	32	137.9 ± 16.8	32	10.5 ± 1.3
2003	NB	26	888.7 ± 152.4	26	35.5 ± 4.8	26	24.8 ± 4.9
	NRB	4	676.8 ± 66.1	4	49 ± 2.8	3	17.62 ± 1.2
	SB	35	1010.14 ± 106.9	35	106.9 ± 9.1	35	20.84 ± 5.5
2004	NB	22	773.6 ± 193	22	52.02 ± 9.3	22	13.7 ± 6.8
	NRB	4	791.28 ± 147.5	4	71.57 ± 11.9	4	16.87 ± 10.6
	SB	24	890.27 ± 64.6	24	99.91 ± 16.3	24	6.69 ± 0.8
2006	NB	24	672.71 ± 53.5	24	38.17 ± 4.1	24	14.34 ± 3.7
	NRB	3	1158.33 ± 23.5	3	79.33 ± 7.3	3	44.57 ± 16.4
	SB	22	1195.45 ± 83.3	22	142.5 ± 10.7	22	17.27 ± 4
2011	NB	31	645.94 ± 45	31	29.25 ± 1.8	31	12.02 ± 4.8
	NRB	5	846.83 ± 42.4	5	68.67 ± 7.9	5	1.66 ± 0.4
	SB	30	1150.21 ± 76.3	30	134.54 ± 15.2	30	5.26 ± 0.9

Table 3.5 One-Way ANOVA Analysis for each year across basins (NB, Nar, SB) using Total Phosphorus (TP), Total Nitrogen (TN) and Chlorophyll-a (CHLA)

One - Way ANOVA			
	TN	TP	CHLA
1969	*	**	**
2002	**	**	**
2003		**	
2004			
2006	**	**	
2011	**	**	

* indicates p-value < 0.05

** indicates p-value < 0.01

Table 3.6 Tukey's HSD Test across basins (NB, Nar, SB) for each year using Total Phosphorus (TP), Total Nitrogen (TN) and Chlorophyll-a (CHLA) for each year

Tukey Analysis							
	BASINS	1969	2002	2003	2004	2006	2011
TN	Nar-NB						
	SB-NB	*	**			**	**
	SB-Nar						
TP	Nar-NB						
	SB-NB	**	**	**		**	**
	SB-Nar		*				
CHLA	Nar-NB						
	SB-NB	*					
	SB-Nar						

* indicates p-value < 0.05

** indicates p-value < 0.01

Table 3.7 One-Way ANOVA Analysis results between years 1969, 2002, 2003, 2004, 2006, and 2011 for North Basin (NB), Narrow Basin (Nar), and South Basin (SB) using Total Phosphorus (TP), Total Nitrogen (TN) and Chlorophyll-a (CHLA)

One-Way ANOVA			
	NB	Nar	SB
TN	**	**	**
TP	**	*	**
CHLA			**

* indicates p-value < 0.05
** indicates p-value < 0.01

Table 3.8 Tukey's HSD Test between years that in North Basin (NB+Nar) and South Basin, using Total Phosphorus (TP), Total Nitrogen (TN) and Chlorophyll-a (CHLA) for each year.

	NB			SB		
	TN	TP	CHLA	TN	TP	CHLA
2002 - 1969	*	*		*	*	
2003 - 1969	*	*		*	*	*
2004 - 1969	*	*		*	*	
2006 - 1969	*	*		*	*	
2011 - 1969	*	*		*	*	
2003 - 2002						
2004 - 2002						
2006 - 2002						
2011 - 2002						
2004 - 2003						*
2006 - 2003						
2011 - 2003						*
2006 - 2004						
2011 - 2004						
2011 - 2006						*

* indicates p-value < 0.05

Table 3.9 Densities (mean ± SE) of Cladocera (CLAD), calanoid copepods (CAL), and cyclopoid copepods (CYC) groups between 1969 and 2002 in North Basin (NB), Narrow Basin (Nar) and South Basin (SB) for all open water seasons

		SPRING			
		n	CLAD (ind/L)	CAL (ind/L)	CYC (ind/L)
NB	1969	19	0.5 ± 0.2	3.4 ± 0.4	15.5 ± 3.4
	2002	12	17.9 ± 6.4	24.3 ± 4.8	13.4 ± 2.2
Nar	1969	3	1.8 ± 0.8	7.2 ± 2.6	6.6 ± 1.3
	2002	2	57 ± 0.4	30.9 ± 4.2	25.2 ± 5.3
SB	1969	14	0.7 ± 0.1	6.3 ± 0.98	11.5 ± 1.7
	2002	8	25.9 ± 10.1	38.8 ± 2.5	27.3 ± 3.2

		SUMMER			
		n	CLAD (ind/L)	CAL (ind/L)	CYC (ind/L)
NB	1969	25	6.9 ± 0.9	6.8 ± 0.7	21.2 ± 2.5
	2002	11	68 ± 27	23.3 ± 3.8	31.3 ± 6
Nar	1969	5	3.2 ± 0.8	14.6 ± 2.2	5.8 ± 1.5
	2002	3	6.5 ± 1.5	23.8 ± 5.4	18.1 ± 9.9
SB	1969	21	12.5 ± 1.9	26.4 ± 2.5	11.3 ± 2.1
	2002	11	6.2 ± 1.3	28.4 ± 3.4	6.1 ± 1.4

		FALL			
		n	CLAD (ind/L)	CAL (ind/L)	CYC (ind/L)
NB	1969	23	4.8 ± 1	5.2 ± 0.6	12.2 ± 1.3
	2002	12	3.6 ± 0.7	3.6 ± 0.7	9.4 ± 1.5
Nar	1969	5	0.3 ± 0.1	3 ± 0.5	1.1 ± 0.3
	2002	3	2.8 ± 0.5	2.2 ± 0.8	3.7 ± 0.7
SB	1969	26	4.5 ± 0.9	14.1 ± 2.7	7.5 ± 1.3
	2002	10	18.6 ± 5.8	4.4 ± 1.1	10.6 ± 1.5

Table 3.10 Two-Way ANOVA comparing Cladocera, calanoid and cyclopoid (including calanaoid and cyclopoid nauplii respectively) density between basins and 1969 (pre-eutrophication) and 2002 (post-eutrophication) across seasons and Tukey's HSD Test (post hoc analysis)

CLADOCERA (ONE-WAY ANOVA)

	Spring	Summer	Fall
YEAR	*	*	*
BASIN			*
BASIN:YEAR	*	*	*

Tukey HSD Test

	Spring	Summer	Fall
YEAR	*	*	*
BASIN			
NRB-NB			*
SB-NB			*
SB-NRB			*

* indicates p-value < 0.05

CALANOID COPEPODS (ONE-WAY ANOVA)

	Spring	Summer	Fall
YEAR	*	*	
BASIN	*	*	*
BASIN:YEAR	*	*	

Tukey HSD Test

	Spring	Summer	Fall
YEAR	*	*	
BASIN			*
NRB-NB			
SB-NB	*	*	*
SB-NRB			

* indicates p-value < 0.05

CYCLOPOID COPEPODS (ONE-WAY ANOVA)

	Spring	Summer	Fall
YEAR	*		
BASIN		*	*
BASIN:YEAR	*		

Tukey HSD Test

	Spring	Summer	Fall
YEAR	*		
BASIN			
NRB-NB		*	*
SB-NB		*	*
SB-NRB			

* indicates p-value < 0.05

Table 3.11 Correlation coefficients between Total Phosphorus (TP), Total Nitrogen (TN), Chlorophyll-*a* (CHLA) variables and density of cladoceran species (ind/L) for North Basin, Lake Winnipeg in the years 1969, 2002, 2003, 2004, 2006 and 2011

	CHLA	TP	TN
CHLA	1.00		
TP	0.95	1.00	
TN	0.97	0.99	1.00

	D. retrocurva	*D. mendotae*	*D. longiremis*	*C. quadrangulata*	*C. sphaericus*	*B. longirostris*	*D. birgei*	*E. coregoni*
CHLA	0.20	0.53	0.60	-0.10	-0.80	0.03	0.53	0.35
TP	0.31	0.40	0.74	0.16	-0.93	0.28	0.39	0.47
TN	0.33	0.51	0.66	0.07	-0.90	0.22	0.44	0.54

Table 3.12 Correlation coefficients between Total Phosphorus (TP), Total Nitrogen (TN), Chlorophyll-*a* (CHLA) variables and density of cladoceran species (ind/L) for South Basin, Lake Winnipeg in the years 1969, 2002, 2003, 2004, 2006 and 2011

	CHLA	TP	TN
CHLA	1.00		
TP	0.21	1.00	
TN	0.24	1.00	1.00

	D. retrocurva	*D. mendotae*	*C. quadrangulata*	*B. longirostris*	*D. birgei*	*D. pulex*
CHLA	0.18	0.79	-0.18	0.18	0.19	0.85
TP	-0.64	0.76	-0.99	-0.75	-0.47	0.28
TN	-0.62	0.77	-0.99	-0.73	-0.43	0.30

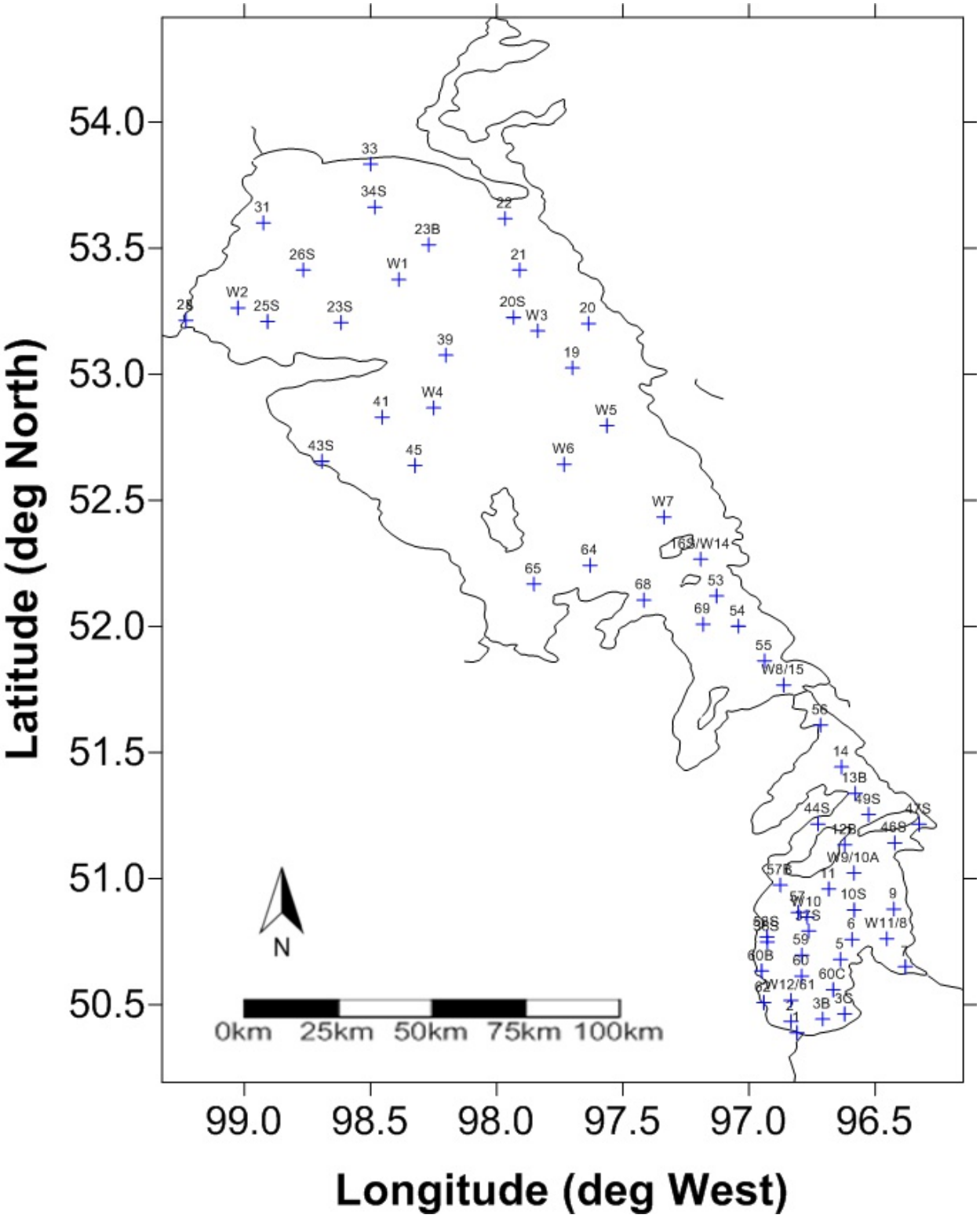


Figure 3.1 Map of Lake Winnipeg showing all 69 stations where water, zooplankton, benthos sampling and fish trawling are conducted. The stations are the same from 1969 to 2011.

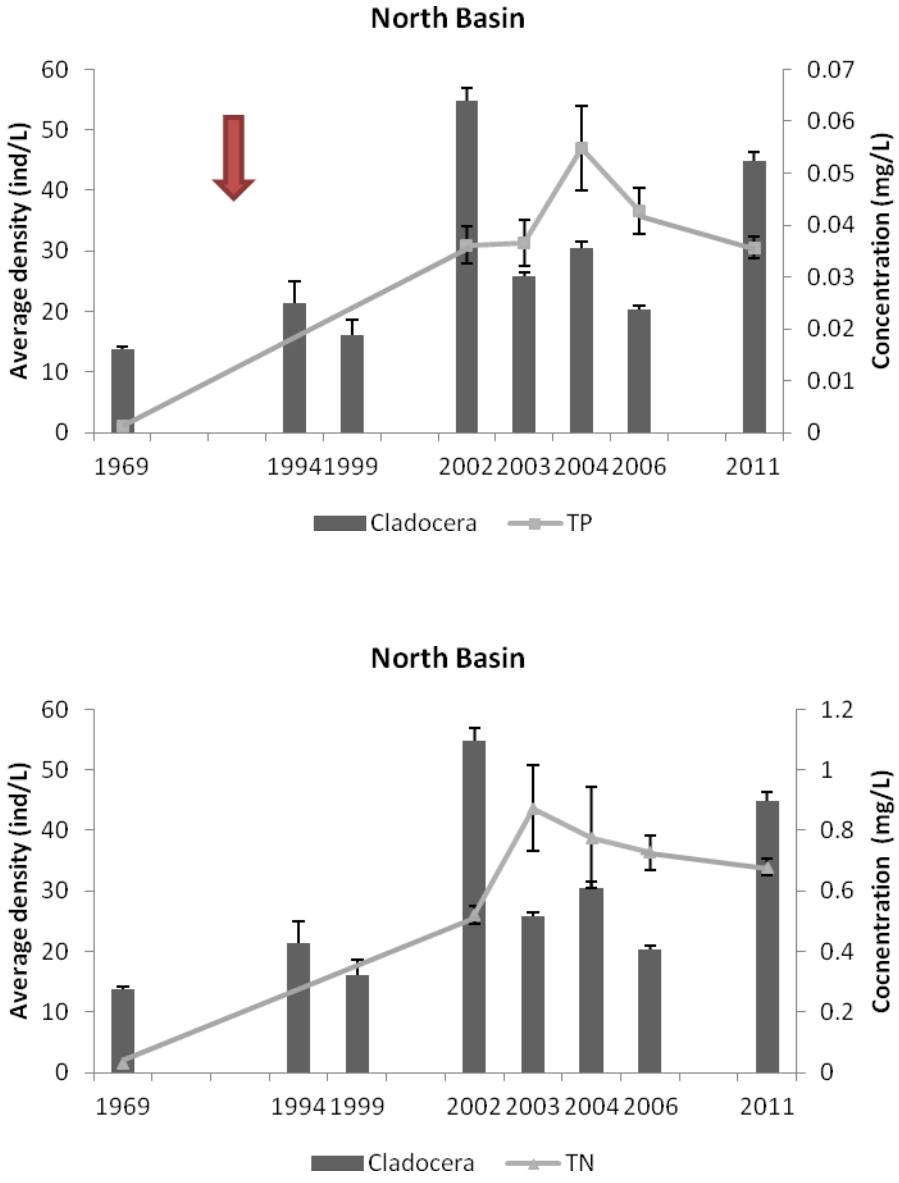
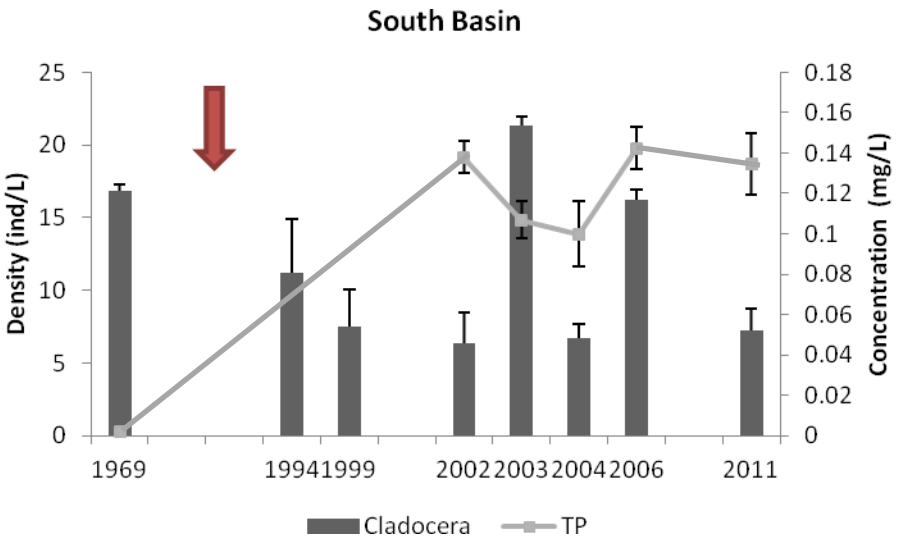


Figure 3.2 North Basin (NB + Nar) - Density of Cladocera (mean ± SE) through the years with the mean (± SE) values of TN and TP. Red arrow indicates invasion of Rainbow Smelt around 1990.



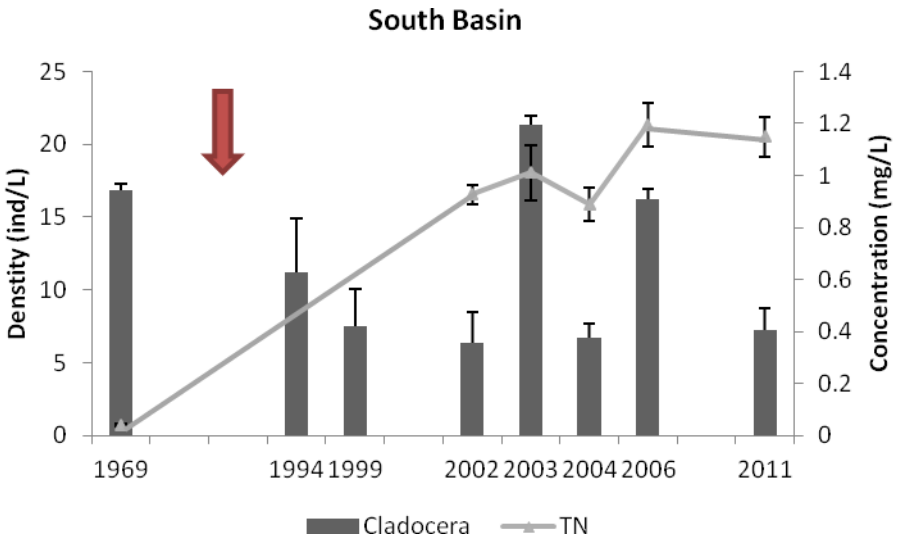


Figure 3.3 South Basin - Cladocera (mean ± SE) through the years with the mean (± SE) of TN and TP. Red arrow indicates invasion of Rainbow Smelt around 1990.

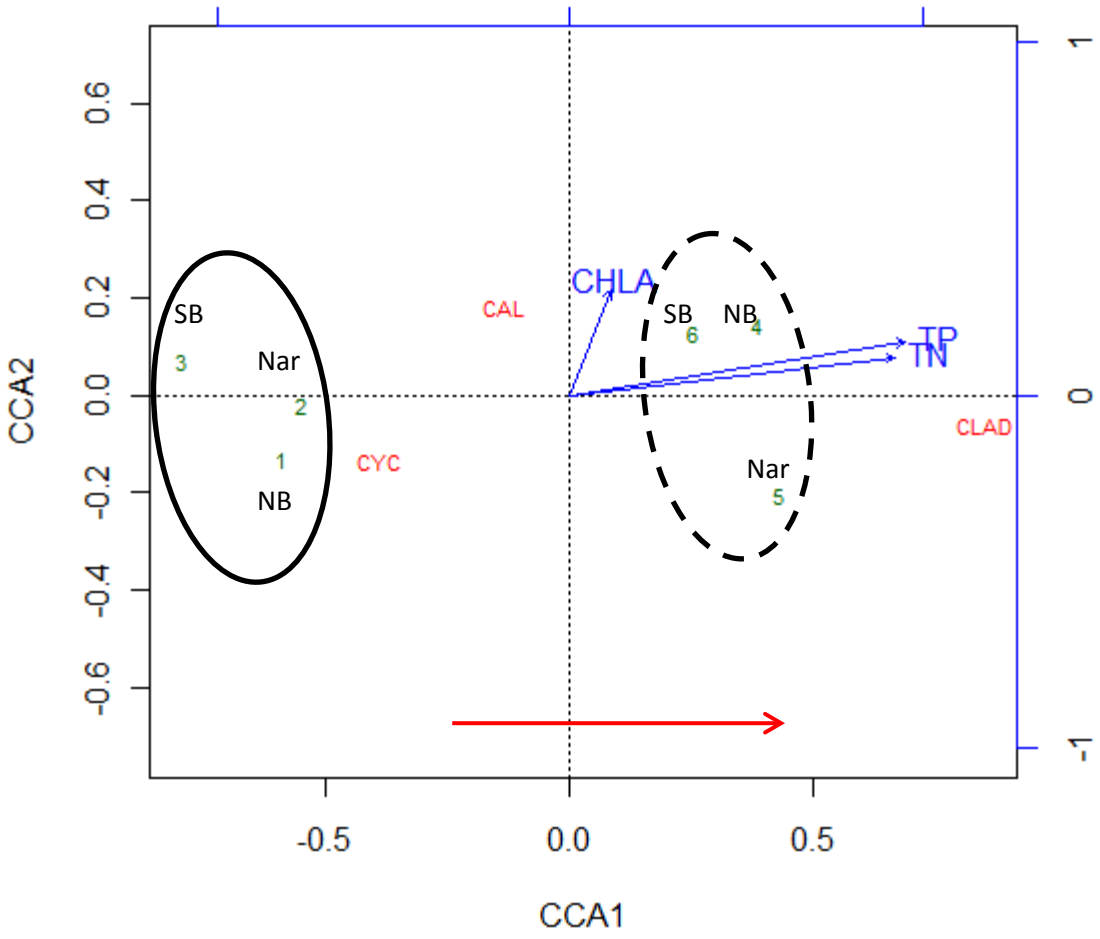


Figure 3.4 Canonical Correspondence Analysis (CCA) of Cladocera (CLAD), calanoid (CAL) and cyclopoid (CYC) copepods including their respective nauplii in 1969 (solid line) and 2002 (dash line) and each basin – Spring Season. Red arrow indicates the direction of eutrophication in the ordination diagram.

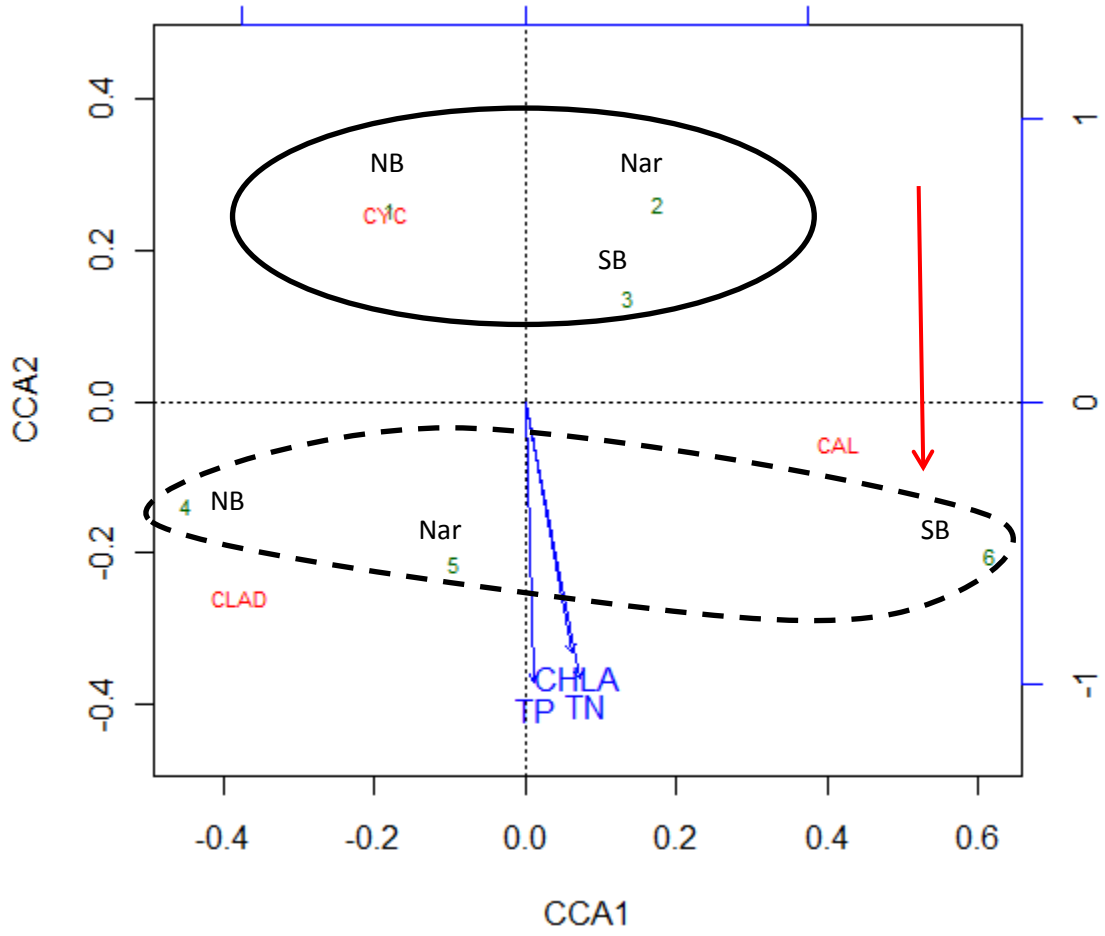


Figure 3.5 Canonical Correspondence Analysis (CCA) of Cladocera (CLAD), calanoid (CAL) and cyclopoid (CYC) copepods including their respective nauplii in 1969 (solid line) and 2002 (dash line) and each basin – Summer Season. Red arrow indicates the direction of eutrophication in the ordination diagram.

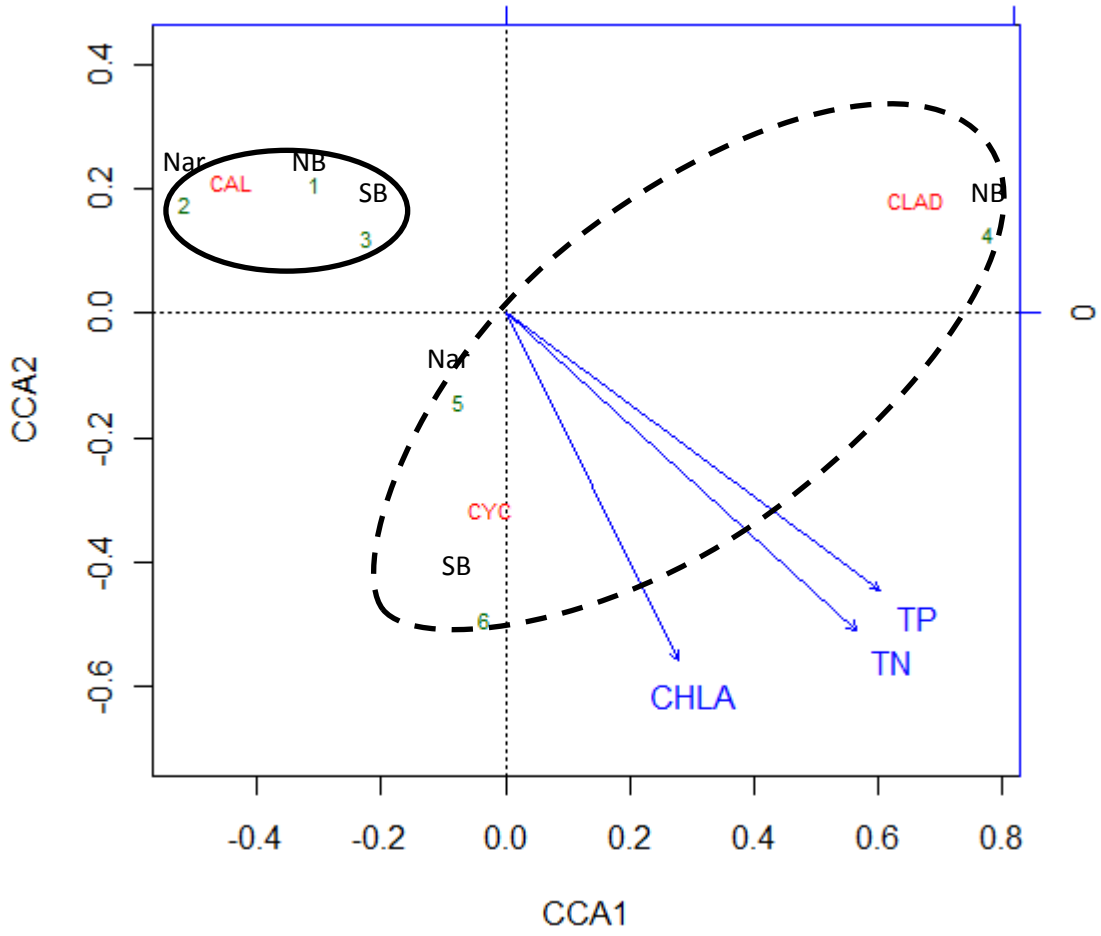


Figure 3.6 Canonical Correspondence Analysis (CCA) of Cladocera (CLAD), calanoid (CAL) and cyclopoid (CYC) copepods including their respective nauplii in 1969 (solid line) and 2002 (dash line) and each basin – Fall Season. Red arrow indicates the direction of eutrophication in the ordination diagram.

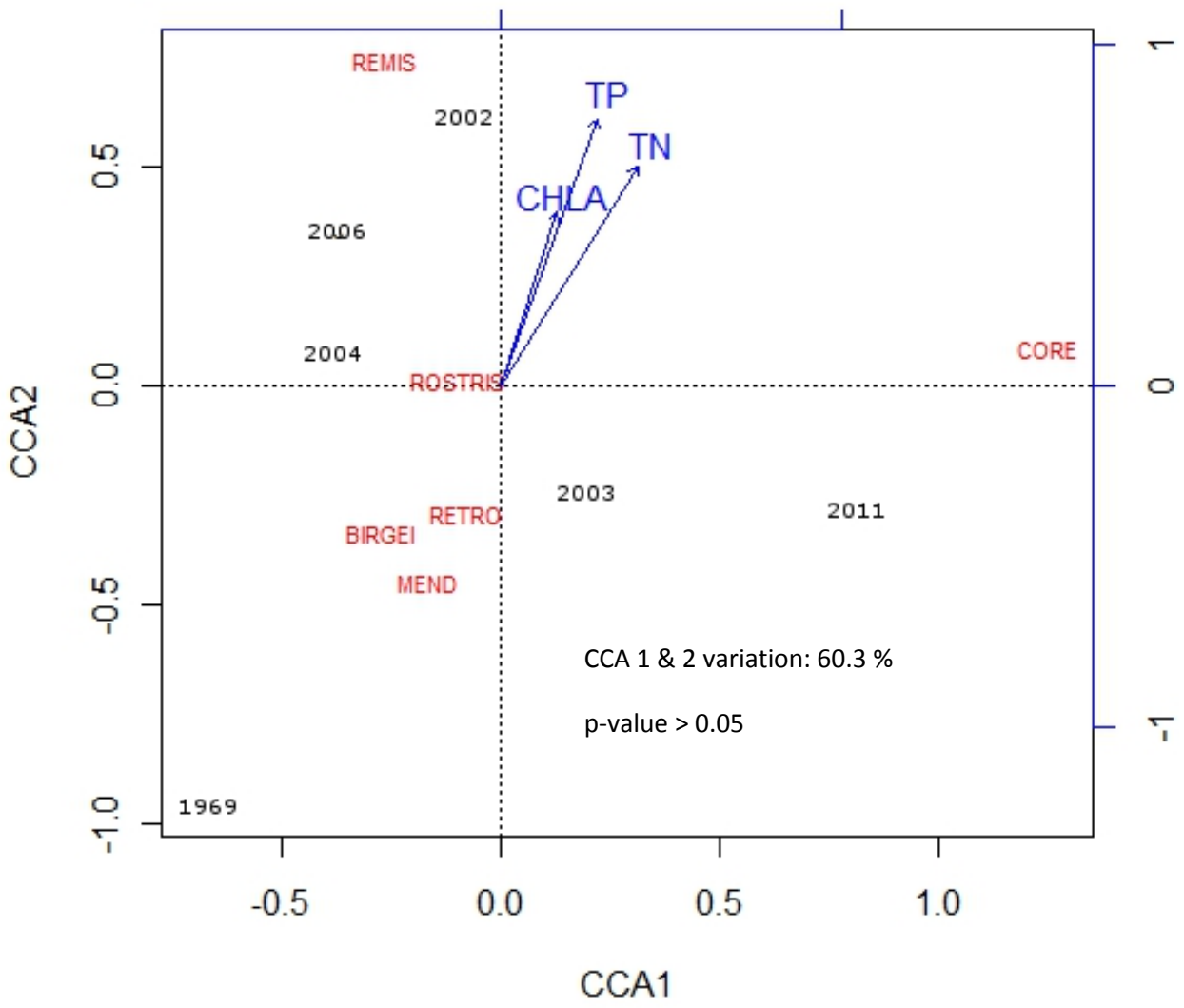


Figure 3.7 Canonical Correspondence Analysis (CCA) with the Cladocera community and Total Nitrogen (TN), Total Phosphorus (TP), Chlorophyll-*a* (CHLA) in summer of 1969, 2002 – 2006 and 2011 – North Basin. CORE=*Eubosmina coregoni*, REMIS=*Daphnia longiremis*, RETRO=*D. retrocurva*, MEND=*D. mendotae*, BIRGEI=*Diaphanosoma birgei*, ROSTRIS=*Bosmina longirostris*.

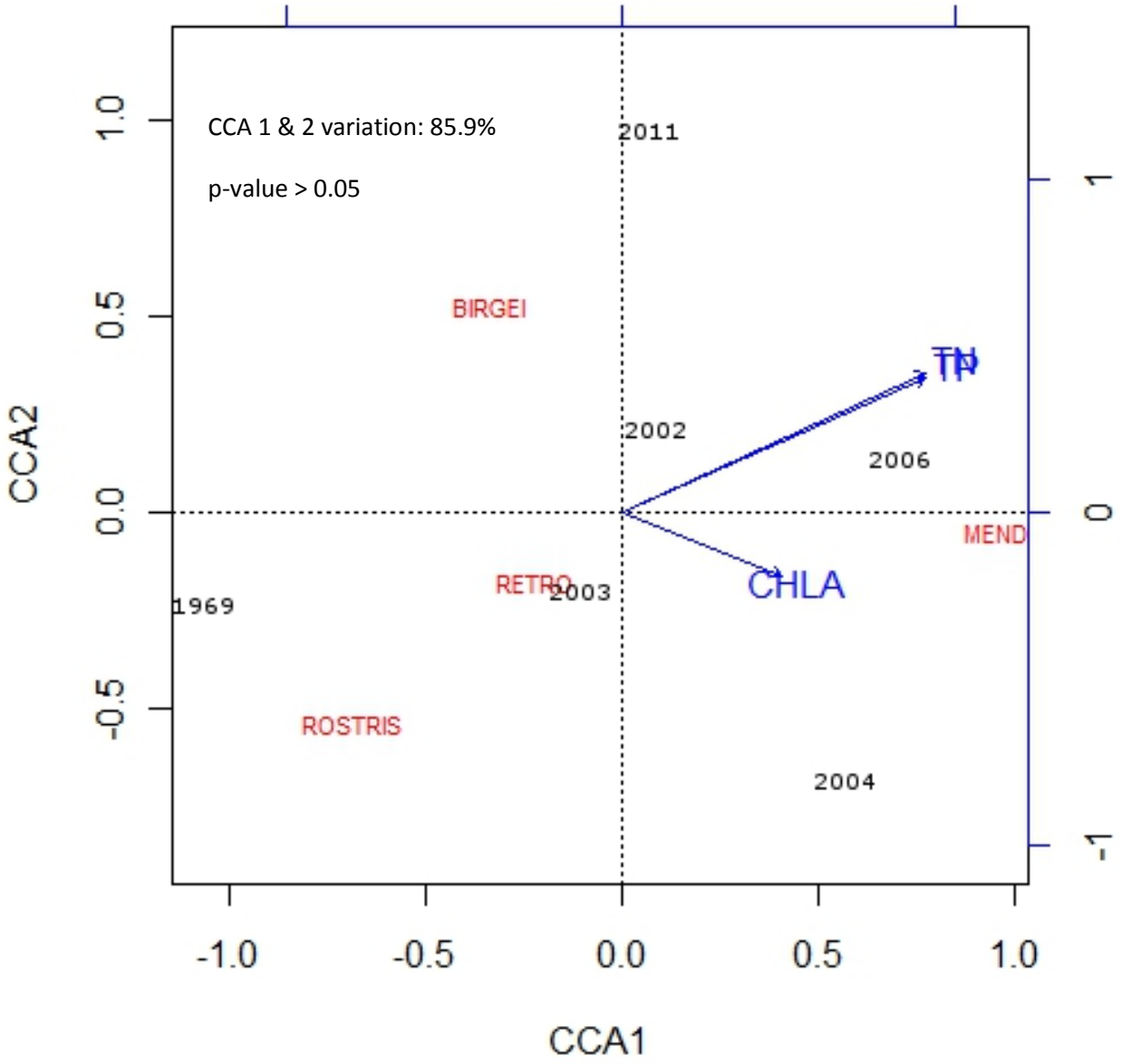


Figure 3.8 Canonical Correspondence Analysis (CCA) with the Cladocera community and Total Nitrogen (TN), Total Phosphorus (TP), Chlorophyll-*a* (CHLA) in summer of 1969, 2002 – 2006 and 2011 – South Basin., CORE=*Eubosmina coregoni*, REMIS=*Daphnia longiremis*, RETRO=*D. retrocurva*, MEND=*D. mendotae*, PUL=*D.*pulex, , BIRGEI=*Diaphanosoma birgei*, ROSTRIS=*Bosmina longirostris*.

CHAPTER 3: APPENDIX

In North Basin, *Daphnia retrocurva*, *D. mendotae*, *D. longiremis*, *Ceriodaphnia quadrangula*, *Chydorus sphaericus*, *Diaphanosoma birgei*, *E. coregoni*, and *D. pulex* showed variation between years (One-way ANOVA, Table A3.1). *Bosmina longirostris* and *L.kindti* density variation was found to be not significant. *D. retrocurva* density from 2011 was significantly higher than 1969 and 1999 (Tukey HSD Test, Table A3.1/Figure A3.1). *D. mendotae* density was higher in 2011 than in 1969 (Tukey HSD Test, Table A3.1/Figure A3.1). *D. longiremis* density in 2002 was significantly higher than 1969, 1994, 1999 and 2003 (Tukey HSD Test, Table A3.1/Figure A3.2). *Chydorus sphaericus* density in 1999 was significantly higher (Tukey HSD Test, Table A3.1/Figure A3.3). *Diaphanosoma birgei* presented significant variation throughout all years (Tukey HSD Test, Table A3.1/Figure A3.3). *Ceriodaphnia quadrangula* showed a similar pattern as *D. longiremis*, where 2002 density was significantly higher (Tukey HSD Test, Table A3.1/Figure A3.33). *Eubosmina coregoni* density for 2011 was highest in comparison to all years (Tukey HSD Test, Table A3.1/Figure A3.4). *D. pulex* density in 2004 was significantly higher in this basin (Tukey HSD Test, Table A3.1/Figure A3.5). The Narrows data were pooled into North Basin.

In South Basin, *D. retrocurva*, *D. mendotae*, *Bosmina longirostris*, *D. pulex* and *L.kindti* presented variation between years (One-way ANOVA, Table A3.2). For *D. retrocurva*, 1969 density was the only one significantly higher than 1994 and 1999. Its density in the first decade of 2002 was not significant (Tukey HSD Test, Table A3.2/Figure A3.1). *D. mendotae* had significant high densities in 1994, 1999 and 2006 (Tukey HSD Test, Table A3.2/Figure A3.1). *B. longirostris* density showed a significant decrease since 1969 (Tukey HSD Test, Table

A3.2/Figure A3.2). *L. kindti* density of 1969 was significantly lower than most years recorded in the basin (Tukey HSD Test, Figure A3.4). *D. pulex* densities in 2004 and 2011 were significant (Tukey HSD Test, Table A3.2/Figure A3.5).

Table A3.1 One-Way ANOVA results using density of Cladocera species (ind/L) for Summer Season through years 1969, 1994, 2002 – 2006, 2011 for North Basin (NB + Nar), Lake Winnipeg and Post-Hoc Analysis (Tukey's HSD Test)

One-Way ANOVA / NORTH BASIN

	D. retrocurva	*D. mendotae*	*D. longiremis*	*C. quadrangula*	*B. longirostris*	*C. sphaericus*	*D. birgei*	*E. coregoni*	*D. pulex*	*L. kindti*
Years	*	*	*	*		*	*	*	*	

Tukey's HSD Test / NORTH BASIN

	D. retrocurva	*D. mendotae*	*D. longiremis*	*C. quadrangula*	*B. longirostris*	*C. sphaericus*	*D. birgei*	*E. coregoni*	*D. pulex*	*L. kindti*
1994-1969										
1999-1969						*	*	*		
2002-1969			*	*			*			
2003-1969							*			
2004-1969									*	
2006-1969							*			
2011-1969	*	*						*		
1999-1994										
2002-1994			*	*						
2003-1994							*			
2004-1994										
2006-1994							*			
2011-1994								*		
2002-1999			*	*		*				
2003-1999						*	*			
2004-1999						*			*	
2006-1999						*				
2011-1999	*					*	*	*		
2003-2002			*	*			*			
2004-2002				*						
2006-2002				*						
2011-2002								*		
2004-2003							*			
2006-2003										
2011-2003							*	*		
2006-2004										
2011-2004								*	*	
2011-2006								*		

Table A3.2 One-Way ANOVA results using density of Cladocera species (ind/L) for Summer Season through years 1969, 2002 – 2006, 2011 for South Basin, Lake Winnipeg and Post-Hoc Analysis (Tukey's HSD Test)

One-Way ANOVA / SOUTH BASIN

	D. retrocurva	*D. mendotae*	*D. longiremis*	*C. quadrangula*	*B. longirostris*	*D. birgei*	*D. pulex*	*L. kindti*
Years	*	*			*		*	*

Tukey's HSD Test / SOUTH BASIN

	D. retrocurva	*D. mendotae*	*D. longiremis*	*C. quadrangula*	*B. longirostris*	*D. birgei*	*D. pulex*	*L. kindti*
1994-1969	*	*			*			*
1999-1969	*				*			
2002-1969					*			*
2003-1969					*			*
2004-1969					*			
2006-1969		*			*			
2011-1969	*				*			*
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2011-2006		*					*	

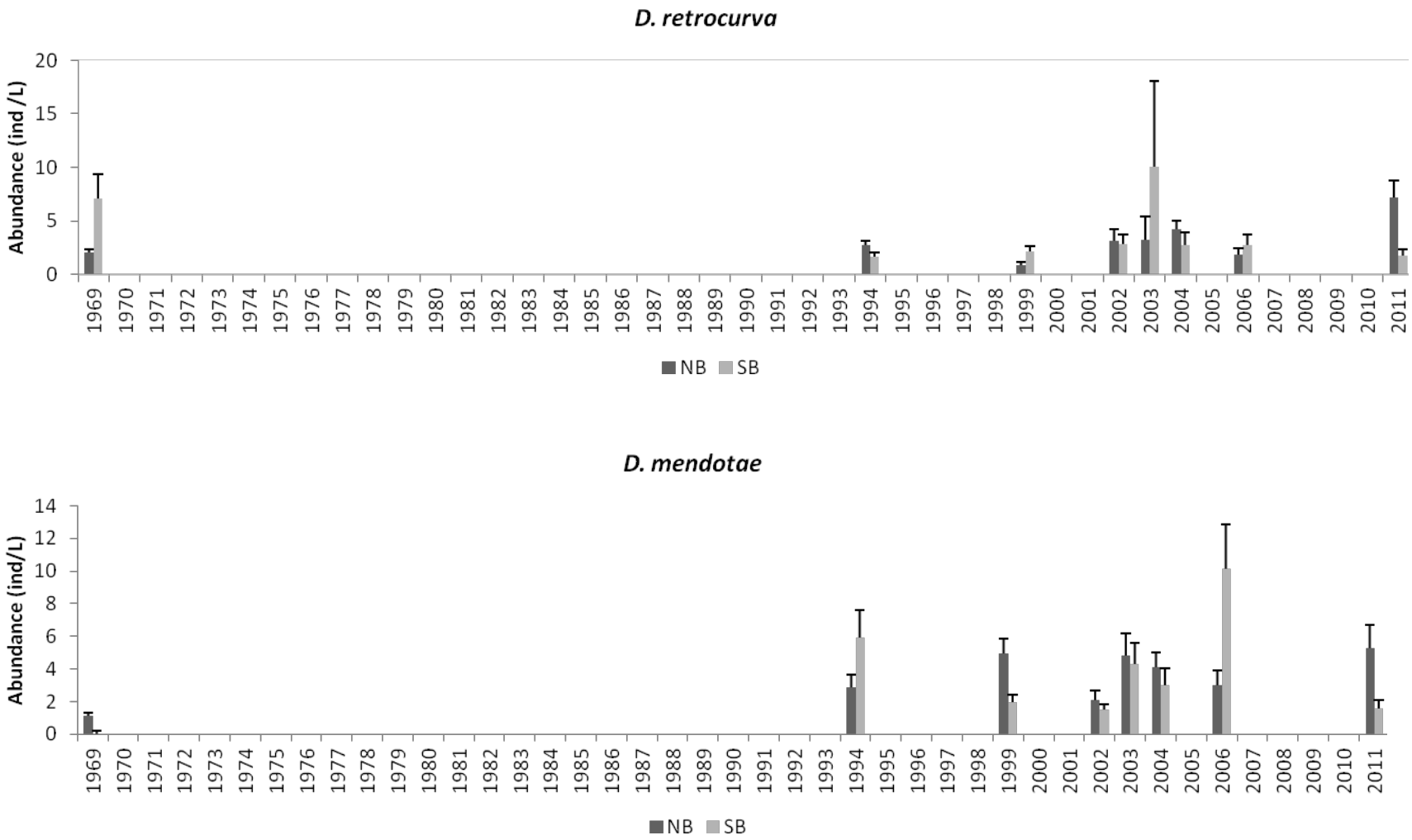

Figure A3.1 Densities (mean ± SE) of Cladocera species in North Basin (NB + Nar) and South Basin of Lake Winnipeg in Summer

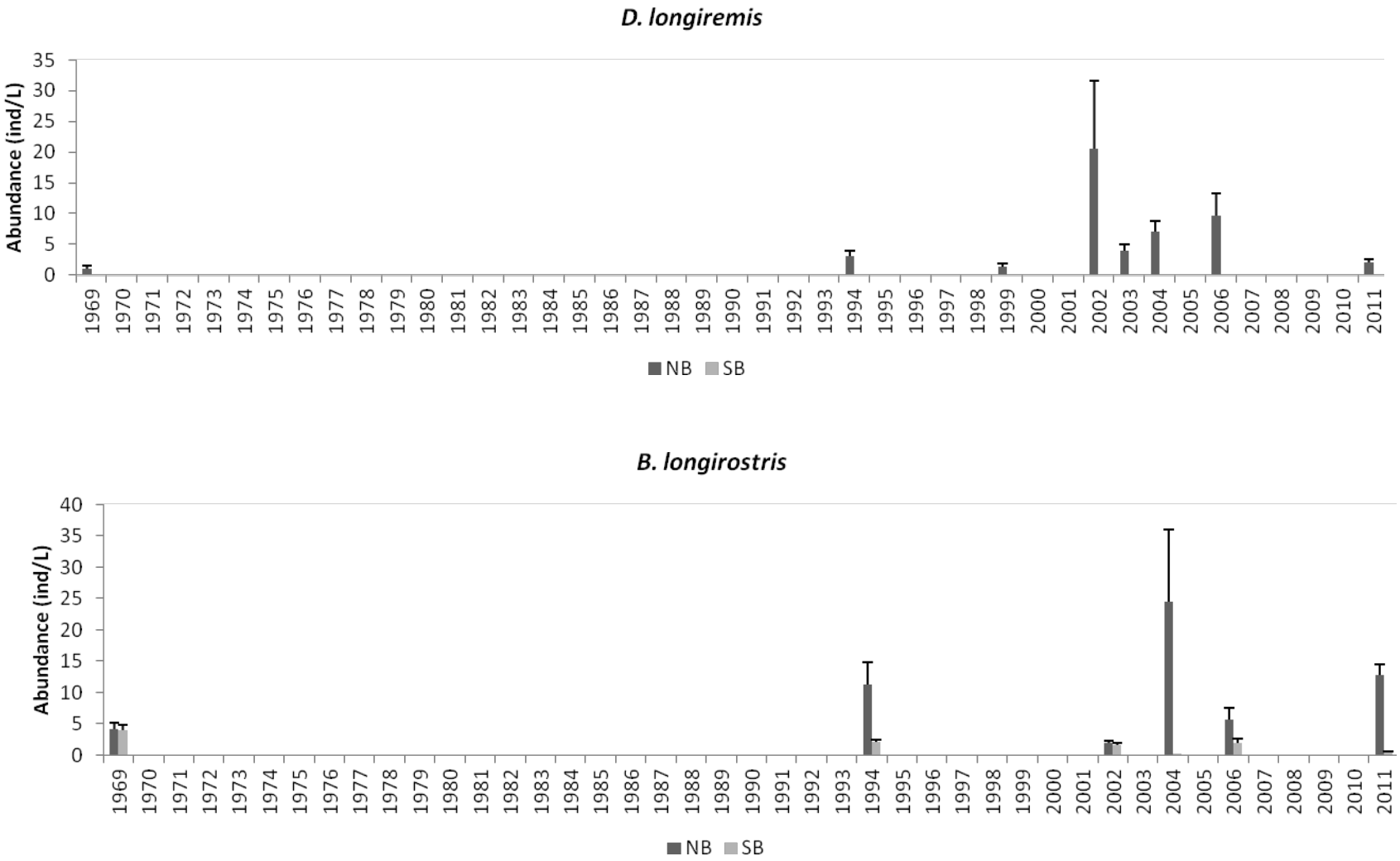

Figure A3.2 Densities (mean ± SE) of Cladocera species in North Basin (NB + Nar) and South Basin of Lake Winnipeg in Summer

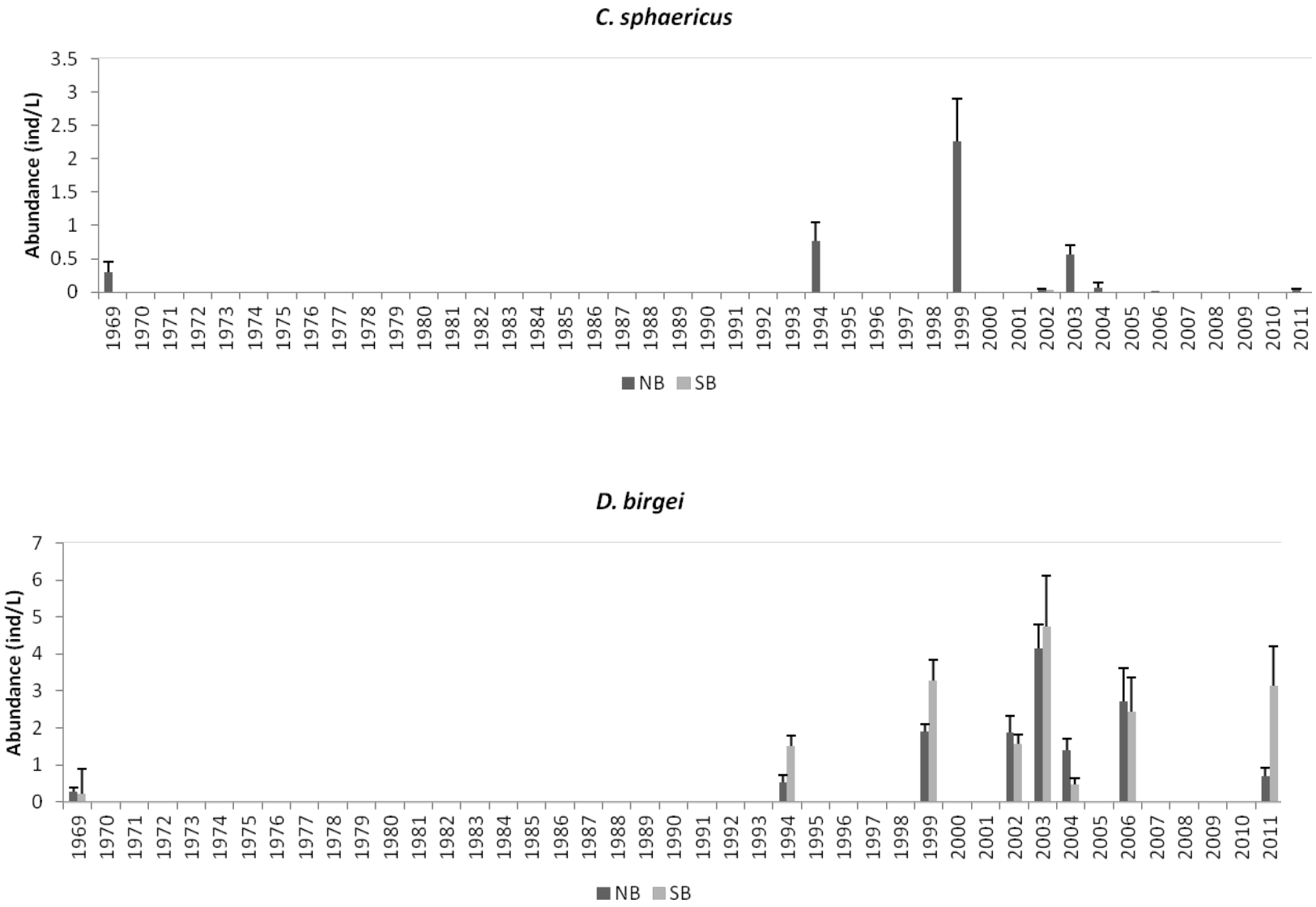

Figure A3.3 Densities (mean ± SE) of Cladocera species in North Basin (NB + Nar) and South Basin of Lake Winnipeg in Summer

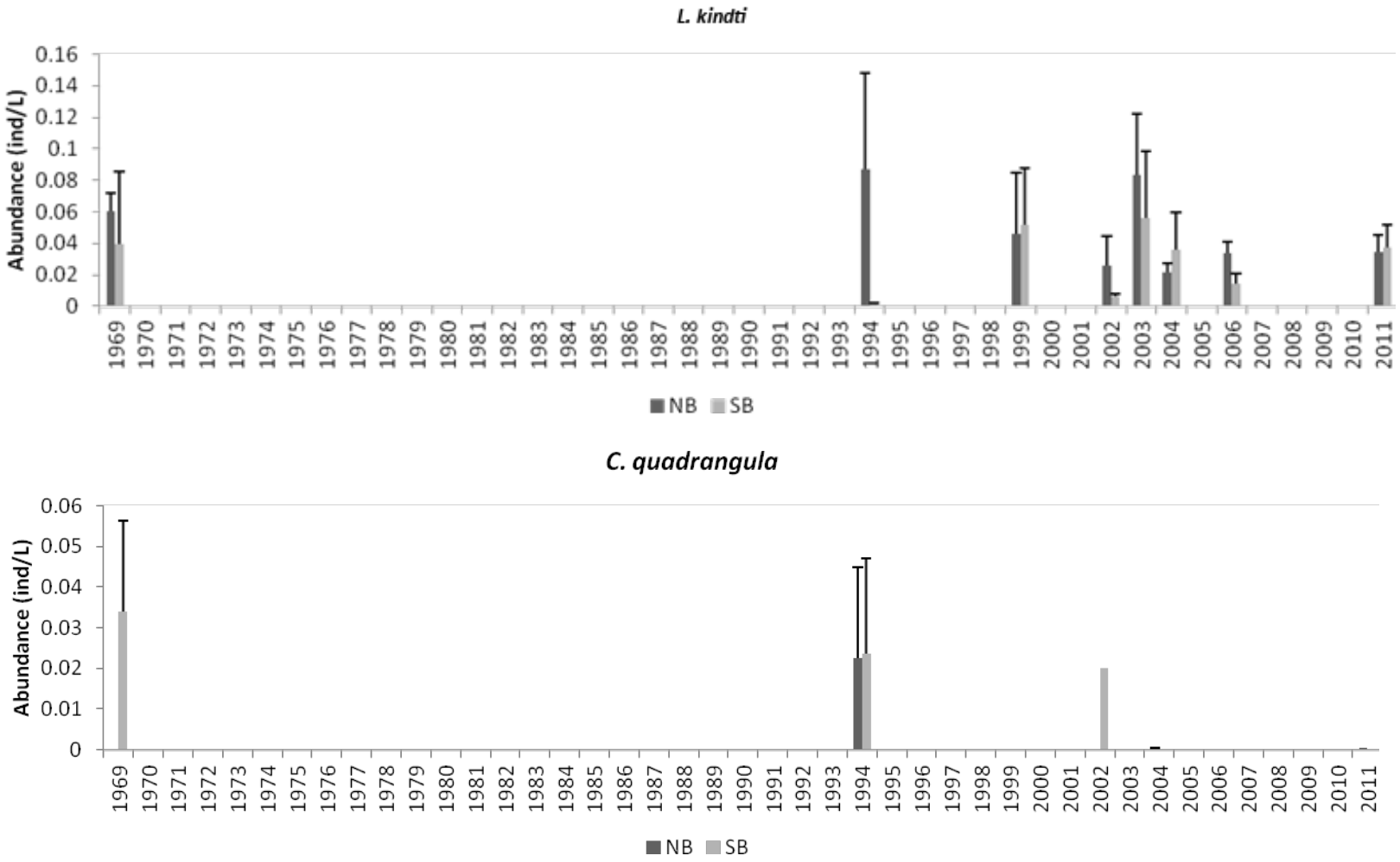

Figure A3.4 Densities (mean ± SE) of Cladocera species in North Basin (NB + Nar) and South Basin of Lake Winnipeg in Summer

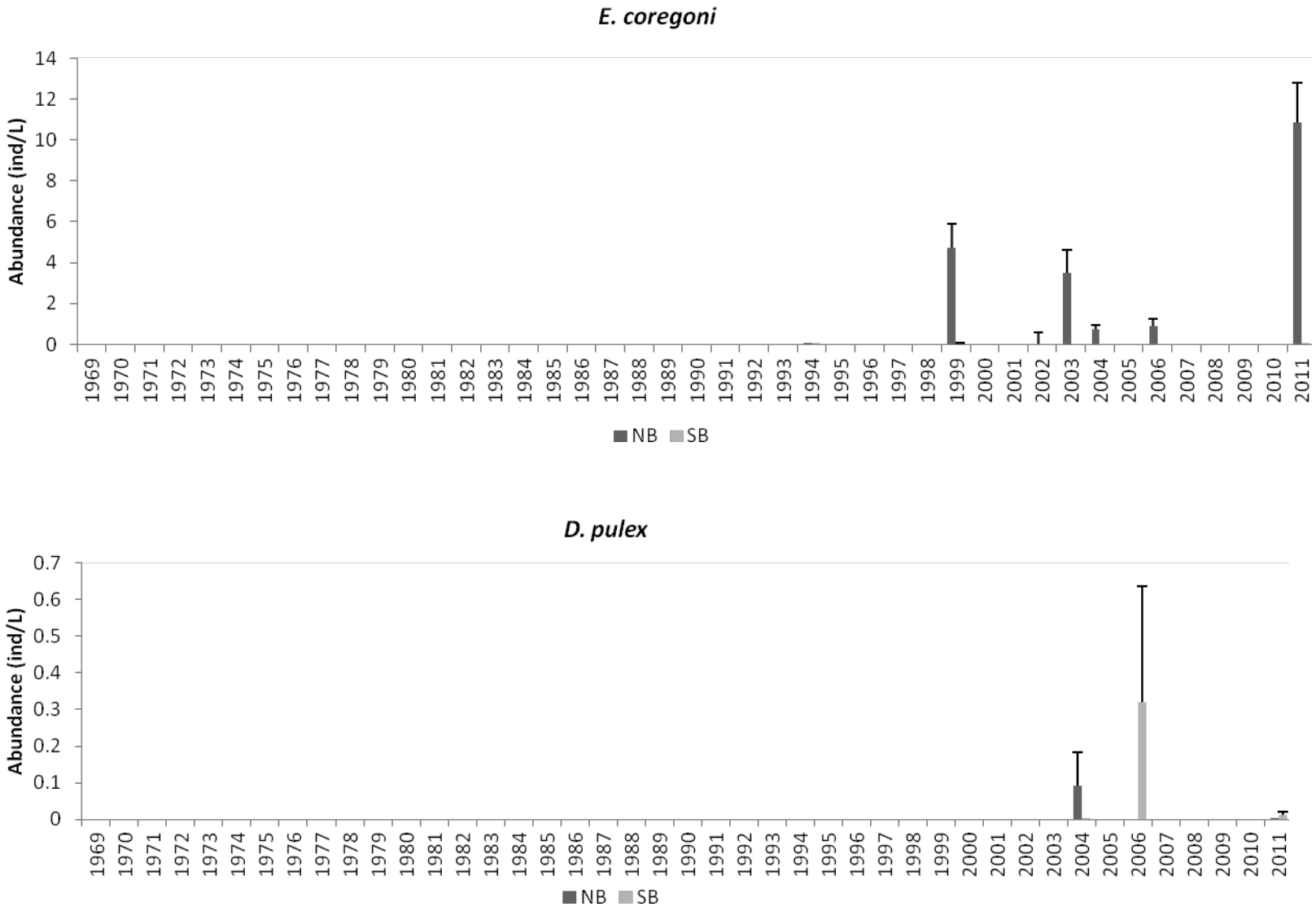

Figure A3.5 Densities (mean ± SE) of Cladocera species in North Basin (NB + Nar) and South Basin of Lake Winnipeg in Summer

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CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

- Research showed weak diel vertical migration, mainly for Cladocera in the North Basin. Evidence of reverse vertical migration was also found for most taxa which is indicative of invertebrate predator pressure. *L.kindti* a known invertebrate predator is present in Lake Winnipeg, and its absence within stratified samples might hint to errors during the sample collection with the net.
- Although Schulze (2011) indicated the influence of fish visual predation on zooplankton in turbid shallow lakes, light seemed to be the only factor responsible for DVM in Lake Winnipeg, as there were no consistent patterns observed when comparing zooplankton and fish vertical distribution between sites. Comparison with fish vertical distribution data might not be adequate as depth gaps between the stratified fish trawls exist (Lumb *et al.*, 2012)
- Other factors found relevant to DVM in shallow lakes such as turbulence (Baranyai and Lazlo, 2010) must be taken into account. As certain zooplankton groups are more sensitive to turbulence that affects their motility and filtering rates (Baranyai and Lazlo, 2010).
- Although research done by Livings *et al*. (2010) showed that column are more precise in determining zooplankton density than integrated sampling in well-mixed lakes, our t-test resulted in only a few significant difference between the two sampling methods. To make a more robust comparison and determine the dynamics of DVM in Lake Winnipeg, a

temporal and spatial sampling in a larger scales is paramount (Downing and Rigler, 1984; Livings *et al.*, 2010).

- Total phosphorus, total nitrogen and chlorophyll-*a* concentrations increased significantly in comparison with pre-eutrophication conditions (1969) and were highly correlated with the changes encountered in post-eutrophication conditions (2002) within the zooplankton community of Lake Winnipeg. Throughout the data of years available, Total Phosphorus and Total Nitrogen present significant variation in all basins and Chlorophyll-*a* only for South Basin in specific years. These results represent a clear indication of eutrophication phenomenon in Lake Winnipeg similar to other well studied lakes (McCullough *et al.*, 2012; Johansson *et al.*, 2000; Barbiero *et al.*, 2001; Conroy *et al.*, 2005).
- According to environmental variables used as proxies for eutrophication, the Narrows did not differ from North Basin in all years studied.
- All major zooplankton groups showed a significant higher density in post-eutrophication (2002) conditions than in 1969 (pre-eutrophication) with a high degree of variability depending on the season and basin which has been observed in other lakes under eutrophication (Conroy *et al.*, 2005; Barbiero *et al.*, 2001). Calanoid copepods and Cladocera were the groups to be affected in terms of species composition.
- There were basin differences on the basis of variation within the Cladocera community affected throughout the years in the summer season, with *Daphnia longiremis* and *Daphnia mendotae* showing a positive correlation with high nutrient levels, in North Basin and South Basin respectively. This might be an indication of large-bodied Cladocera being able to survive in eutrophic conditions while small-bodied Cladocera are

unable to ingest large phytoplankton (Vijverberg and Boersma, 1997; Holm *et al*., 1983). . These results were not statistically significant.

- The role of invasive species such as Rainbow Smelt on the changes within the Cladocera community must be taken into account as it has established itself successfully in the North Basin since the 1990s (Suchy *et al.*, 2010) and shown to be feeding upon large-bodied Cladocera (Sheppard *et al.*, 2012). However, no long-term fish community composition and density data are available.
- The time scale gap within the available data is an issue to give a more robust picture of the effects of these nutrients to the zooplankton community of Lake Winnipeg. Furthermore, previously documented (Patalas and Salki, 1992) intra-basin variation must be included into long-term effects of eutrophication to the zooplankton community.
- Despite the present issues with data set and lack of fish community data, the variation present within the zooplankton community, specifically Cladocera were found to be highly correlated and significant with the proxies for eutrophication in Lake Winnipeg.

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