Zooplankton community dynamics in the Trent Seven Waterway along Lake Simcoe and Lake Couchiching

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Abstract

The Trent Severn Waterway (TSW) is a 386km channel that connects Lake Ontario to Georgian Bay. There is a lack of information on the water quality in the nearshore region of the TSW specifically in regards to the planktonic communities. Zooplankton are key components of aquatic ecosystems since they graze on phytoplankton and are preyed upon by planktivorous fish. Therefore, changes to their community will have significant impacts to lower and upper trophic levels. The main objectives of this research are to (i) provide baseline data on zooplankton community dynamics in the nearshore region of the TSW; and (ii) determine if zooplankton can effectively be used as water quality indicators in this area.

Plankton and limnologic data sampling occurred at eight nearshore sites in Lake Simcoe and Lake Couchiching along the TSW over ten months from 2015-2016. A deepwater transect in Lake Couchiching was sampled over three seasons at varying depths. Environmental variables were collected to indicate water quality and included temperature, dissolved oxygen, chlorophyll *a*, pH, conductivity, total suspended load and nutrients (nitrate and total phosphorous). This study demonstrated that nearshore regions of the TSW exhibit a range of environmental conditions, varying from mesotrophic (N = 25.12 µg/L, TP = 10.83 µg/L, CHL *a* = 1.58 mg/m^3) to mesoeutrophic conditions (N = 78.92 µg/L, TP = 31.31 µg/L, CHL *a* = 13.29 mg/m^3).

Additionally, zooplankton community composition exhibited significant variation spatially and temporally and water quality reflected the degree of anthropogenic disturbance. The highly disturbed sites (TR, LC, MB, PO) experienced higher nutrient concentrations, conductivity, zooplankton biomass and density with lower dissolved oxygen concentrations characteristic of degraded water quality. Zooplankton density ranged from 1.11×10^7 – 1.43×10^8/L among all sites. Biomass varied from 26.09 – 221.16 µg/L with richness varying from 6.2 – 9.4. Diversity did not differ substantially (1.23 – 1.69). RDA found some species level response to environmental variables although multiple regression explained more variance in the data. High abundance of *Bosmina longirostris* in highly disturbed sites indicates more eutrophic conditions which supports other research. Long term monitoring of zooplankton can provide baseline data that localized effects from specific anthropogenic stressors can be compared.

Lay summary

Lakehead University's Department of Biology mission statement is "faculty and students in the Department of Biology are bound together by a common interest in explaining diversity of life, the fit between form and function, and the distribution and abundance of organisms". The main focus of this study is on zooplankton dynamics and as such contributes to one of the principal themes in the mission statement, being the relationship between organisms and their environmental functions. This study advances our understanding of zooplankton dynamics and the environmental variables that influence this community along the nearshore region of the Trent Severn Waterway. Zooplankton can be utilized as water quality indicators because they are impacted by temporal, spatial, biological, chemical and physical processes occurring in lakes. The major research questions explored were: 1. What is the zooplankton community composition in this truly dynamic area of study? 2. Does the zooplankton community composition vary with respect to season, environmental variables and sampling location? 3. Are there certain species or groups of zooplankton that could be used as biological indicators of water quality in this area? Results suggest that zooplankton dynamics did vary temporal, spatially and with changing environmental conditions. Zooplankton biomass and density proved to be good indicators of anthropogenic stress with both being substantially higher in highly disturbed sites compared to less disturbed sites which support other research. Long term zooplankton monitoring serves as a source to compare to localized impacts from anthropogenic disturbances and can provide useful information about changing water quality conditions.

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Chapter 1 Introduction

1.1 Overview

Freshwater ecosystems provide humans with drinking water, food, transport routes, recreation and spiritual fulfillment. The Lake Simcoe watershed generates revenue of over $ 200 million annually and therefore contributes significantly to the local and provincial economy (LSEMS, 2008). This would generally explain as to why humans first settled around water bodies. Anthropogenic activities occurring within and surrounding water bodies can result in degradation to water quality including the collapse of fisheries and decreased biodiversity (Kanavillil et al., 2012). The Trent Severn Waterway (TSW) is no exception encountering intensive agriculture and urbanization along its shorelines leading to anthropogenic impacts such as nutrient enrichment, climate change, metal and organic pollutants and invasive species (Palmer et al., 2011). Impairment to water quality can be detected in lower trophic levels, for instance planktonic species are impacted by subtle changes in environmental conditions (Gannon & Stemberger, 1978). Therefore, the purpose of this research is to provide baseline information on the zooplankton community dynamics in the TSW and determine the effectiveness of using zooplankton as water quality indicators in this area.

The general morphology and ecology of the three main classes of zooplankton will be discussed. A literature review concluded that there are limited studies on zooplankton communities within the nearshore region of the TSW. Furthermore, the exploration of zooplankton as indicators of water quality is not well understood. This study thus addresses this gap by studying zooplankton community dynamics in the TSW where it flows through Lake Simcoe and Lake Couchiching specifically in the nearshore region.

1.2 What are Zooplankton?

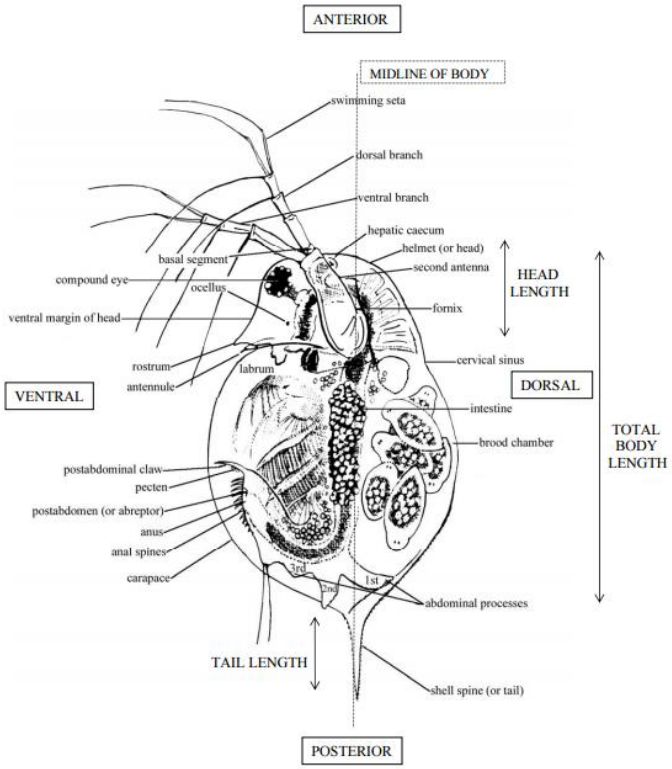
Zooplankton are microscopic animals that live suspended within the water column. They are the foundation of most aquatic food webs and as such they play a pivotal role in the transfer of energy to higher trophic levels from lower i.e. from the primary producers to higher order consumers like aquatic insects, juvenile fish, and planktivorous fish (Balcer et al., 1984; Welch & Jacoby, 2004). Therefore any environmental disturbances such as nutrient enrichment, thermal stability, pollutant loading or invasive species, which alter the zooplankton community dynamics, would likely impact other trophic levels. Freshwater zooplankton are comprised mainly of protozoans, rotifers and crustaceans (cladocerans and copepods) (APHA, 2005). The protozoan community has not been well studied in most aquatic ecosystems due to difficultly in identification (over 2000 species) (Witty, 2004). Therefore, this group will not be discussed in this research.

1.2.1 Cladocerans

The group Cladocera refers to the four orders Anopoda, Ctenopoda, Haplopoda, and Onychopoda (Witty, 2004) and the 11 families that fall under these orders such as Bosminidae, Chydoridae, Daphniidae, Leptodoridae, etc. Cladocerans are typically referred to as water fleas and range in size from 0.2 – 3.0 mm in length (Balcer et al., 1984; APHA, 2005). Their bodies are not distinctly segmented and are enclosed in a hardened shell like structure called a carapace which opens ventrally (Fig. 2). One of the main distinguishing features of this group is the presence of a single compound eye (Balcer et al., 1984).

Cladocerans are commonly filter feeders. They use their thoracic legs to bring water currents containing suspended food particles to their antennules (Balcer et al., 1984; Welch &

Figure 1. General morphology of a cladoceran (Witty, 2004)



Jacoby, 2004). Swimming hairs (setae) on their legs separate larger particles that cannot be consumed from the smaller ingestible particles such as algae, bacteria, protozoa, and other organic matter usually referred to as seston (Balcer et al., 1984; Welch & Jacoby, 2004).

They reproduce primarily through parthenogenesis in favourable conditions; female offspring develop from unfertilized eggs that are released after molts of mature females (Balcer et al., 1984). In unfavourable conditions such as overcrowding, decreasing food or temperature, high accumulation of metabolic waste or light intensity changes parthenogenetic eggs produce males. These males mate with females creating fertilized diploid eggs encased in an ephillium case that protects the eggs while they stay in a resting phase for more favourable conditions (Balcer et al., 1984; Welch & Jacoby, 2004).

This species undergoes cyclomorphosis throughout the year which results in changes to morphology such as helmet shape, eye size and shell spine and antennule length (Balcer et al., 1984). During the late fall, winter and early spring *Daphnia* have short, round helmets and as the population grows in the late spring into summer the head of each succeeding generation becomes longer and larger until late fall where they return to the a more rounded shape (Balcer et

al., 1984). Up until the seventies researchers believed plankton underwent cyclomorphosis due to sinking rate regulation; varying temperature affects viscosity which was thought to alter sinking speed (Lagergren et al., 2000). Currently, the explanation of why cladocerans undergo cyclomorphosis differs for each species and could include variations in water temperature, turbulence, light intensity, genetics and predation (Balcer et al., 1984). For example, Black (1980) observed that seasonal patterns of cyclomorphosis in *Bosmina longirostris* is likely the result of temporal predators (copepod *Epischura nevadensis*).

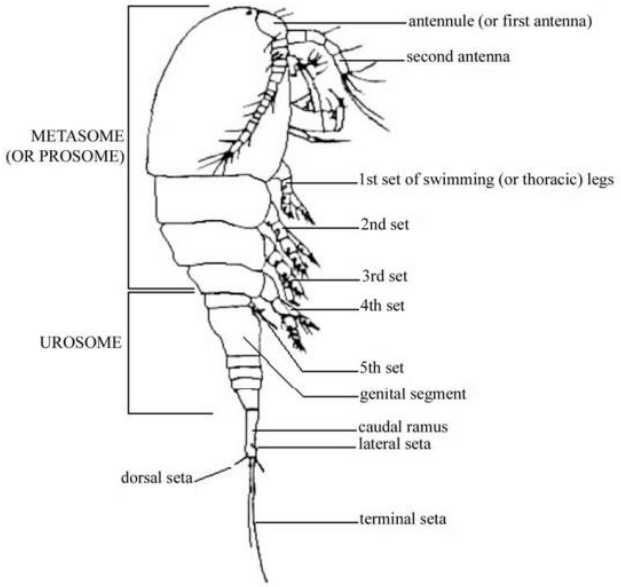
Another phenomenon many cladocerans undergo is vertical migration. This is where species migrate vertically in the water column in diurnal cycles. At dusk populations migrate to the surface from the deep and then travel downward again at dawn (Balcer et al., 1984). Light intensity changes are the major trigger for this behaviour although other factors such as age and species size, food supply, day length, oxygen concentration or turbulence can have an impact as well (Balcer et al., 1984). Distances covered in one diurnal cycle can range from 1 – 25 m. This behaviour is believed to be a result of predator avoidance in larger crustacean species. They spend daylight hours in the deeper, darker hypolimnion to avoid visual predators such as fish and migrate to the epilimnion at night where they are less likely to be seen (Welch & Jacoby, 2004).

1.2.2 Copepods

Copepods are generally referred to as oarsmen and comprise the three suborders Calanoida, Cyclopoida, and Harpacticoida (Balcer et al., 1984; Welch & Jacoby, 2004). They show greater species diversity in nearshore regions as there are a greater variety of habitats (Balcer et al., 1984). Their bodies are elongated, cylindrical and clearly segmented (Figure 2) ranging in length from 0.3 – 3.2 mm (Balcer et al., 1984; Welch & Jacoby, 2004). They are easily distinguishable from cladocerans due to the presence of a single, small pigmented eye and

multiple pairs of swimming appendages (Balcer et al., 1984; Welch & Jacoby, 2004) along with their segmented bodies.

Figure 2. General morphology of a copepod (Witty, 2004)



Copepods are mainly filter feeders; they move their second antenna and mouth parts to create water currents that bring food to the feeding appendages. Calanoids are able to selectively filter algae using satae on their maxillae and grasp algae or smaller zooplankton using modified maxillipeds (Balcer et al., 1984; Welch & Jacoby, 2004). Cyclopoid species can be considered herbivores, omnivores, or carnivores depending on their food preferences (detritus, algae, protozoans, cladocerans, or other copepods) (Balcer et al., 1984). Since harpacticoids are benthic dwellers they mainly consume detritus.

Unlike reproduction in cladocerans, copepods only reproduce sexually. A male transfers a packet of sperm (spermatophore) from their genital pore into a female genital segment and a fertilized egg is released from the genital tract (Balcer et al., 1984; Welch & Jacoby, 2004). Most female copepods brood the eggs in sacs although some calanoid species release eggs directly into the water (Balcer et al., 1984; Welch & Jacoby, 2004). Eggs hatch into small larvae termed nauplii which have three pairs of appendages they continue to molt and add appendages through six naupliar stages where they molt and enter the last copepodid stage (mature adults) (Welch & Jacoby, 2004). Interestingly, because they do not reproduce parthenogenetically female cyclopoids and harpacticoids can store sperm producing fertile clutches for extended

periods of time in the absence of males (Welch & Jacoby, 2004). They are able to adapt to unfavourable conditions by reducing their metabolic rate and enter diapause where they become dormant near the bottom; some species utilize this adaptive strategy in the winter where others in warm water temperatures (Balcer et al., 1984).

Similar to cladocerans copepods undergo diel vertical migration. They are capable of migrating up to 100 m because they are very strong swimmers (Balcer et al., 1984; Welch & Jacoby, 2004). According to Ringelberg (2009), the underlying mechanisms behind diel vertical migration have not been well examined as most studies last only a few days with only a few sample times and depth thus they lack sufficient detail on temporal development. Longer term studies have been conducted in Lake Constance and Lake Maarsseveen both located in Europe although the findings do not substantiate one another. Stich and Lampert (1981) and Geller (1986) both conducted extensive studies in Lake Constance on diel vertical migration a few years apart documenting similar migration patterns although their interpretations were vastly different (Ringelberg, 2009). Geller (1986) proposed that low food concentration and temperature were responsible for the occurrence of migration where Stich and Lampert (1981) opined that visually predating fish were the main driving force. It is generally accepted that crustacean zooplankton migrate to the deep during the day to avoid visual predation by fish (Lampert, 1993). Until the essential physiological and behavioural mechanisms are better understood considerable debate will most likely continue on the primary and causal causes of diel vertical migration in zooplankton.

1.2.3 Rotifers

Rotifers originated in freshwater with less than 5% of the 2,500 species occurring in brackish or marine waters (Smith, 2001). They are microscopic in size ranging from 40 µm – 2.5 mm and are divided into three classes: Seisonidea, Bdelloidea and Monogononta (Smith, 2001). They are typically referred to as wheel animals because their ciliated corona looks like a rotating wheel when viewed from the anterior (Figure 3). Of the three zooplankton groups rotifers are found in the most diverse habitats including deep regions of lakes, small puddles, damp soil, mosses or vegetable debris, from the Antarctic to Arctic to trophic and even in eaves troughs (Smith, 2001).

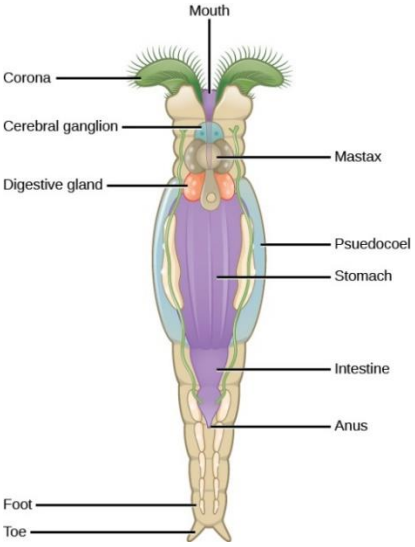


Figure 3. Anatomy of a bdelliod rotifer (University of California, 2016)

Approximately 90% of the known rotifer species fall under the Class Monogonota being characterized by a single ovary, non-branching mastax, and the presence or absence of a secreted tube (lorica) (Smith, 2001). Across the entire rotifer species there is great variation in morphological features and adaptations. The three body regions head, trunk and foot can typically be used to distinguish between species. The corona surface is ciliated with density of ciliation varying among species, although they serve the purpose of movement and feeding (Smith, 2001). The mastax is located between the esophagus and pharynx and is very unique because there is no other comparable structure within the animal kingdom (Smith, 2001). This structure contains translucent jaws (trophi) that tear and grind food into digestible portions (Smith, 2001).

As previously mentioned the cilia are used for feeding. They move back and forth creating a current that concentrates algae and detritus particles towards the mouth similar to the mouth appendages in crustacean zooplankton. There are several common genera that feed on other rotifers including *Asplanchna* sp., *Dicranophorus* sp., *Ploesoma* sp. and *Trichocerca* sp. (Smith, 2001).

Bdelliods reproduce solely through parthenogenesis where with Monogononta some species are exclusively parthenogenetic and other species reproduce sexually (Smith, 2001; Welch & Jacoby, 2004). The occurrence of males in these species is generally restricted to a few weeks. Ploimate rotifers have two distinctive types of females although they are morphologically interchangeable (with the exception of two to three species) (Smith, 2001). The first type are termed amictic and they reproduce asexually with both the body cells. Amictic eggs have the same number of diploid chromosomes. The second type, mictic females, only appear during changing environmental conditions with eggs undergoing double meiotic division becoming haploid (Smith, 2001). If these eggs are become fertilized by males they become resting eggs that are thick walled and highly resistant to adverse environmental conditions such as higher or lower temperatures and desiccation (Smith, 2001).

The oxygen requirements for most plankton and nearshore rotifers is quite high although there are some species that are able to withstand anaerobic conditions for short periods and low dissolved oxygen concentrations (0.1 – 1.0 ppm) for longer periods (Smith, 2001). *Asplanchna, Filinia*, *Ployartha* and *Keratella*, all limnetic plankton, are generally found in the oxygen deficient hypolimnion in lakes (Smith, 2001). Similarly, trickling filters in wastewater treatment plants contain oxygen deficient bottom layers of mud that usually contain *Lecane* sp., *Lepadella*

sp. and bdelliods species (Smith, 2001). The cilia are thought to provide adequate amounts of oxygen to rotifers in these environments through generation of water currents (Smith, 2001).

Rotifers exhibit seasonal variation similar to other plankton species. Some species have been identified as being monocyclic, dicyclic, polycyclic, acyclic or perennial based on what their annual population curves look like (one, two, multiple or no pronounced peaks) (Smith, 2001). *Brachionus angularis* and *Keratella cochlearis* commonly show dicyclic population patterns with a spring and fall maxima although sometimes they are perennial (Smith, 2001). Pennak (1949) stated it best that "cycles of abundance for plankton species are highly variable within each species, from year to year within a single lake, and especially variable from one small lake to another". Therefore, without conducting longer term zooplankton monitoring it would be difficult to estimate the probable population patterns of multiple or singular species.

Cyclomorphosis is evident among rotifer species resulting in variation of species among habitats. Size and lorica development among species seems to differ substantially from one habitat to another (Smith, 2001). All levels of development in posterior spines in *Brachionus calyciflorus* have been observed, from well-developed long spines to very short ones or no spines (Smith, 2001). The development of spines in the species is predominately a result of starvation, low temperatures, and chemical substances emitted by predatory *Asplanchna* sp. (Smith, 2001). These variations were thought to describe different species up until 1915. *Keratella* sp. is an extreme example as it is known to show 13 different forms (Smith, 2001).

The overall abundance and dispersal of zooplankton within aquatic ecosystems will be influenced by tolerance to abiotic parameters and interactions with other organisms (biotic). Some of the major influencing factors as described below.

1.2.4 Biotic Influences

Initially ecologists were focused on abiotic factors that influence zooplankton communities (Davidson & Andrewartha, 1948; Grinnell, 1917). The transition to study biotic influences occurred with the reasoning that the physiology and behaviour of organisms are impacted by abiotic factors, which can affect the outcome of biotic interactions resulting in community changes (Dunson & Travis, 1991).

1.2.4.1 Predation and Competition

Planktivorous fishes have been shown to significantly impact zooplankton community composition. The factors that determine if a particular species is vulnerable to fish predation are visibility, evasiveness and habitat overlap. Visibility of zooplankton to predators is dependent upon the presence or absence of eggs, size and pigmentation (Welch & Jacoby, 2004). It is interesting to point out that zooplankton found in lakes containing predatory fish are typically translucent where in lakes without these predators similar species show pigmentation (Welch & Jacoby, 2004). The stomach contents of fish have been found to be strongly populated by egg bearing *Daphnia* even in habitats where they only encompass a small portion of all the crustacean zooplankton (Welch & Jacoby, 2004). Certain species employ vastly different evasive techniques to avoid fish predators. For instance, cyclopoid copepods and rotifers possess well developed jump responses which allow them to quickly move away from predators in comparison to slower moving species such as *Daphnia* (Welch & Jacoby, 2004). Another method some species utilize to escape predation is diel vertical migration which was previously discussed.

Zooplankton communities subject to intense predation by fish are typically dominated by smaller evasive zooplankton species such as rotifers, small cladocerans and cyclopoid copepods

(Arnott & Vanni, 1993; Brooks & Dodson, 1965; Hrbacek et al., 1962; Lynch, 1979; Pont et al., 1991; Welch & Jacoby, 2004). This is mainly attributed to their size, larger zooplankton are more easily seen by visual predatory planktivorous fish (Arnott & Vanni, 1993). In aquatic systems where fish predation is absent the zooplankton assemblage is usually dominated by larger taxa, for example *Daphnia* species (Brooks & Dodson, 1965; Welch & Jacoby, 2004). There are two hypotheses proposed as to why this is.

The first being size-selective predation by large, predatory invertebrates on smaller zooplankton. Predation by invertebrates on smaller zooplankton is more intense in the absence of fish because fish prey on larger invertebrates reducing their abundance (Arnott & Vanni, 1993). Therefore, when fish predation is scarce there are more invertebrates to prey on smaller zooplankton (Dodson, 1974; Lynch, 1979); they potentially prey more intensely on smaller taxa in comparison to larger species because their small size aids in easier handling and digestion (Swift & Fedorenko, 1975; Williamson, 1987). *Chaoborus*, an invertebrate predator, reaches much higher densities in the absence of fish predation and can significantly impact smaller zooplankton populations (Fedorenko, 1975; Yan et al., 1991).

The second hypothesis is competitive suppression of small zooplankton by large, herbivorous zooplankton. This hypothesis is based upon the fact that larger zooplankton such as *Daphnia* are believed to be better competitors compared to smaller, less efficient filter feeders (Arnott & Vanni, 1993). Brooks and Dodson (1965) proposed the size efficiency hypothesis of increasing zooplankton size causing resource acquisition to increase disproportionately faster than the metabolic requirement. In other words, the net energy increases (resource intake minus metabolic cost) as zooplankton size increases (Arnott & Vanni, 1993). Larger zooplankton species have a competitive advantage in conditions of fluctuating resources with periods of high

resource concentrations and extended periods of starvation because they are able to survive longer periods of resource depletion compared to smaller species (MacIsaac & Gilbert, 1991). MacIsacc and Gilbert (1991) conducted serval laboratory experiments using *Keratella* sp. and *Daphnia* and found that under high food regimes *Daphnia* did not prey on *Keratella* sp. but when food supply was low *Daphnia* preyed on *Keratella* sp. Although there have been sufficient experiments conducted in lakes, mesocosms and microcosms, debate still continues on whether predation or competition are more important determinants of zooplankton composition in lakes.

1.2.4.2 Food Availability

Availability of food is another biotic factor that impacts zooplankton community dynamics. Food abundance and quality affect growth rates and female fertility (Welch & Jacoby, 2004). In food rich conditions zooplankton reach the first reproductive event faster at larger sizes and produce larger clutches (Welch & Jacoby, 2004). Herbivorous zooplankton consume a wide range of suspended particulates, seston, comprised of phytoplankton, bacteria, detritus and microzooplankton (rotifers and ciliated protozoans). Their ability to utilize seston as a food resource depends on how nutritionally adequate it is. For instance, some species of phytoplankton possess hardened or gelatinous cell walls so they are able to pass through zooplankton digestive systems intact (Welch & Jacoby, 2004).

The growth response of zooplankton to increasing amounts of seston follows the same model of Michaelis-Menton for phytoplankton growth rate versus nutrient supply (Welch & Jacoby, 2004). During low seston concentrations (<0.5 mg/L) *Daphnia* grow linearly related to food quantity; as food concentrations increase eventually ingestion rates become saturated and growth reaches the maximum potential for that given food source (Welch & Jacoby, 2004). Additionally, food availability significantly varies on an inter-annual basis in lakes (Shade et al.,

2007). Therefore, strong seasonal peaks of zooplankton biomass is associated with peak food concentrations when food is restricted in a given year but when food is abundant zooplankton abundance is sustained throughout the year (McCauley & Murdoch, 1987). Chang et al. (2014) discovered that the association between zooplankton and phytoplankton species composition was stronger compared to zooplankton species composition and physicochemical parameters. It can be concluded that while physical factors are the main drivers for seasonal succession, food quality (phytoplankton species composition) determines the success of zooplankton species in competition and thus the overall amplitude of species shifts (Chang et al., 2014).

1.2.5 Abiotic Influences

In addition to biotic factors, abiotic factors such as light and temperature have been shown to impact zooplankton community dynamics. A brief description of influence of important abiotic factors are given below.

1.2.5.1 Temperature

Seasonal variation of temperature impacts zooplankton species composition and total biomass. Typically, zooplankton biomass increases in spring to mid-summer peaking in June to July and then starts to decrease in fall (Chang et al., 2014; Pothovern & Fahnenstiel, 2015). Total phosphorous, chlorophyll *a*, zooplankton density and biomass all showed significant seasonal variation patterns in Lake Ontario (Hall et al., 2003).

The initiation of seasonal succession in temperate lakes has been largely attributed to changes in water temperature (Yoshida et al., 2001). It has been found that increasing temperature changed the number of generations from one to two per year in some zooplankton species (Winder et al., 2009). Furthermore, zooplankton growth and egg development are strongly temperature dependant (Welch & Jacoby, 2004). Temperature reportedly affects

Daphnia as temperature increases signal them to emerge earlier (Carvalho & Kirika, 2003) and shortens their life spans (Bottrel, 1974). In laboratory tests average life span for *Daphnia magna* was found to be 40 and 56 days when exposed to 25ºC and 20ºC, respectively (APHA, 2005).

Elevated temperatures can also cause less water (evaporation) to enter lakes through stream and from the groundwater. This leads to a decreased dissolved organic carbon concentration in water thereby elevating ultraviolet-B penetration in lakes (Schindler et al., 1996). This increase induces changes in the molecular mechanisms that daphniids utilize to repair UV damage such as photoenzymatic repair and light independent nucleotide-excision repair (Rautio & Tartarotti, 2010). There are conflicting findings on whether DNA repair occurs more optimally at higher or lower temperatures (Altshuler et al., 2011). Pinel-Alloul et al. (1995) carried out a survey on the abiotic and biotic factors influencing zooplankton heterogeneity in Québec lakes discovering that both factors contributed to explaining 48% of the variability in zooplankton although abiotic factors explained more variance.

1.2.5.2 Light Intensity

Light intensity is thought to be most prominent trigger of diel vertical migration in zooplankton (Balcer et al., 1984; Brierley, 2014). This is because of the high amount of activity in migrating zooplankton populations at dawn and dusk. Interestingly, varying cloud cover and phases of the moon also support a varying light intensity (Brierley, 2014). The depth that species travel becomes less dependent on light and more dependent on other factors including water temperature and chemical hormones emitted form predator fish (Balcer et al., 1984). Furthermore, zooplankton populations are impacted by anthropogenic disturbances.

1.3 Zooplankton Community Structure Variability due to Anthropogenic Disturbances

Anthropogenic activities surrounding watersheds have long been depicted as causing negative impacts on water quality (Kanavillil et al., 2012). The main anthropogenic stressors which impact Lake Simcoe and Lake Couchiching and subsequently the TSW zooplankton community dynamics are invasive species, climate change, salinity, calcium decline and nutrient enrichment (Young & Jarjanaiz, 2015).

1.3.1 Invasive Species

Most introductions of invasion species into waterbodies can be linked back to humans. For instance, the introduction of Ponto-Caspian invaders into the Great Lakes was due to ballast water dumping from transoceanic ships containing the invaders (*Dreissena polymorpha* and *Dreissena bugensis*, *Neogobius melanostomus*, and *Bythotrephes longimanus*) (Young & Jarjanaiz, 2015). From there the invaders were able to travel throughout the Great Lakes attached to boats or by simply swimming into connecting waterways. A study by Weisz and Yan (2010) conclusively demonstrated a strong correlation between *Bythotrephes longimanus* occurrence and anthropogenic activities in lakes with shoreline coverage of cottages being the strongest predictor of its occurrence compared to other physical and chemical variables.

Invasive *Bythotrephes longimanus* (spiny water flea) is a generalist cladoceran predator that invaded the Laurentian Great Lakes in the 1980s from Europe (Sprules et al., 1990). It first appeared in Lake Simcoe during 1993 and became well established by 1994 (Young & Jarjanaiz, 2015). It can be assumed that Lake Couchiching would have been invaded around the same time based on its proximity to Lake Simcoe. *Bythotrephes longimanus* is able to thrive in a wide range of temperature, salinity and pH gradients (Grigorovich et al. 1998) making it a resilient competitor for native zooplankton that possess smaller tolerance ranges. It captures its prey

using its large thoracic legs, dismembers the body, and then proceeds to drink the liquid innards (Burkhardt & Lehman, 1994). *Bythotrephes longimanus* prefers slow moving, visible prey such as *Bosmina* and *Daphnia* species (Grigorovich et al. 1998) and can consume up to 75% of its body weight in food per day (Lehman et al. 1997). Therefore, *Bythotrephes longimanus* caused significant reductions in zooplankton abundance and richness because it primarily feeds on cladocerans (Azan et al, 2015; Yan et al., 2002).

It has been reported that this predator occupies the epilimnion during the day (Young & Yan, 2008) thus it induces vertical migration of its prey to the lower hypolimnetic layer where their growth is inhibited (Pangle et al., 2007). The effects of *Bythotrephes longimanus* on zooplankton abundance, composition and vertical migration can have impacts to other trophic levels. For example, rotifers appear to benefit from the competitive and predator release as native crustacean zooplankton populations decline (Hovius et al. 2006, 2007). The loss of herbivorous zooplankton may impact phytoplankton populations because copepods are less efficient grazers leading to increased abundance of algae; findings have been mixed (Azan et al., 2015). Nutrient cycling may also be affected because copepods sequester less phosphorous and uptake more nitrogen than daphniid species (Williamson & Reid, 2009). Overall, in most lakes throughout North America crustacean zooplankton abundance has declined as a result of the invasion (Azan et al., 2015). One study showed increased zooplankton abundance in Lake Huron although this was attributed to the higher density of fast swimming copepods and their nauplii post invasion due to less competition and predation from cladocerans and their ability to escape predation by *Bythotrephes longimanus* (Fernandez et al. 2009). Cladoceran species richness declined by 36% following invasion in Harp Lake (Boudreau & Yan, 2003), 26 Canadian lakes (Kelly et al., 2012) and 10 lakes in the Muskoka district (Strecker et al., 2006).

Bosmina has been indicated as the most sensitive cladoceran species to predation as it was found to decline in 12 out of 15 studies; *Leptodora kindtii*, and *Daphnia retrocurva* are the second and third most sensitive species, respectively (Azan et al., 2015).

The effects of predation by invasive *Bythotrephes longimanus* on copepods species have not been detected in the majority of North America studies (Azan et al., 2015). It is proposed that the impact could be at the species level with more tolerant species replacing sensitive ones that perform similar ecological functions (Azan et al., 2015). *Bythotrephes longimanus* does predate on copepods but copepods have better developed escape responses than large *Daphnia* species thus they move out of the sensory range of this predator (Vanderploeg, 2011). Copepods also exhibit diel vertical migration travelling deeper with increasing *Bythotrephes longimanus* abundance.

The Lake Simcoe Regional Conservation Authority (Young & Jarjanaiz, 2015) first began monitoring *Bythotrephes longimanus* in 1999 and have concluded that its abundance has been declining since 2006 possibly due to increased predation by planktivorous fish and invasive round goby. Further research is required to determine the exact cause.

Another group of invasive species that could potentially impact zooplankton community dynamics are Dreissenids (zebra and quagga mussels). Dreissenids increase water clarity by filtering out algae and other suspended solids (Evans, 2007). Clearer water results in increasing light penetration, which can result in proliferation of submerged aquatic vegetation in shallow waters (Ginn, 2011). Increase in aquatic vegetation abundance/biomass can lead to changes in the community of phytoplankton, zooplanktons' main food resource, directly through changes to nutrient cycling, increased sedimentation, shading and allelopathy and indirectly via increasing grazing from limnetic zooplankton (Winter et al., 2011). It is also hypothesized that this invader

redirects the flow of nutrients and phytoplankton away from the offshore into shallow water where they inhabit thus impacting the pelagic zooplankton communities (Young & Jarjanaiz, 2015).

Studies which examined zooplankton communities over the entire ice-free season were better able to capture changes in the community compared to single sampling events, irrespective of sample design (Azan et al., 2015). The introduction of other aquatic invasive species into this watershed would likely cause changes to zooplankton communities dynamics as evident from impacts caused by current invaders. Another anthropogenic influence likely to impact zooplankton is climate change.

1.3.2 Climate Change

Climate change is largely anthropogenically driven due to increases from greenhouse gas emissions (IPCC, 2007). The effects from climate change on aquatic ecosystems are expected to influence water quality through decreasing water availability, concentrating pollutants and increasing salinity (Schindler, 2001). Climate change is expected to impact keystone species such as *Daphnia* through temperature fluctuations which may lead to cascading effects up and down the aquatic food web (Jeppenson et al., 2010). Daphniid species are expected to shift from more thermophobic to thermophilic as climate increases and in some cases this could provide an ideal environment for invasive species to flourish such as thermotolerant *Daphnia lumholtzi* (Lennon et al., 2001). *Bythotrephes longimanus* is able to thrive in a range of temperatures from 4 – 30 ºC thus this species would most likely be able to survive a few degree increase in temperatures and could potentially replace other *Daphnia* species which are more sensitive to even slight fluctuations in temperature.

For every rising degree in temperature, predation by cladoceran *Leptodora kindtii* and planktivorous fish on *Daphnia galeata* begins markedly earlier in the season by 13.0 and 6.5 days, respectively which means that *Daphnia galeata* has less time to build-up its population resulting in lower species density (Wagner & Benndorf, 2007). There are some studies that suggest metals become more toxic at increased temperatures because bioaccumulation increases (Cairns et al., 1975). Changes in rates of metabolism and feeding due to climate change will affect the rate of uptake and detoxification as well as the sensitivity of organisms to toxins. A prime example, the rate of cadmium uptake in *Daphnia* spp. significantly increased at higher temperatures potentially caused by increased ventilation rates in response to higher metabolic rates and oxygen demand (Cairns et al., 1975; Heugens et al., 2003).

The bulk of research focuses on increasing temperature effects as a result of climate change although there are other impacts from climate change that may induce changes in zooplankton communities such as increased storm intensity and duration. Increased storm duration and intensity may directly impact zooplankton distribution because they are free floating and move with changing currents and winds also indirectly through introducing increased nutrients into aquatic systems which in turn can lead to increased phytoplankton growth and thus increasing food resources for zooplankton. Significant changes to the food web could potentially be induced by climate change with some effects not yet fully recognized. An emergent anthropogenic contaminate entering waterways that will impact zooplankton is salinity.

1.3.3 Salinity

LSRCA (2015) has recorded increasing levels of chloride in Lake Simcoe and its tributaries since monitoring began in the seventies. Because salinity concentrations are increasing rapidly in tributaries directly adjacent to roads and seasonal patterns are evident

(highest chloride concentrations in the winter and early spring), application of road salt for winter operations is thought to be the main culprit (LSRCA, 2015). Ontario applies the most road salt out of all Canadian provinces with rock salt (sodium chloride) being the most common deicing agent utilized due to its cost effectiveness, ease of application and suitability for this climate (Roe & Patterson, 2014). Salinity affects osmotic regulation in aquatic organisms (Silver et al., 2009) thus declines in salt intolerant species lead to biomass reductions and possible massive changes to communities (Corsi et al., 2010).

Increasing salinity concentrations have been shown to cause decreases in zooplankton densities with significant declines observed at 50 – 150 ppm salinity (Dalinksy et al., 2014). Higher chloride has been linked to reducing average copepod populations by 85% and cladoceran density by 94% (Meter et al., 2011). Community level effects are still not fully understood with some studies showing that increased road salts in stormwater and natural ponds decreases zooplankton grazing pressure on algae and as such tadpoles indirectly benefit through the loss of competitions for algal resources (Meter et al., 2011). Chloride is considered an increasingly common pollutant of aquatic ecosystems with scientific research still needed to investigate the direct and indirect effects within communities and how this will effect trophic level interactions. The effect of decreasing calcium will cause detrimental impacts to crustacean zooplankton.

1.3.4 Calcium Decline

Calcium is an essential macronutrient for most organisms' thus subsequent declines in calcium will likely have implications on aquatic organisms (Azan et al., 2015). Declining calcium inputs into lakes through atmospheric deposition has been found (Keller et al. 2001; Likens et al. 1998; Watmough et al. 2005) as well as decreased hydrologic inputs potentially

attributed to reforestation of previously logged watershed (growing trees uptake plentiful amounts of calcium) (Likens et al. 1998; Piirainen et al. 2004; Watmough et al. 2003). Dissolved ionic calcium is especially important for cladocerans because they utilize it to form their protective carapace (Cowgill et al. 1986). Lakes with lower calcium concentrations typically report comprised remineralization of carapaces (Greenaway, 1985) thus survival, growth and reproduction of daphniids become threatened (Ashforth & Yan, 2008). Calcium declines lead to the loss of daphniids in pelagic communities in several studies (Cairns 2010; Hessen et al., 1995).

Furthermore, affects to zooplankton community distribution and size have been noted as species that require high amounts of calcium (for example, *Daphnia pulex*, *Daphnia pulicaria*, and *Daphnia mendotae*) are generally located in lakes with high calcium concentrations (Wærvågen et al. 2002). Cairns (2010) claimed that the optimum calcium concentration for larger daphniid species ranged between 2.76 – 16.1 mg/L with reproduction and anti-predator defences becoming impaired in the range of 1.26 – 1.69 mg/L for differing species of the same size. Paleolimnological studies have shown concurrent evidence of declining calcium and decreasing relative abundances of daphniids from the *Daphnia longispina* complex (*Daphnia ambigua, Daphnia dubia, Daphnia mendotae, Daphnia longiremis,* and *Daphnia retrocurva*) from post-industrial sediments in 36 of 37 lakes examined (Jeziorski et al. 2012). Declining calcium most likely will cause detrimental effects to zooplankton community dynamics and ultimately planktivorous fish since they prey on large cladoceran species.

1.3.5 Nutrient Enrichment

Nutrient enrichment is another anthropogenic disturbance that influences zooplankton composition. Numerous studies have shown that climate change will impact more than just

temperatures it may also intensify the impact of eutrophication due to the loss of fish to oxygen starvation (Feutchmayr et al., 2009; McKee et al., 2003; Moran et al., 2010). In a study performed using freshwater mesocosms it was hypothesized that phytoplankton abundance increased with higher nutrient loading leading to peaks occurring later in the season and therefore higher and later peaks in zooplankton grazers (Feutchmary et al., 2010). This was confirmed with higher nutrient levels causing a later peak in *Daphnia* spp. (a major predator of phytoplankton) abundance which corroborated another study by Jäger et al. (2008) that found an increased peak *Daphnia* spp. biomass with phosphorous enrichment. Overall, it was found that chlorophyll *a*, phytoplankton and crustacean zooplankton peak abundance responded strongly to nutrient enrichment and elevated temperature (Feutchmary et al., 2010).

Another study looked at the variability in plankton under varying nutrient regimes. Pan et al. (2014) suggested that the stability of the phytoplankton and zooplankton relationship fluctuates with nutrient concentrations because of phytoplankton possessing nutrient dependent morphology and inferred that extremely low or high nutrient loading may disrupt this relationship. It is understandable that nutrient deficiency especially of nitrogen and phosphorous limits the growth of phytoplankton thereby impeding the transfer of energy to higher trophic states (Perhar et al., 2013) and decreasing growth rates of grazers (Grover et al., 2003; Verschoor et al., 2004).

When nutrient enrichment occurs it can induce strong population oscillations within the aquatic food chain potentially causing the loss of both phytoplankton and zooplankton (Davis et al., 2010; Van Donk et al., 2011). One reason for this is phytoplankton expresses a non-linear morphological response to changes in nutrient such as forming long filaments and increases colonies in high nutrient environments. This is thought to be due to the off-set of the surface

area to volume ratio by the increase in nutrient availability (Kruskopf, 2006). Longer filaments and larger colonies may infer with the feeding appendages of zooplankton inadvertently creating a defence mechanism (Gilwicz et al., 1990; Van Donk et al., 2011). Nonetheless, in extremely high nutrient conditions it may cause fragmentation of these newly formed filaments and colonies due to high concentrations of inorganic salts (can cause cell apoptosis) (Ning et al., 2002; Wu et al., 2003). Ultimately, the relationship between phytoplankton and zooplankton appears to be less variable under medium nutrient concentrations compared to low or high concentrations (Fussmann et al., 2000).

1.4 Previous Zooplankton Studies in Lake Simcoe and Lake Couchiching

1.4.1 Lake Simcoe Water Quality

Water quality issues in Lake Simcoe first became apparent in the 1970's due to the excessive growth of aquatic macrophytes and algae as well as the recruitment failure of popular cold water fish lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis)* (Palmer et al., 2011). These impacts were attributed to increases in phosphorous loading from anthropogenic sources such as wastewater treatment plant effluent, runoff from agriculture and urban areas and aerial deposition of phosphorous enriched soil particles. Phosphorous levels within the lake have been reduced to 72 tons/yr from over 100 tons/yr in the seventies; an overall reduction of 30% (Winter et al., 2002). The Lake Simcoe Protection Plan (LSPP) specified a minimum target of 7 mg/L for hypolimnetic dissolved oxygen concentration to support the natural recruitment of cold fisheries (LSPP, 2009). Therefore, total phosphorous would need to be further reduced to an estimated 44 tons/yr to meet the above target for dissolved oxygen concentrations (Young et al., 2011).

More recently, other stressors have begun to affect Lake Simcoe and the surrounding watershed, for instance climate change, metal and organic pollutants and invasive species. There are approximately 16 known invasive species in Lake Simcoe which means it is likely they also inhabit Lake Couchiching and the Trent Severn Waterway since Lake Simcoe flows north into Lake Couchiching and these two lakes are connected as part of the Trent Seven Waterway. Examples of aquatic invasive species include zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena rostriformis bugensis*), spiny water flea (*Bythotrephes longimanus*), and Eurasian watermilfoil (*Myriophyllum spicatum*). One major issue with invasive species is that when there are multiple invasive species introduced from the same region it can produce an "invasional meltdown" in the invaded lake (Ricciardi, 2001); one invasive species assists with invasions by other species.

Potential effects from *Dreissenid* mussels include increasing water clarity and decreasing total phosphorous and phytoplankton which indirectly influences other trophic levels (Higgins & Vanderzanden, 2010). In a large scale survey of the lake from 1980 – 2007 Winter et al. (2011) found that total algal biovolume and community composition positively correlated with lake water total phosphorous concentration and genus and species shifts were correlated with *Dreissena* establishment along with nutrient concentrations and lake clarity.

The effects of climate change on lake chemistry and biota are not well understood in this region. Warmer June and September air temperatures by 2.5 and 2ºC, respectively are thought to likely cause earlier development of thermal density gradients and prolonged fall mixing resulting in stratification of the main basin and Kempenfelt Bay to last approximately 33 days longer and 55 days longer in Cook's Bay (Stainsby et al., 2011). This will have serious implications to coldwater fish species directly because warmer air temperatures will cause the epilimnion to

extend deeper decreasing the volume of coldwater habitat and subsequently dissolved oxygen concentrations. Indirectly this may impact them as well through reducing the temporal overlap between zooplankton and phytoplankton (Winder & Schindler, 2004), which could lead to less abundance of zooplankton for planktivorous fish to prey upon.

Excessive nutrients, total suspended solids, chloride, metals, biological pathogens, organic chemicals, pharmaceuticals and emerging contaminants of concern can all negatively impact water quality. Sediment surveys carried out in Lake Simcoe every 5 years show high concentrations of organic and metal contaminants. Some metals have decreased (e.g. chromium) due to changes in wastewater practices and industrial activities while others such as zinc have remained elevated due to the primarily source being uncontrolled storm water runoff (Young & Jarjanaiz, 2015). It is important to study and monitor the effects of current and emerging stressors to restore and prevent further degradation to aquatic ecosystems.

The majority of research and monitoring in Lake Simcoe is performed by with the Ministry of Environment and Climate Change (MOECC) and the Lake Simcoe Regional Conservation Authority (LSRCA). The 2014 Lake Simcoe Monitoring Report summarises data collected on the lake from the 1980's to 2012 and was produced in partnership of the MOECC and LSRCA. In general total phosphorous has been declining since the eighties as a result of several reduction strategies although subwatersheds that receive more urban (East Holland River) or agricultural (Maskinonge and West Holland River) runoff had higher concentrations from 2009-2012 (Young & Jarjanaiz, 2015). Unsurprisingly, Cook's Bay has the highest levels of total phosphorous ranging between 17-24 µg/L with concentrations gradually decreasing northward to Atherely Narrows where they were 9 µg/L; likely attributed to cycling and sedimentation of phosphorous (Young & Jarjanaiz, 2015). At all of the eight open lake stations

there was no significant change in phosphorous since decreasing in the eighties it has been variable but consistent due to mixing and sedimentation.

Total nitrogen another limiting nutrient in aquatic systems is comprised of nitrite and nitrate, ammonium and organic nitrogen. Similar to total phosphorous, total nitrogen has been more variable in tributaries overtime compared to the lake where concentrations were lower and the highest concentrations observed in Cook's Bay (0.52 mg/L). Predictably, nitrogen concentrations varied and were synchronous with phosphorous increases and decreases further signifying that the primary source of both nutrients is runoff from the watershed (Young & Jarjanaiz, 2015).

Other indicators of water quality include water clarity, dissolved oxygen levels and aquatic pollutants. Water clarity was found to have significantly increased in all open water stations. Increases in water clarity earlier on coincided with the diversion of sewage from the Holland River and the establishment of the invasion zebra mussel (Young & Jarjanaiz, 2015). Both of these events lead to decreasing nutrients and phytoplankton in the water column improving water clarity. In more recent times, water clarity has been more stable and even showed signs of decreasing. Minimum volume weighted hypolimnetic dissolved oxygen (MWVHDO) has increased from an average of approximately 3 mg/L in the 1980's to 5 mg/L in 2012. Although this still falls short of the LSPP target of 7 mg/L coldwater fish species have shown signs of recovery (Young & Jarjanaiz, 2015).

Aquatic pollutants include total suspend solids (TSS), chloride, metals, organic chemicals, pharmaceuticals, and other emerging contaminants of concern. The three tributaries East Holland Creek, Lovers Creek and North Schomberg River continuously exceed the Canadian Water Quality Guidelines for chronic exposure to chloride of 120 mg/L. All three

tributaries drain from urban areas. Average annual lake concentrations of chloride are much lower varying from 42-50 mg/L but have been increasing significantly overtime (Young & Jarjanaiz, 2015). Conductivity which is the measure of waters ability to pass an electrical current has significantly increased as well and is influenced by negative ions such as chloride.

The biological community in Lake Simcoe has been monitored extensively at open water stations. Total abundance of phytoplankton has generally been the greatest in Cook's Bay where higher total phosphorous concentrations were found. Diatoms typically comprise the largest group of phytoplankton in the lake. Nutrients were found to be a major factor influencing phytoplankton biovolume. The biovolume witnessed a massive decrease after diverting sewage from reaching in the lake (Young & Jarjanaiz, 2015). Herbivores such as zebra mussels impacted the phytoplankton community by declining the phytoplankton biovolume and changing their species composition. Chlorophyll *a* and phytoplankton biovolume increased during 2004-2010 then started to decrease in 2011 and 2012 (except for in shallower Cook's Bay stations) consistent with the declines in total phosphorous (Young & Jarjanaiz, 2015). There have been no substantial difference detected in the abundance of oligotrophic taxa (*Cyclotella* sp., *Bicosoceca* sp., *Chrysolykos* sp. and *Kephyrion* sp.).

Zooplankton is another biological entity found throughout Lake Simcoe. The abundance of zooplankton was similar at all three open water stations where they were collected with an exception of a very large peak in small cladocerans and immature copepods at Cook's Bay in 1995 (Young & Jarjanaiz, 2015). Overall, zooplankton abundance has decreased significantly at all stations after the establishment of *Bythotrephes longimanus* (spiny water flea) in 1994. Cladocerans, primary prey of *Bythotrephes longimanus*, were impacted the most with the average number of cladoceran species being reduced in half from 6 to 3 from 1993-1994. Since

the 2000's cladoceran richness showed an increase (Young & Jarjanaiz, 2015). Furthermore, the rarity of coldwater species *Daphnia longiremus* and *Leptodiatomus sicilis* since the 2000's is likely attributed to increasing water temperatures (Young & Jarjanaiz, 2015). Currently, the MOECC is studying the factors affecting the zooplankton community and its effects on lower and higher trophic orders (Young & Jarjanaiz, 2015).

1.4.2 Lake Couchiching Water Quality

Monitoring of Lake Couchiching occurs in the offshore region. The first open lake survey was carried out in 1997 by a consulting company. This report provided a comprehensive baseline of water quality for the offshore examining environmental variables such as nutrients, chlorophyll *a*, phytoplankton and zooplankton (Kilgour et al., 2000). From this report it was concluded that this lake could be considered a nutrient poor (oligotrophic) to moderately enriched (mesotrophic) lake based on the water clarity, total phosphorous concentrations and biological community (Sherman, 2005). Seven Sound Environmental Association began monitoring Lake Couchiching at the request of the Mnjikaning First Nation, City of Orillia, Township of Severn, and Township of Ramara in 2003. They have conducted sampling every five years from 2003-2013 during the ice free season. Samples were collected and analysed for changes in trophic status, basic chemistry, phytoplankton community, zooplankton community, hydrologic parameters. The results from the last sampling period in 2013 concluded that total phosphorous has not changed since 1997 while total nitrogen decreased (K. Sherman, personal communication, September 2014). Interestingly, sodium and chloride concentrations were both found to have increased, similar to Lake Simcoe. Zooplankton biomass, phytoplankton biovolume and chlorophyll *a* all decreased compared to previous years. Since water clarity has increased and nutrient and chlorophyll *a* concentrations have decreased overtime it suggests that

the open water area of Lake Couchiching is becoming more oligotrophic (K. Sherman, personal communication, September 2014).

Lakehead University has conducted research on the water quality of both Lake Simcoe and Lake Couchiching encompassing various topics such as phytoplankton community composition, microbial indicators, anthropogenic indicators of water quality (caffeine), biofilms, periphyton community structure, wetland macrophytes as biological indicators and microplastics to name a few. A water quality study was conducted in Lake Couchiching from June – October 2014 through Lakehead University in collaboration with the local community by active stewardship and public training. The findings from this report have yet to be published but the initial findings were that both total phosphorous and total nitrogen were below provincial guidelines and soft green algae and diatoms dominated phytoplankton samples indicating enrichment (D. Balika, personal communication, September 2014).

The lack of available literature on zooplankton in the nearshore region of both Lake Couchiching and Lake Simcoe should be addressed to determine if they would be a suitable proxy of the water quality in these areas and along the TSW.

1.5 Zooplankton as Biological Indicators of Water Quality

Biological indicators are used "to monitor environmental changes, assess the efficacy of management and provide warning signals for impending ecological shifts" (Siddig et al., 2016). There has been a great deal of research completed on the effectiveness of phytoplankton as indicators of water quality compared to zooplankton as indicators. Zooplankton are larger and more easily identified than phytoplankton therefore training and subsequent identification take much less time (Gannon & Stemberger, 1978). Zooplankton are impacted by subtle changes in environmental conditions and react more quickly to changing conditions compared to fish

(Gannon & Stemberger, 1978). As previously discussed zooplankton communities are influenced by both abiotic and biotic factors. The majority of species do occur under a wide range of physiochemical parameters although certain species possess tolerance to temperature, dissolved oxygen, salinity and other factors where metabolic processes become inhibited once the conditions fall out of their optimal tolerance limit (Gannon & Stemberger, 1978).

Table 1 displays the species and groups of zooplankton found to be used as indicators in the past studies. Pollutants, both natural and unnatural, impact some species more than others which will lead to changes in temporal and spatial zooplankton distributions. For instance, blue-green algae blooms are known to inhibit zooplankton growth (Arnold, 1971) and high densities of *Chlorella*, a type of green algae, is toxic to rotifer species *Branchionus calciflorus* (Halbech & Halbech-Keup, 1974).

Table 1. Zooplankton indicator species

Species	**Indicator**	**Author**
Cladocerans		
Bosmina longispina	Oligotrophic	Hasler, 1947; Minder, 1938
Bosmina longirostris	Eutrophic	Hasler, 1947; Minder, 1938
Chydorus sphaericus	Eutrophic	Fryer, 1968
Daphnia galeata	Acid sensitivity	Anas et al., 2013
Copepods		
Diaptomus sicilis	Eutrophic	Gannon, 1972; Gannon, 1974
Cyclops vernalis	Eutrophic	Gannon, 1972; Gannon, 1974
Epischura lacustris	Acid sensitivity	Anas et al., 2013
Limnocalanus macrurus	Oligotrophic	Gannon & Beeton, 1971
Leptodiaptomus minutus	Acidification	Anas et al., 2013
Senecella calanoides	Oligotrophic	Gannon & Beeton, 1971
Rotifers		
Anuraeopsis fissa	Eutrophic	Gannon & Stemberger, 1978
Brachionus calciflorus	Green algae (*Chlorella*)	Halbech & Halbech-Keup, 1974; Gannon & Stemberger 1978
Brachionus sp.	Eutrophic	Gannon & Stemberger, 1978
Keratella sp.	Eutrophic	Gannon & Stemberger, 1978
Keratella cochlearis	Acidification	Anas et al., 2013
Keratella longispina	Acidification	Anas et al., 2013
Trichocerca sp.	Eutrophic	Gannon & Stemberger 1978

Lake trophic level may have substantial influence on zooplankton community dynamics. Oligotrophic lakes typically display smaller zooplankton biomass comprised of a diverse group of species while eutrophic lakes have greater biomass with fewer species (Gannon & Stemberger, 1978). These differences can be complicated in larger lakes as water currents can distribute species to unfavourable environments (Gannon & Stemberger, 1978). Another phenomenon observed in eutrophic lakes is that rotifers (*Brachionus* sp., *Euchlanis* sp., *Platyias* sp., *Lecane* sp., *Monostyla* sp., *Lepadella* sp., *Trichocera* sp. and some Digonata species) that are primarily seen in nearshore habitats become abundant in limnetic regions (Gannon & Stemberger, 1978).

Limnocalanus macrurus and *Senecella calanoides*, both calanoid copepods, are exceptional indicators of oligotrophic conditions as they inhabit cold, well-oxygenated lake bottoms (Gannon & Stemberger, 1978). They are not usually found in areas with temperatures above 15ºC and dissolved oxygen concentrations <0.6 mg/L (Dadswell, 1974; Gannon & Beeton, 1971). Therefore, changes in the abundance of these species may indicate changes to trophic levels. In Lake Erie during the late 1920's *Limnocalanus macrurus* was very abundant and by the late 1950's they were rarely detected (Gannon & Beeton, 1971).

Paleolimnological studies conducted in Switzerland and North America have found shifts from the oligotrophic *Bosmina longispina* to more eutrophic species *Bosmina longirostris* indicative of eutrophic transitions in lakes (Alberta et al., 2010; Hasler, 1947; Minder, 1938). The value of some species as indicators are restricted by region. For example, *Diaptomus sicilis* and *Cyclops vernalis* are good indicators of eutrophic conditions in the Laurentian Great Lakes as they typically characterize eutrophic embayment areas such as Green Bay, Saginaw Bay and

Lake Huron (Gannon, 1974). *Cyclops vernalis* may not be a good indicator in other regions because of its high variability in dispersal and cryptic speciation (Gannon & Stemberger, 1978).

Rotifers exhibit high population turnover rates and thus respond more quickly to environmental changes than crustacean zooplankton (Gannon & Stemberger, 1978). Indicators of eutrophic conditions in North America are *Anuraeopsis fissa*, *Brachionus* sp., *Keratella* sp., and *Trichocerca* sp. (Gannon & Stemberger, 1978). *Anuraeopsi fissa* is found in highly productive bogs in northern Michigan and eutrophic nearshore habitats of the Great Lakes.

Changes in the relative proportions of crustacean zooplankton group have also been found to be a useful indicator of trophic conditions. For instance, cladocerans and cyclopoid copepods are typically more plentiful in eutrophic waters compared to calanoid copepods (Gannon & Stemberger, 1978). This pattern has been observed in Lake Michigan, Superior, Huron, Erie and Ontario (Gannon, 1972, 1974, 1975; Patalas, 1972). Rotifer species associated with highly eutrophic conditions were found to in high abundance near the outlet of Saginaw River indicating that there was nutrient enrichment from the river (Gannon & Stemberger, 1978). These changes in composition may be beneficial to monitoring water quality in inland lakes as well.

Absence and presence indicators of acid-stress were used in a study conducted on Albertan lakes. Highly acidified lakes were characterized by the presence of acid tolerant species (*Leptodiaptomus minutus*, *Keratella cochlearis* and *Keracotia longispina*) and absence of acid sensitive species (*Daphnia galeata*, *Epischura lacustris*, and *Diacyclops thomasi*) (Anas et al., 2013). The method is useful because acid tolerant species can be found in stress free aquatic systems therefore the presence of these species and absence of acid sensitive species is a more reliable indicator (Anas et al., 2013). A long-term field study confirmed that land use in

watersheds strongly affects crustacean zooplankton species. A strong significant adverse effect of agriculture land use was present with species richness averaging 6.4 taxa for impacted sites and 9.5 taxa for less impacted sites (Dodson et al., 2007).

Following the recovery of eight Sudbury lakes from metal contamination (nickel, copper and aluminium) and acidification, zooplankton species richness was reported to recover as well (Keller & Yan, 1991). Species richness was negatively correlated with metal concentrations ($r = -0.80$) and positively correlated with pH ($r = 0.84$) and generally showed greatest improvement in larger, deeper lakes with many inflows and considerable water quality improvements (Keller & Yan, 1991). Therefore, zooplankton community dynamics can also be used as a measure of recovery in contaminated lakes.

There is a lack of research on zooplankton community dynamics as indicators in the TSW. By monitoring the zooplankton community for one year during the ice free period it may provide insight into if specific species or groups of species are good indicators for this area.

1.6 Specific Aims and Research Rationale

1.6.1 Research Objectives and Questions

This research will address the gap in research on zooplankton in nearshore regions of the TSW by utilizing an ecological approach to determine the following objectives:

1. Provide baseline knowledge on the zooplankton community dynamics in the Trent Seven Waterway where it flows through Lake Simcoe and Lake Couchiching; and
2. Determine the effectiveness of zooplankton community dynamics as water quality indicators in this area.

From these objectives several research questions have been proposed:

1. What is the zooplankton community composition in this ecologically important study area?

2. Does the zooplankton community composition vary with respect to season, environmental variables and sampling location (level of exposure to anthropogenic activities)?
3. Are there any species or groups of zooplankton that could be used as biological indicators of water quality in this area?

1.6.2 Research Rationale

There are several reasons to justify this study. The main justification being that a literature review concluded that there is limited studies on zooplankton communities within the nearshore region of the TSW. Zooplankton monitoring in Lake Simcoe and Lake Couchiching occurs primarily in the offshore region. One reason for this might be due to the fact that the nearshore area experiences greater fluctuations in chemical, physical and biological components, as a result of disturbances to water quality, compared to the offshore region. Therefore the offshore region provides a better overall measure of environmental condition within a lake. This study proposes to address this gap by studying zooplankton community dynamics in the TSW where it flows through Lake Simcoe and Lake Couchiching specifically in the nearshore region. Secondly, the nearshore zone of water bodies typically exhibit signs of water quality impairment first because impacts from runoff are less diluted compared to pelagic regions. Lastly, the nearshore zone is substantial to juvenile fish production because they rely predominantly on zooplankton communities in shallow waters for feeding (Nicholls & Tudorancea, 2001). This research may lead to increased understanding of zooplankton interaction with higher and lower trophic levels in the nearshore zone.

The rest of this thesis is divided into 4 chapters. Chapter 2 outlines the methodology used in this study. Chapter 3 describes the spatial and temporal variation of zooplankton community composition and environmental parameters from eight nearshore and three

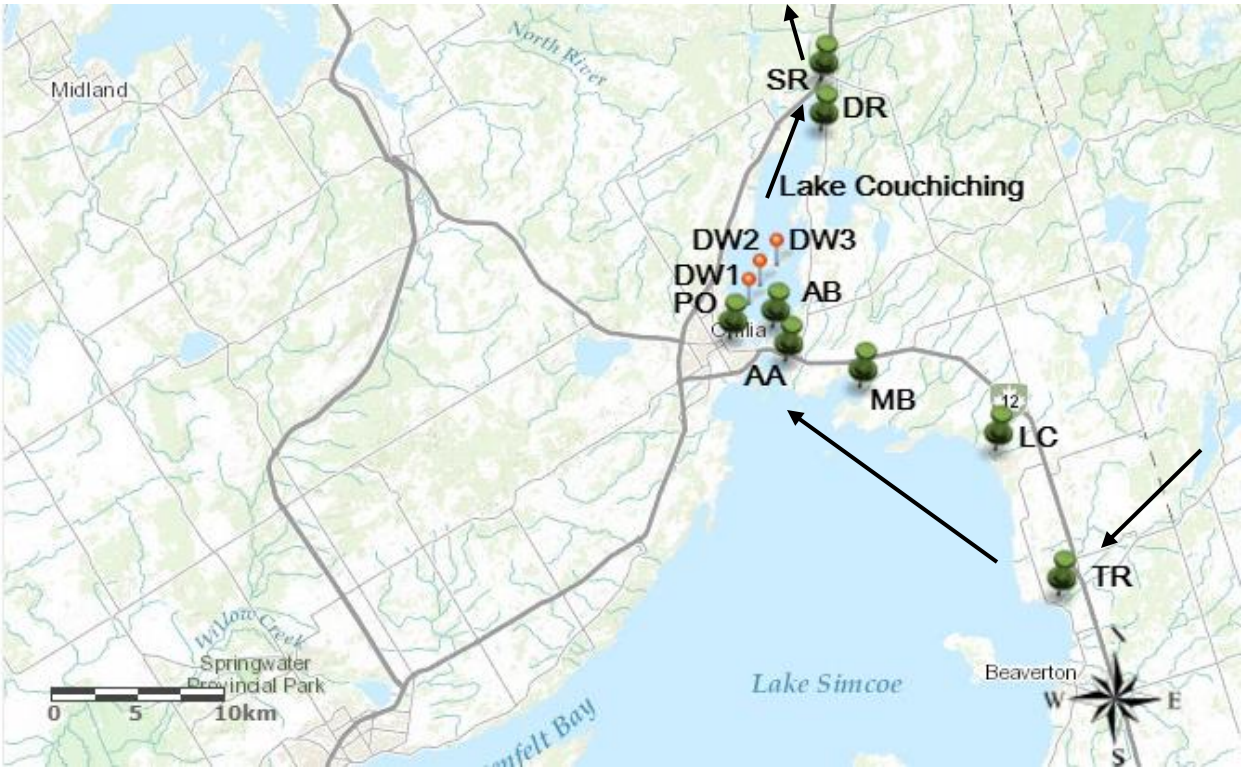
open water sites along the TSW exposed to varying anthropogenic disturbances. Chapter 4 illustrates zooplankton community composition as an indicator of water quality. The final chapter presents an overall summary, conclusion and future research recommendations.

Chapter 2 General Methodology

2.1 Study Location

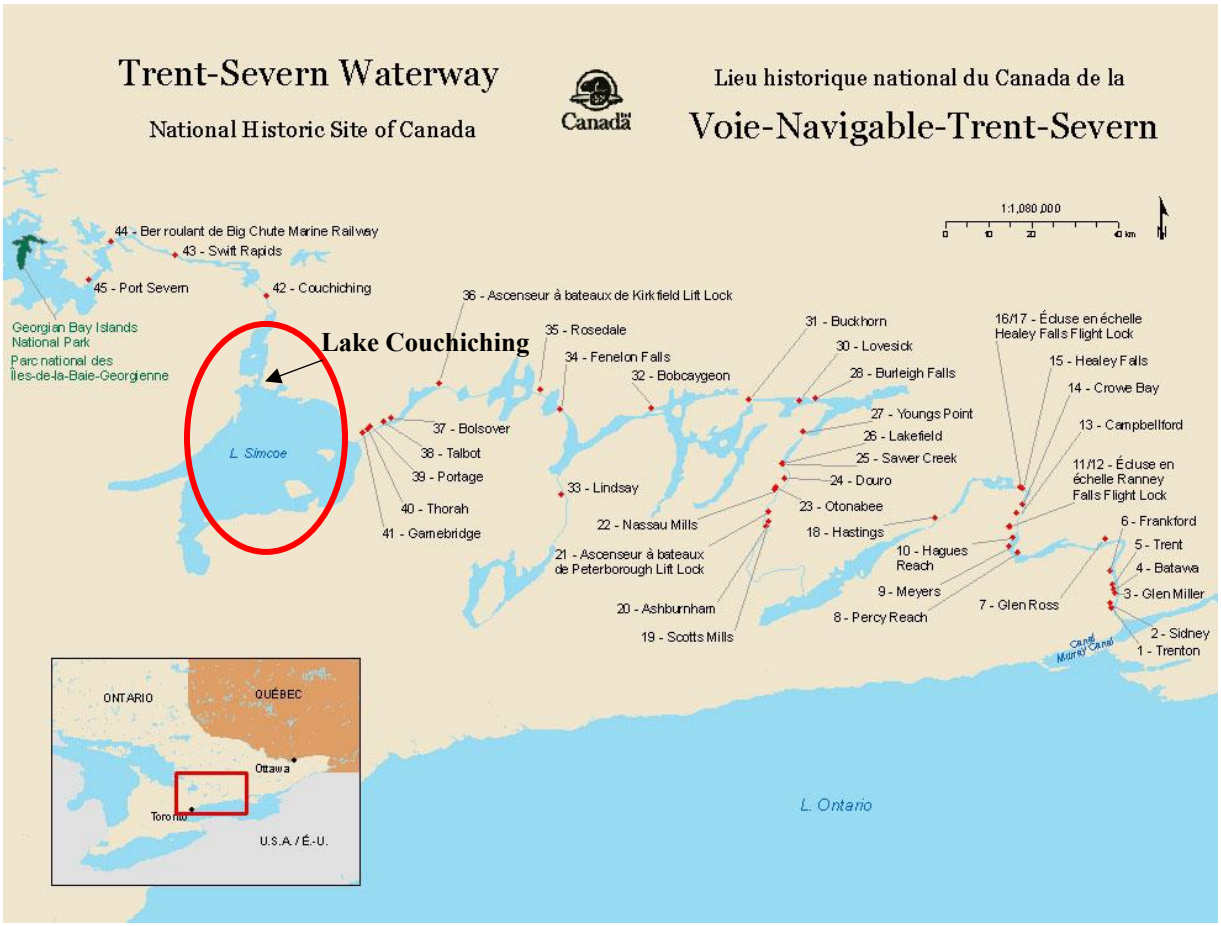
The study was conducted at eleven sampling sites (Figure 1) located in the northern reaches of Lake Simcoe and Lake Couchiching along the TSW. The sites sampled were Talbot River (TR), Gamebridge, ON (44°28'21.0216, 079°10'08.6772"); Lagoon City (LC), Brechin, ON (44°32'57.6960", 079°13'05.2860"); McPhee Bay (MB), Orillia, ON (44°35'01.7160", 079°18'35.8128"); Atherley Narrows A (AA) (44°36'02.1816", 079°22'16.5216") and Atherley Narrows B (AB) (44°36'21.7440", 079°22'12.0216"), Orillia, ON; Port of Orillia (PO), Orillia, ON (44°36'44.0280", 079°24'43.3260"); Severn River (SR), Washago, ON (44°44'54.2364", 079°20'34.3191"); Dock rd (DR), Washago, ON (44°42'56.7684", 079°20'30.7648"); and Deepwater 1 (DW1) (44°37'01.5024", 079°24'22.8609"), Deepwater 2 (DW2) (44°37'09.8652", 079°24'04.3453") and Deepwater 3 (DW3) (44°37'21.9864", 079°22'46.0962").

Figure 1. Map of sampling sites in Lake Simcoe and Lake Couchiching. Map generated through ERSI ArcGIS. Arrows indicate flow of water through the TSW.



The TSW is a 386 km canal connecting Lake Ontario to Georgian Bay (Parks Canada, 2015). Historically this waterway was used by fur traders while in modern times it serves as an important economic resource. The Severn River watershed comprises the last section of the TSW before it enters Georgian Bay and contains both Lake Simcoe and Lake Couchiching among several other lakes and rivers (Figure 2). The drainage area for the Lake Simcoe-Couchiching basin is predominantly located in rolling farmland resulting in lower runoff rates and higher evaporation losses from lake and land surfaces (Parks Canada, 2015).

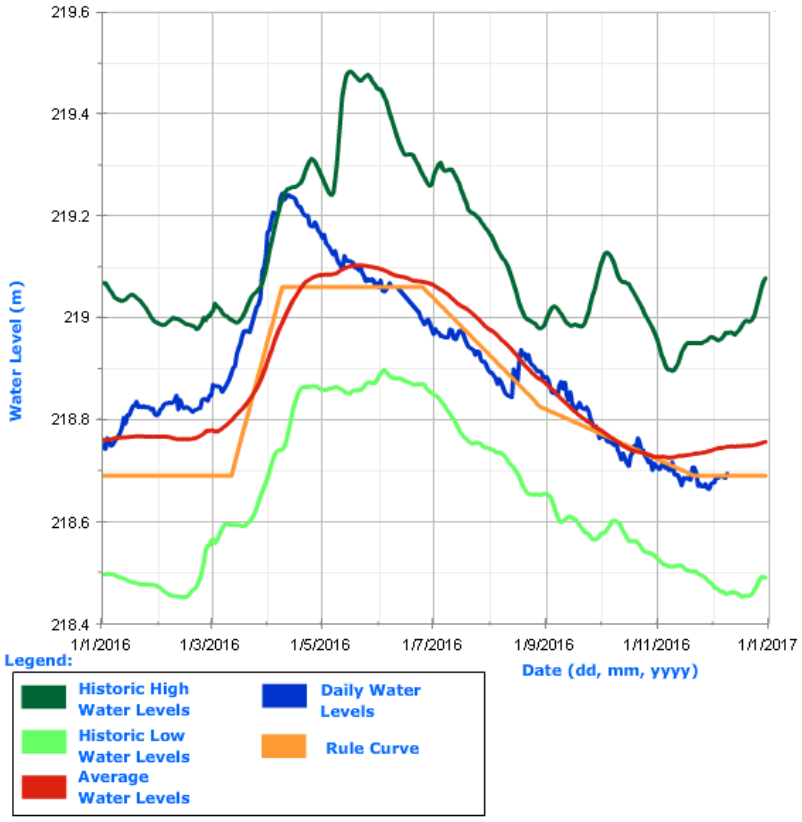
Figure 2. The Trent Seven Waterway (Parks Canada, 2015)



Water levels in TSW are maintained by Parks Canada through a Water Management Program to ensure safe navigation along the canal while taking into consideration the seasonal fluctuation in water level, watershed characteristics and fish spawning (Parks Canada, 2012). In the spring, the main objective is to store as much water as possible to maintain water levels for navigation through the summer while trying to reduce or eliminate flooding from the highly variable Black River. Lake Simcoe and Lake Couchiching water levels are managed using a rule curve (Figure 3), which specifies the most desirable water level for a given day of the year for a specific water body (Parks Canada, 2012). The size of the lakes and limited inflow and outflow relative to high evaporation rates makes it difficult to reverse a trend if the water level departs

the rule curve (Parks Canada, 2012). For instance, if water levels drop shutting down the Washago dams would not cause water levels to rebound nor if levels increased would operating the dams at max flow.

Figure 3. Lake Simcoe water levels as controlled by a rule curve (Parks Canada, 2015)



Lake Simcoe is a dimictic mesotrophic lake with a mean total phosphorous concentration of 14 µg/L (Palmer et al., 2011). It has a surface area of 722 km^2 and a total watershed area of 2899 km^2 (Palmer et al., 2011). The lake is broken down into three areas geographically: Cook's Bay comprising the southern tip of the lake (mean depth 13 m, maximum depth 15 m, surface area 44 km^2), Kempenfelt Bay located adjacent to Barrie, Ontario (mean depth 14 m, maximum depth 42 m, surface area 34 km^2), and the main basin covering the northeastern region (mean depth 14 m, maximum depth 33 m, surface area 643 km^2)(Winter et al., 2007; Young &

Jarjanazi, 2015). Land use within this watershed is continuously changing; currently, agriculture constitutes 36% of land use, urban 8% with natural heritage features such as wetlands and woodlands comprising over 51% (Source Water Protection South Georgian Bay Lake Simcoe Protection Region, 2013). Water from Lake Simcoe flows through one outlet in the northern end at Atherley Narrows and drains into Lake Couchiching (Dittrich, et al., 2012).

Lake Couchiching in comparison is a smaller lake having a surface area of 45 km^2 and mean depth of 6 m; it is considered a nutrient poor oligotrophic to moderate mesotrophic lake (SSEA, 2005). Land use adjacent to the lake includes rural and agricultural activities, shoreline recreational and permanent residences, and urban centres along Cumberland Beach and the City of Orillia shorelines (Armstrong, 2000). There are seven water filtration plants that draw approximately 36,283 m^3/d of water from the lake at intakes ranging in depth from 2-7 m (SSEA, 2005). The main inlet of Lake Couchiching is Atherley Narrows with water being transported into the TSW through Severn River.

2.2 Sampling Site Descriptions

Eight nearshore sites were chosen based on their exposure to varying anthropogenic stressors and accessibility (require dock or some type of structure to lower plankton trap from). The anthropogenic stressors include intense shoreline development (TR, LC, MB), frequent boating activity (AA, AB, SR, PO) and a relatively less impacted docking area (DR). Table 1 below summarises the overall rank given to each site based upon the anthropogenic stressors operating at each of the sites. The anthropogenic activities occurring at each site were ranked from 0 to 2 based on stressor occurrence; absent (0), moderate (1) or high (2). An overall ranking was calculated by dividing the total additive effect of each stressor by the maximum

rating (ie. for AA there are 5 stressors and the maximum for each stressor is 2 giving 10 and the total additive effect is 6; 6 out of 10 gives an overall rating of 1.2 out of 2).

Table 1. Anthropogenic stressors and subsequent disturbed rating for sampling sites

Anthropogenic Activities	**TR**	**LC**	**MB**	**AA**	**AB**	**PO**	**SR**	**DR**
Boating	2	2	2	2	2	2	2	1
Urban runoff	2	2	2	1	1	2	1	1
Boat ramp	0	2	0	1	1	2	0	1
Shoreline alteration	2	2	2	1	1	2	2	1
Wastewater effluent discharge	0	2	0	0	0	0	0	0
Public access	2	2	1	1	1	2	1	1
Overall Rating	***2***	***2***	***1.75***	***1.2***	***1.2***	***2***	***1.5***	***1***

Site 1: Talbot River (TR), Gamebridge

The first site is located in the outlet of the Talbot River in Gamebridge part of Severn Township. This region serves as an access point for boats to enter Lake Simcoe from the TSW. The area immediately surrounding the river consists of constructed (concrete) canals and a lift bridge which is highly frequented by travelling boats and fisherman most months of the year. Farmland and forested regions border the river with residential dwellings located east and west of the lift bridge. Shoreline alteration and urban runoff are assumed to be high since the canal is paved for a great distance and there is no shoreline vegetation present to act as a buffer and filter. The overall rating of this site is 2 out of 2 meaning that it is considered to be highly disturbed.

Site 2: Lagoon City (LC), Brechin

The second site is situated on the northwestern shores of Lake Simcoe in Brechin where the Lagoon City canals outlet into Lake Simcoe. Prior to the seventies this area was a wetland but it was converted to a residential area with the population currently sitting at approximately

2,500 residents (2016). Channels were dredged 6ft deep to accommodate boats and dwellings constructed roughly 10 meters from the canal edge. Fertilizer runoff from lawns, effluent discharge from the sewage treatment facility and excessive boating are the main anthropogenic impacts. Based on this assessment LC is believed to be highly disturbed and this is reflected in the 2 out of 2 rating for anthropogenic influence.

Site 3: McPhee Bay (MB), Uptergrove

The next site is located in McPhee Bay Ramara Township on the northwestern shores of Lake Simcoe. This site was accessed from a local marina. The marina itself is quite large offering storage, docking and services to over 150 boats. The biggest anthropogenic stressors at this site mostly come from boating and nutrient enrichment from urban runoff. This site is considered highly disturbed and had a rating of 1.75 out of 2 for anthropogenic influence.

Site 4: Atherely Narrows A (AA), Atherely

Site four is located in the outlet of Lake Simcoe (Atherely Narrows) in Atherely part of Ramara Township. Construction was occurring during most of the sampling periods which included dredging of the nearshore area and removal/replacement of old docks. Best care was taken to sample on the opposite side of the siltation fences. This region was the deepest of all sites sampled and received the most wind action. The shoreline has been altered with large boulders to prevent erosion and gardens located within five meters of the shore. Boat traffic is high through the narrows and therefore acting as the greatest anthropogenic stressor. Overall this site had a rating of 1.2 and is considered to be one of the moderately disturbed.

Site 5: Atherely Narrows B (AB), Atherely

Situated on the northern side of the narrows in Lake Couchiching is site 5 also found in Ramara Township. Permission was granted to access the site in a marina. This marina is much

smaller in comparison to the marina at site 3 housing approximately 50 boats at a time. This site provides habitat for Canada geese and several duck species. These populations of birds contributed substantial amount of feces on grass and docks and therefore into the water. The shoreline consists of manicured grass and docking structures. Similar to AA the main anthropogenic disturbance is boating although the overall rating is 1.2 and it is considered a moderately disturbed site.

Site 6: Port of Orillia (PO), Orillia

The Port of Orillia is located in downtown Orillia in southern side of Lake Couchiching. This is the main port in Orillia and therefore boat traffic is very heavy during summer season. The shorelines are constructed thus allowing high runoff rates during storm events. There is a berm across the docks providing shelter for the area from high winds. The trail system running along the shoreline, proximity to Couchiching Beach and presence of boating ramps renders this area highly accessible to the public. This site is considered a highly disturbed site and received a rating of 2 out of 2.

Site 7: Severn River (SR), Washago

Site 7 is located in Fawcett Reserve along the Severn River in the northern region of Lake Couchiching. Lake Couchiching flows into Severn River with water travelling the rest of the TSW into Georgian Bay. This area is noted to be frequently travelled by boaters and a popular spot among local fishermen. Highway 11 transects the river just 20 meters from the sampling site. This is a signnificant arterial highway connecting central Ontario with the Muskoka region. It is possible that this highway would be a major source of anthropogenic disturbances such as runoff from winter road salting/sanding applications. During the last four sampling periods

construction was occurring on the bridge which would have likely introduced other solids and chemicals into the river. This site is rated as a moderately disturbed one with a rating of 1.5.

Site 8: Dock Rd (DR), Washago

The last nearshore sampling site is located along the northwestern shores of Lake Couchiching in Ramara Township. Permanent and seasonal cottages surround the shoreline of this area. Septic beds and holding tanks are the main forms of sewage treatment for this area. Small numbers of personal watercrafts and boats were noted especially due to the presence of a public dock and boat ramp. Larger vessels would have a difficult time utilising the dock because the water is very shallow (<1 m) and there are exposed rock formations throughout the coastline. This site is considered to be the least impacted site compared to the other seven sites because it is accessible to only a smaller portion of the population. Therefore, it is described as the least disturbed site with a rating of 1 indicating that there are still some anthropogenic disturbances present.

Deepwater sites: Deepwater (DW1, DW2, DW3), Lake Couchiching

DW sites 1, 2, and 3 are situated along a transect from the Port of Orillia to Heron Island. Three depths were sampled at each site when lake level permitted. Lake Couchiching is a shallower lake (mean depth 6m) therefore it was believed that the limnologic characteristics would be similar among the three sites. These sites were not included in the above table because they were only sampled seasonally and they experience different anthropogenic stressors compared to nearshore sites. Boating activity would be considered the most substantial or main anthropogenic disturbance in this region of Lake Couchiching.

2.3 Zooplankton Analysis

Zooplankton samples were collected using a 30 L capacity Schindler-Patalas Plankton Trap with mesh size 80 μm. Samples were drawn from 1 m depth where possible in nearshore sites and three depths (1m from the surface, mid depth and 1 m off bottom) at offshore sites. Samples were concentrated down to 10 mL using 70% alcohol and stored in the laboratory until analysis. Samples were counted and identified in to species level (or Genera) for cladocerans, rotifers and copepods with juvenile copepods being distinguished as either nauplii of copepodid stages using dichotomous keys from Haney et al. (2013) or Witty (2004). Zooplankton density, richness, diversity and biomass (from length-dry weight regression relationships) were determined. Triplicate samples were collected at one station per sampling period to understand the repeatability of sampling episodes. The methods for determining each of the zooplankton community parameters are presented below:

(a) Species density

Density (#/mL) = (Number of individuals counted/fraction counted)/original volume 30L x 1000mL

(b) Species diversity

Shannon Wiener Diversity index: $H' = \sum - (Pi \times \ln Pi)$
H = the Shannon diversity index
Pi = fraction of the entire population made up of species i
S = numbers of species encountered
$\sum$ = sum from species 1 to species S

(c) Species richness

Richness = Count all of the different species or genera present in a sample

(d) Species biomass

$Ln(W) = (Ln(\alpha) + \beta Ln(L)) \times 15$ (to bring up to condensed volume 45mL)/30L (original volume)
W = dry weight in μg
L = length in mm
α = intercept
β = slope

2.4 Phytoplankton Analysis

Phytoplankton samples were collected at 1m depth in the nearshore and three varying depths in offshore sites using a Van Dorn water sampler. One litre water was stored in clean plastic bottles. Bottles were stored in a cooler while transportation to the laboratory where they were stored in a refrigerator until analysis. During analysis the 1 L original sample was concentrated down to 5mL through a series of centrifuge cycles (15 minutes at 2500 rpm). The condensed samples were stored in the fridge until enumeration. Phytoplankton were enumerated using a haemocytometer, and identified with the aid of identification keys and manuals (Spaulding et al., 2010). Counts were performed in replicates and phytoplankton density per litre was determined using a dilution factor (Public Health England, 2013). Phytoplankton density, richness, and biomass were determined. The above calculations for zooplankton were applied to phytoplankton counts.

2.5 Environmental Variables

The environmental variables monitored were dissolved oxygen, temperature, conductivity, pH, chlorophyll *a*, and nutrients (total suspended solids (TSS), total phosphorous and nitrate). These samples were collected using a Van Dorn sampler at 1 m depths for nearshore sites and varying depths for offshore sites. Dissolved oxygen, pH, conductivity, and temperature were measured in-situ using a dissolved oxygen probe (VWR Symphony H10D), pH probe (VWR Symphony SP70P), and hydrolab (VWR Symphony SB9 0M5), respectively.

For total phosphorous and nitrate estimations water samples were collected in 250 mL clean polyethylene bottles and frozen until analysis. Total phosphorous was analysed following the standard Ammonium persulfate digestate method (APHA, 2005) then performing HACH (2003) and measuring light absorbance at 807 nm with a Beckton Dickson Spectrometer

(DU700). Nitrate was determined using the cadmium reduction method measuring the absorbance of light at 507 nm with same spectrometer mentioned above (HACH, 2015). Both analyses were run in replicate and concentrations expressed in mg/L. The accuracy of the method was verified by sending random samples to Lakehead Analytical Services where the samples were analysed using an auto analyzer. Low standard deviations were found between the different sampling methods.

Chlorophyll *a* was determined using the APHA standard method (APHA, 2005). This included filtering 1 L of sample through a vacuum pump using a 42.5 mm Whatman GF/B glass fibre filter and extracting the chlorophyll *a* with 90% acetone for 16-24 hrs. The following day the samples were condensed by centrifugation for 15 min at 4200 rpm before measuring the absorbance of supernatant in a spectrometer. Results for chlorophyll *a* are in expressed in mg/m^3. The TSS measurement was done by filtering 1 L of water through a pre-weighed filter paper (Whatman GF/C) and drying the filters in an incubator at 50ºC for 24-48 hours. The filters were reweighed and difference between the initial and final weights of the paper gave TSS. Final TSS values were expressed as mg/L. Triplicate samples were collected at one station per sampling period for environmental parameters and zooplankton to understand the repeatability of sampling episodes. Comparisons between triplicates yielded low standard deviations.

2.6 Statistical Analysis

2.6.1 Temporal and Spatial Differences

Statistical analyses was performed using the statistical program R version 3.2.3. Analysis of variance (ANOVA) techniques were used following a general linear model. Two factor ANOVA without replication was performed to determine if environmental variables and varied temporal and spatial. The null hypothesis tested was there are no differences in environmental variables among sites controlling for month effects. Assumptions of normal distribution and

homogenous variance were tested using residual plots and normality tests. A Sequential Bonferroni correction was performed on p-values to adjust for running multiple tests to preserve the statistical power of all the tests. This involved ranking the unadjusted p-values from largest to smallest than adjusting p-values using the experiment error rate of 0.05 and number of multiple tests. Where significance was found Tukey's HSD multiple comparison of means compared all possible pairwise comparisons of means to determine directionality of differences. When data could not be normalised using transformations Friedman's and Kruskal Wallis nonparametric tests were utilised. This same analysis was used to find temporal and spatial differences in zooplankton assemblages (density, diversity, richness, biomass) and performed for both nearshore and openwater habitats.

A two sample t test (two tailed) assuming equal variance was performed to determine differences between zooplankton community dynamics (biomass, density, richness and diversity) in least, moderately and highly disturbed sites.

2.6.2 Influence of Environmental Variables on Species Composition

Multiple regression analysis was carried out to study the relationship between environmental variables and zooplankton composition in nearshore habitats. The null hypothesis of the multiple regression is that all partial slopes are zero and the alternative that at least one partial slope is not zero. The distribution of the response and predictors were analyzed with histograms and transformed to better fit the assumption of normality if they did not follow normal distribution. A correlogram was used to check for correlation among predictor variables and then predictors were plotted with the response variable in scatterplots. The model was ran and the variance inflation factors (VIF) were checked and removed if greater than 2 (Zuur et al., 2010). Partial regression plots were used to indicate direction and significance of correlations

between predictors and response. Multiple regression was also utilized to determine how zooplankton richness and diversity correlated with environmental variables in least, moderately and disturbed sites.

2.6.3 Detecting Species Level Changes with Ordination Analysis

Canonical ordination techniques were used to determine the relationship between zooplankton species presence and environmental variables. This analysis indicates whether or not zooplankton composition would be a good indicator of water quality in the TSW. Redundancy Analysis (RDA), a constrained linear canonical ordination technique (Van der Wollenberg, 1977), was performed in CANOCO 4.56 software. The model was run with only the environmental variables causing significant influence on species presence.

To determine the suitability of the zooplankton community as biological indicators of local environmental conditions multivariate analysis using ordination techniques were employed. This allowed for the analysis of relationships between environmental variables mentioned above and zooplankton biomass and density at a species level at highly, moderately and least disturbed sites. To determine what ordination technique suits the best detrended correspondence analysis (DCA), a form of indirect gradient analysis, was ran to estimate the amount of heterogeneity in species data. If gradient lengths displayed weak unimodal distribution, Redundancy Analysis (RDA), a constrained linear canonical ordination technique, was utilized. For each analysis, variables were centered and standardized because each variable had different units. The Monte Carlo permutation test (499 permutations, $p<0.05$) was run to determine the significance of the explanatory effect of environmental variables (Reyes et al., 2013). Environmental variables not having a significant explanatory effect were removed from the final model as well as those species that occurred infrequently (<10 times throughout the entire sampling year).

Chapter 3 Zooplankton Composition and Environmental Variables in the Nearshore Regions of the Trent Severn Waterway

3.1 Introduction

A literature review found that there is a lack of studies conducted on nearshore zooplankton communities along the TSW specifically in Lake Simcoe and Lake Couchiching. However, studies have been completed within the Great Lakes. Studies conducted in the seventies on the Great Lakes focused on describing the nearshore community with comparisons to offshore stations. Gannon (1975) looked at large-scale variation in crustacean zooplankton along a horizontal transect in Lake Michigan and found that species were relatively uniform during spring, fall and winter. In summer there were distinct patterns in species abundance with *Diacyclops thomasi*, *Eurytemora affinis*, *Bosmina longirostris*, *Eubosmina coregoni*, and *Chydorous sphaericus* present in significantly greater abundance in nearshore areas (0 – 18 km) compared to the open lake. Conversely, *Leptodiaptomus sicilis*, *Leptodiaptomus minutus*, *Skistodiaptomus oregonensis*, and *Daphnia mendotae* were significantly prevalent in the offshore (Gannon, 1975). Another study conducted in southeastern Lake Michigan supported these findings with large species of copepods dominating offshore stations resulting in greater zooplankton biomass at offshore stations compared to inshore stations (Hawkins & Evans, 1979).

A more in-depth study carried out from the nearshore areas of Lake Michigan from 1971 – 1977 found strong seasonal and depth trends in zooplankton abundance (Evans et al., 1980). Zooplankton community composition was largely driven by water depth from mid-spring to mid-fall. Abundance was lowest in the 5 – 10 m deep area dominated by nauplii, *Asplancha* sp., and *Bosmina longirostris* and highest from 20 – 50 m consisting of larger copepods species (*Diacyclops* spp., *Leptodiaptomus* spp., and *Daphnia* spp.). In fall cladocerans and copepods

dominated in nearshore and offshore regions, respectively. These seasonal differences were thought to be impacted by temperature regimes, phytoplankton abundance and predation by fish and invertebrates (Evans et al., 1980).

Into the nineties, a transition in research occurred. During this period research focused on describing the nearshore community and their impacts on other trophic levels, and impacts of invasive species on nearshore zooplankton species. Bridgeman et al. (1995) conducted zooplankton grazing experiments at three sites in Saginaw Bay, Lake Huron. Two of the sites were located in the inner bay where conditions were eutrophic due to input from the Saginaw River at a mean depth of 5 m and the other site in the outer bay consistent with oligotrophic conditions at a depth of 14 m. They measured weight specific zooplankton filtering rates during maximum abundance before (June 1991) and after (June 1992), the establishment of *Dreissena polymorpha* (zebra mussel). Biomass specific filtering rates ranged from 0.24 – 0.33 mL µg dry wt^{-1} d^{-1} for the inner bay and 1.27 – 1.83 mL µg dry wt^{-1} d^{-1} for the outer bay between years. Large decreases in biomass, 40% and 70% for inner and outer bay, respectively, resulted in an average of 58% decline in community filter rates between years. It was concluded that decreases in phytoplankton productivity and abundance during the sampling period could not be attributed to zooplankton grazing and more likely a result of the recent *Dreissena polymorpha* colonization (Bridgeman et al., 1995).

Johannsson et al. (1999) evaluated the use of a zooplankton mean size index developed by Mills et al. (1987) to assess the fish community in nearshore and offshore sites in Lake Erie. It was found that the index could be used to accurately describe the fish community in western Lake Erie during 1993. When the index was used to describe the fish community structure from 1988 – 1990 in the same region it was unreliable. This was thought to be due to oversights in

accurately measuring the fish populations from the lakes the model was derived and the ratio of crustacean of zooplankton which can drastically alter the index (Johannsson et al., 1999). In another study carried out in Lake Erie they measured the impact of dreissenids on primary and secondary production. They found that dreissenids impact zooplankton production through decreasing algal biomass and primary production and removing rotifers from the water column (decreasing zooplankton biomass and abundance). Furthermore, they produce veligers which can contribute to 10 – 25% of zooplankton production resulting in a change in zooplankton species assemblage (Johannsson et al., 2000).

Research into the 2000's focused on similar issues as the previous decade looking at anthropogenic impacts, trophic level interactions and invasive species effects. Offenberg & Baker (2000) looked at determining the impact of elevated urban atmospheric pollutants on nearshore surface waters in Lake Michigan. They found that polychlorinated biphenyl (PCB) concentrations in zooplankton were greater in the winter and polycyclic aromatic hydrocarbon (PAH) concentrations were consistent regardless of season. Suggesting that PAHs are deposited at a constant rate throughout the year and PCBs are not. Goforth & Carman (2005) studied the role of shoreline geomorphology and land cover on nearshore biological communities. It was determined that in developed mid-bluff shorelines or areas with less stable substrates, the waters were experiencing lower zooplankton densities (Goforth & Carman, 2005).

Dettmers et al. (2003) studied patterns of nearshore zooplankton community fluctuations and age 0 yellow perch abundance in the southwest basin of Lake Michigan from 1988 – 89 and 1996 – 98. They found that zooplankton density, biomass and mean size decreased between sampling periods which may have explained a reduced rate of yellow perch recruitment. A strong positive linear relationship was found between zooplankton density in June, time of first

arrival of feeding larvae and catch unit per effect of age 0 perch. This supports their hypothesis that greater zooplankton density results in increased perch larvae survival (Dettmers et al., 2003). Predatory zooplankton can cause impacts to zooplankton composition. Pothoven & Höök (2014) collected data on zooplankton including predatory *Bythotrephes longimanus* and *Leptodora kindtii* at four sites in inner Saginaw Bay, Lake Huron from 2009 – 2010. Zooplankton production and biomass increased greatly from May to June due to the appearance of larger *Daphnia* spp. Predatory cladocerans were noted to potentially cause large impacts on zooplankton in a short time (1 – 2 months) specifically in July and August where prey consumption was a large portion of or exceeded prey production. Consumption by *Bythotrephes longimanus* was found to be much higher than that of *Leptodora kindtii* and as such should be taken into consideration when evaluating the flow of energy within the Great Lakes food web (Pothoven & Höök, 2014).

Warner et al. (2006) reported on the changes of seasonal abundance of nearshore zooplankton community in Lake Ontario from 1995 – 2000 as a direct result of *Cercopagis pengoi* (fishhook water flea). Early summer zooplankton densities were similar among years however the late summer to fall densities were significantly lower from 1998 – 2000 compared to 1995 – 97. Since there were no significant changes in temperature or chlorophyll *a* concentrations and the decrease coincided with the arrival of *Cercopagis pengoi* in 1998, the peak seasonal abundance of *Cercopagis pengoi* was thought to be the main reason for this decrease. Patterns of zooplankton abundance in Lake Champlain were examined from 1992 – 2010. A decrease in rotifers abundance was noted in the mid-nineties due to the establishment of invasive species *Dreissena polymorpha* (Mihuc et al., 2012). The community experienced a change again after the colonization of invasive *Alosa pseudoharengus* (alewife). Body length of

Leptodiaptomus sp. and *Daphnia retrocurva* decreased as a predator avoidance technique. The zooplankton community has experienced much change in the last two decades with recent declines in larger zooplankton due to alewife (Mihuc et al., 2012).

Other studies have looked at the impact of hydrologic conditions on zooplankton communities. In 2013, Thomasen et al., evaluated the effects of wave exposure and hydrologic connectivity on the zooplankton community in Long Point Bay, Lake Erie. They created a relative exposure index (REI) by collecting wind and fetch data. It was concluded that zooplankton abundance was greater in sheltered areas (low REI) compared to sites with a high REI further supporting the importance of hydrologic connectivity and wind exposure on zooplankton assemblages (Thomasen et al., 2013).

Recent studies tend to focus on updating the current knowledge of zooplankton communities in the Great Lakes as some of the lakes are shifting to oligotrophic conditions in offshore regions. Lake Michigan, for instance has transitioned to a more oligotrophic state (Evans et al., 2011). Pothoven & Fahnenstiel (2015) studied spatial and temporal trends in zooplankton along a nearshore to offshore transect in Lake Michigan during 2007 – 2012. Zooplankton biomass was observed to be significantly different between nearshore (15 m) and mid depth sites (45 m) but neither differed significantly from offshore sites. *Bythotrephes longimanus* abundance differed at each site with higher abundance at offshore than nearshore sites. Zooplankton assemblage in the nearshore experienced the greatest change between early spring and early to late summer largely attributed to the appearance of larger bodied cladocerans (*Bosmina longirostris*) and copepods (*Diaptomidae* spp. and *Leptodiaptomus* sp.). Copepod abundance decreased seasonally comprising 92%, 60% and 52% of nearshore zooplankton composition in spring, early summer and late summer, respectively. Interestingly, predatory

cladoceran assemblage showed seasonal patterns transiting from a system dominated by *Cercopagis pengoi* in the spring to *Leptodora kindtii* and *Bythotrephes longimanus* in the early to late summer. This research provided insight into the current state of offshore zooplankton communities and differences with nearshore communities.

Thomas et al. (2017) made an integral discovery with their research on macro- and microzooplankton methods in Lake Michigan. Microzooplankton consist of rotifers, nauplii and dreissenids veligers ranging in size from 20 – 200 μm. They are important to nutrient recycling and energy transfer within aquatic ecosystems (Makarewicz & Likens, 1979; Segers, 2008). They compared using traditional plankton nets (64 μm), which generally only collect macrozooplankton to microzooplankton nets (20 μm). The traditional nets were found to greatly underestimate total rotifer density by an order of magnitude, veliger density by almost one order of magnitude and nauplii by threefold. Furthermore rotifers contributed to approximately 51% of total zooplankton biomass. This rather large oversight means that there is substantially greater prey resources available to invasive Asian carp in Lake Michigan and the likelihood that this species could become established is high (Thomas et al., 2017).

From the above description, it is clear that not enough data/studies on zooplankton distribution and dynamics is available from the TSW. In order to fill this gap, zooplankton samples were collected along with environmental parameters from a significant part of TSW, the Lake Simcoe and Lake Couchiching, for a period of one year by completing shoreline and deep water (Lake Couchichiching only) sampling. Thus, this chapter describes the spatial and temporal variance of water quality parameters and zooplankton composition from eight nearshore and three deep water regions in Lake Simcoe and Lake Couchiching exposed to differing anthropogenic influences. Samples were collected for ten months over a one-year

period (except for ice period) from nearshore sites and three times (representing three seasons) from deep water sites to represent temporal and spatial changes in zooplankton community.

This chapter addresses the first objective of this thesis; to collect baseline data on zooplankton community in the TSW and answer the two fundamental questions 1. *What is the zooplankton community composition in these sampling locations*? and 2. *Does the zooplankton community composition vary with respect to time of the year, and sampling location and how is it related to local environmental conditions?* It can be hypothesized that the zooplankton communities will exhibit spatial, temporal variability, and with changes in environmental parameters. The largest differences in zooplankton communities are typically observed between spring and early/late summer due to an increase in total biomass in summer with the appearance of larger species (Pothovern & Fahnenstiel, 2015). Thus it is predicted that highest density and biomass of zooplankton will be in late spring (June). Food availability and water temperature are considered as the two most important factors that affect zooplankton abundance (Palatas, 1972) so it can be speculated that sites with higher mean temperatures and chlorophyll concentrations (as a measure of phytoplankton biomass) will yield greater species densities (TR, LC, MB). Eutrophication has been noted to have considerable effects on species abundance and composition with oligotrophic lakes exhibiting lower biomass consisting of a greater number of species and eutrophic lakes having higher biomass with less species (Gannon & Stemberger, 1978). Hence, sites that are highly impacted by anthropogenic stressors (TR, LC, MB) and have higher total phosphorous, TSS and nitrate concentrations are expected to have higher species abundance and biomass but less diversity and richness. Tolerant species such as rotifers will thrive in highly impacted sites contributing to higher density and biomass compared to less impacted sites (DR, AB, AA) which will have the lowest density and biomass. Moderate sites

(PO, SR) will fall somewhere between. Therefore it is predicted that the zooplankton community differ between locations (exposure to anthropogenic activities) and time of the year. Also since the water is drained from Lake Simcoe to Lake Couchiching, the zooplankton community in Lake Couchiching is influenced by the Lake Simcoe community.

3.2 Methods

3.2.1 Study Location

A detailed general methodology is provided in Chapter 2. In brief, this study was conducted at eleven sampling sites location in northern Lake Simcoe and throughout Lake Couchiching (Figure 1). The sites are as follows: Talbot River (TR), Gamebridge, ON (44°28'21.0216, 079°10'08.6772"); Lagoon City (LC), Brechin, ON (44°32'57.6960", 079°13'05.2860"); McPhee Bay (MB), Orillia, ON (44°35'01.7160", 079°18'35.8128"); Atherley Narrows A (AA) (44°36'02.1816", 079°22'16.5216") and Atherley Narrows B (AB) (44°36'21.7440", 079°22'12.0216"), Orillia, ON; Port of Orillia (PO), Orillia, ON (44°36'44.0280", 079°24'43.3260"); Severn River (SR), Washago, ON (44°44'54.2364", 079°20'34.3191"); Dock rd (DR), Washago, ON (44°42'56.7684", 079°20'30.7648"); and Deepwater 1 (DW1) (44°37'01.5024", 079°24'22.8609"), Deepwater 2 (DW2) (44°37'09.8652", 079°24'04.3453") and Deepwater 3 (DW3) (44°37'21.9864", 079°22'46.0962").

3.2.2 Zooplankton Analysis

Zooplankton samples were collected from each site for a period of one year on a monthly interval. Thus a total of 126 samples (100 nearshore and 26 open water) were collected during the study. Zooplankton were counted and identified using several identification keys (Haney et al., 2013; Witty, 2004). The zooplankton community was described by determining density, diversity, richness and biomass. Density is a measure of the number of zooplankton per volume

of water. Diversity is the measure of different species distribution within a community and the number of individuals within each species group. Richness represents the total number of different species in a community. The mass of all individuals within a community is termed biomass. Biomass was calculated referencing length-weight equations used by Canada's Department of Fisheries and Oceans derived from multiple sources (Bottrel et al., 1976; Culver et al., 1985; Dumont et al., 1975; Hall et al., 1970; Lewis, 1979; McCauley, 1984; Rosen, 1981; Watkins et al., n.d.).

3.2.3 Environmental Variables

Environmental variables monitored in this study include DO, TEMP, COND, pH, phytoplankton and nutrients (CHL *a*, TSS, TP and nitrate*)*. TEMP, DO, pH and COND were measured in-situ. TSS, phytoplankton, *CHL a* were collected in clean 1 L polyethylene bottles and refrigerated until analysis. Phytoplankton density, richness and diversity were calculated. Total phosphorous and nitrate were frozen until analysis.

3.2.4 Statistics

Several statistical tests were run to analyse the data. To determine differences in data spatially (between sites) and temporally (between months or seasons) two factor ANOVAs without replication using a blocked design were computed and when data could not be normalised using transformations, Friedman's and Kruskal Wallis nonparametric tests were utilised. A multiple regression analysis was run to determine the relationship between environmental variables and zooplankton composition. Statistical tests were performed using the statistical program R version 3.2.3. For a more detailed overview of the statistical analysis refer to Chapter 2.

3.3 Results

3.3.1 Environmental Variables

3.3.1.1 Physiochemical Data

Nearshore regions of Lake Simcoe and Lake Couchiching in this study are exposed to a range of environmental conditions, varying from mesotrophic (N = 25.12 µg/L, TP = 10.83 µg/L, CHL *a* = 1.58 mg/m^3) to mesoeutrophic conditions (N = 78.92 µg/L, TP = 31.31µg/L, CHL *a* = 13.29 mg/m^3) (Table 1). Mean TEMP varied minimally between nearshore sites (12.4 – 14.3ºC) with greater differences observed between sampling months (2.1 – 25.2 ºC) (Table 1). There was no significant difference in TEMP among nearshore sites ($F_{7,63} = 0.538$, $p = 0.802$), although month did have a significant impact on TEMP ($F_{9,63} = 6.324$, $p < 0.001$). TEMP data had to be *Sin* transformed in order to meet the assumptions of normality and homogeneous variance. A post hoc Tukey's HSD comparison of means determined that this significance was due to the difference between October and December with the other sampling months (December always being significantly lower and October significantly lower than most months).

Mean nearshore DO differed between sites (9.91 – 12.07 mg/L) and months (9.20 – 13.69 mg/L) (Table 1). These differences were found to be significant (sites $F_{7,63} = 7.053$, $p < 0.001$; months $F_{9,63} = 28.039$, $p < 0.001$). The follow up Tukey's test found significant differences between many of the pairwise comparisons meaning that all months contributed equally to the overall differences. When the differences between site comparisons were analysed MB and TR were found to have significantly lower DO compared to the other sites.

Nearshore conductivity varied more spatially (299 – 510 µS/cm) than temporally (382 – 517 µS/cm) (Table 1). These differences were tested using nonparametric tests and found to be significantly different between sites (Friedman's $X_2 = 37.464$, $p < 0.001$) and months (Kruskal Wallis $X^3_2 = 25.345$, $p < 0.001$). The follow-up test showed that differences between sites were

predominantly caused by TR having much lower conductivity than other sites. Differences among months were due to December and November having significantly higher COND compared to the other months.

Mean nearshore pH ranged from 7.78 – 8.38 among sites and 7.94 – 8.47 among months (Table 1). These differences were statistically significant (site $F_{7,63} = 6.235$, $p < 0.001$; and month $F_{9,63} = 4.632$, $p < 0.001$). The main cause of the significant difference among sites was lower pH at MB than the rest of the sites. Monthly differences were caused by July and August having higher pH compared to April, December and March.

Open water sites in Lake Couchiching experienced less variable environmental conditions compared to nearshore regions. TEMP means ranged from 17.6 – 18.2 ºC among offshore sites and 9.0 – 24.2 ºC among months (Table 1). Differences in TEMP were not significantly different between sites ($F_{7,14} = 1.598$, $p = 0.215$) but were for months ($F_{2,14} = 6552.31$, $p<0.001$). This difference was equally attributed to all sampling periods. Mean DO varied more among months (8.17 – 11.40 mg/L) than sites (9.42 – 10.30 mg/L) (Table 1). DO differed significantly between sites ($F_{7,23} = 6.417$, $p = 0.002$) due to the site 9C having higher DO concentrations compared to the other sites. Monthly DO also differed significantly ($F_{2,23} = 529.53$, $p < 0.001$) influenced by variability between months.

COND varied among months (413 – 456 µS/cm) and less so among sites (431 – 442 µS/cm) (Table 1). COND differed significantly between months ($F_{2,23} = 26.42$, $p < 0.001$) but it did not vary significantly between sites ($F_{7,23} = 0.66$, $p = 0.699$). pH means were 8.23 – 8.33 for sites and 8.22 – 8.36 for months (Table 1). Differences were found to be significant (months $F_{2,23} = 35.91$, $p < 0.001$; sites $F_{7,23} = 2.89$, $p > 0.005$).

Table 1. Environmental variable data, mean values and standard deviation (in parenthesis) of limnologic parameters at nearshore and open water sampling sites during the 2015/2016 sampling period.

	Oct	Nov	Dec	Mar	Apr	May	Jun	Jul	Aug	Sept	Mean
TEMP (ºC)											
Nearshore											
TR	9.7	3.0	2.3	3.2	11.2	16.2	24.6	25.0	25.8	17.1	13.8 (8.87)
LC	8.2	3.1	1.5	3.4	10.0	15.2	24.5	25.9	25.9	16.5	13.4 (9.14)
MB	8.4	3.4	1.2	3.8	13.1	15.6	24.3	25.6	25.4	16.2	13.7 (8.89)
AA	8.4	4.0	2.3	3.5	6.3	12.0	21.0	24.0	25.2	17.1	12.4 (8.36)
AB	8.0	4.5	3.0	2.6	7.6	13.5	24.4	24.5	23.8	17.3	12.9 (8.56)
PO	8.4	2.4	2.6	5.9	9.9	16.3	24.7	25.1	25.0	16.6	13.7 (8.65)
SR	7.5	2.5	1.8	3.5	10.4	17.6	25.1	25.9	25.2	16.4	13.6 (9.24)
DR	8.3	4.0	2.2	4.5	12.3	17.7	25.6	25.8	25.0	17.8	14.3 (8.87)
Mean per SP	8.4 (0.58)	3.4 (0.71)	2.1 (0.55)	3.8 (0.94)	10.1 (2.12)	15.5 (1.83)	24.3 (1.30)	25.2 (0.66)	25.2 (0.60)	16.9 (0.50)	
Open water											
7A	9.0	N/A	N/A	N/A	N/A	N/A	20.1	N/A	23.7	N/A	17.6 (6.26)
7B	9.0	N/A	N/A	N/A	N/A	N/A	20.2	N/A	23.6	N/A	17.6 (6.24)
8A	9.2	N/A	N/A	N/A	N/A	N/A	20.3	N/A	25.1	N/A	18.2 (6.66)
8B	9.1	N/A	N/A	N/A	N/A	N/A	20.4	N/A	24.4	N/A	18.0 (6.48)
8C	9.2	N/A	N/A	N/A	N/A	N/A	20.2	N/A	24.0	N/A	17.8 (6.28)
9A	8.8	N/A	N/A	N/A	N/A	N/A	20.5	N/A	24.4	N/A	17.9 (6.63)
9B	8.8	N/A	N/A	N/A	N/A	N/A	20.4	N/A	24.3	N/A	17.8 (6.58)
9C	8.8	N/A	N/A	N/A	N/A	N/A	20.3	N/A	24.0	N/A	17.7 (6.47)
Mean per SP	9.0 (0.16)						20.3 (0.12)		24.2 (0.45)		
DO (mg/L)											
Nearshore											
TR	10.99	13.16	13.29	12.63	10.37	8.69	7.00	6.97	7.29	9.85	10.02 (2.37)
LC	10.46	12.38	13.09	12.36	12.81	11.89	7.97	8.31	8.71	10.75	10.87 (1.85)
MB	8.38	10.14	12.36	11.20	11.93	10.65	8.76	9.09	8.25	8.35	9.91 (1.48)
AA	10.74	12.17	13.29	14.76	13.73	13.01	8.99	9.81	8.87	9.71	11.51 (2.03)
AB	11.43	12.45	12.39	15.04	13.97	13.52	9.48	10.25	9.94	9.66	11.81 (1.87)
PO	10.94	13.01	13.32	13.01	13.94	13.90	11.11	10.54	6.69	9.39	11.59 (2.20)
SR	11.85	13.71	13.54	14.07	13.04	11.39	9.54	10.42	8.43	11.92	11.79 (1.79)
DR	12.12	13.00	13.45	16.44	12.12	13.08	10.78	9.64	8.53	11.58	12.07 (2.08)
Mean per SP	10.86 (1.07)	12.50 (1.01)	13.09 (0.43)	13.69 (1.59)	12.74 (1.16)	12.02 (1.63)	9.20 (1.27)	9.38 (1.14)	8.34 (0.93)	10.15 (1.11)	
Openwater											
7A	11.27	N/A	N/A	N/A	N/A	N/A	9.64	N/A	7.75	N/A	9.55 (1.44)
7B	11.19	N/A	N/A	N/A	N/A	N/A	9.42	N/A	7.65	N/A	9.42 (1.45)
8A	11.18	N/A	N/A	N/A	N/A	N/A	9.70	N/A	8.08	N/A	9.65 (1.27)
8B	11.19	N/A	N/A	N/A	N/A	N/A	9.81	N/A	8.06	N/A	9.69 (1.28)
8C	11.13	N/A	N/A	N/A	N/A	N/A	9.75	N/A	7.92	N/A	9.60 (1.31)

9A	11.76	N/A	N/A	N/A	N/A	N/A	9.74	N/A	8.37	N/A	9.96 (1.39)
9B	11.76	N/A	N/A	N/A	N/A	N/A	9.78	N/A	8.46	N/A	10.00 (1.36)
9C	11.75	N/A	N/A	N/A	N/A	N/A	10.08	N/A	9.08	N/A	10.30 (1.10)
Mean per SP	11.40 (0.28)						9.74 (0.17)		8.17 (0.43)		
COND (µS/cm)											
Nearshore											
TR	254.5	343	405	323	324	298	244	224.7	284.8	287.6	299 (50.23)
LC	536	505	573	572	470	462	396	567	406	417	490 (66.55)
MB	468	714	602	493	460	482	402	407	657	414	510 (104.17)
AA	451	485	496	415	433	427	436	368	509	454	447 (39.67)
AB	442	482	527	450	439	451	426	354	441	453	447 (41.11)
PO	433	508	503	422	449	407	373	441	427	436	440 (38.48)
SR	432	495	522	465	414	435	392	401	391	408	436 (42.65)
DR	438	455	508	448	426	442	389	407	622	417	455 (63.36)
Mean per SP	431.8 (74.26)	498.4 (95.60)	517.0 (54.48)	448.5 (66.22)	426.9 (42.40)	425.5 (52.60)	382.3 (55.59)	396.2 (89.04)	467.2 (115.5	410.8 (49.44)	
Openwater											
7A	476	N/A	N/A	N/A	N/A	N/A	430	N/A	419	N/A	442 (24.69)
7B	478	N/A	N/A	N/A	N/A	N/A	430	N/A	412	N/A	440 (27.86)
8A	448	N/A	N/A	N/A	N/A	N/A	443	N/A	413	N/A	435 (15.46)
8B	478	N/A	N/A	N/A	N/A	N/A	434	N/A	414	N/A	442 (26.73)
8C	447	N/A	N/A	N/A	N/A	N/A	430	N/A	415	N/A	431 (13.07)
9A	441	N/A	N/A	N/A	N/A	N/A	427	N/A	412	N/A	427 (11.84)
9B	445	N/A	N/A	N/A	N/A	N/A	439	N/A	412	N/A	432 (14.35)
9C	440	N/A	N/A	N/A	N/A	N/A	450	N/A	413	N/A	434 (15.63)
Mean per SP	456.6 (16.25)						435.4 (7.45)		413.8 (2.22)		
pH											
Nearshore											
TR	8.56	7.76	7.83	7.86	7.83	7.98	8.11	8.12	8.49	7.97	8.05 (0.26)
LC	8.68	8.11	8.07	7.58	7.67	8.62	8.47	8.25	8.43	8.31	8.22 (0.35)
MB	7.72	7.66	7.96	7.32	7.72	7.87	6.93	8.44	8.37	7.83	7.78 (0.42)
AA	8.40	8.30	8.30	8.22	8.02	8.30	8.54	8.37	8.34	8.18	8.30 (0.13)
AB	8.42	8.47	7.88	8.16	8.02	8.08	8.65	8.32	8.39	8.20	8.26 (0.22)
PO	8.35	8.57	7.96	8.15	8.24	8.49	8.91	8.70	8.36	8.08	8.38 (0.28)
SR	8.38	7.94	7.84	7.82	8.20	8.25	8.75	8.85	8.63	8.50	8.32 (0.35)
DR	8.41	8.66	7.95	8.44	8.16	8.14	8.54	8.67	8.45	8.34	8.38 (0.22)
Mean per SP	8.37 (0.26)	8.18 (0.35)	7.97 (0.14)	7.94 (0.35)	7.98 (0.21)	8.22 (0.24)	8.36 (0.58)	8.47 (0.23)	8.43 (0.09)	8.18 (0.20)	
Open water											
7A	8.32	N/A	N/A	N/A	N/A	N/A	8.15	N/A	8.23	N/A	8.23 (0.07)
7B	8.34	N/A	N/A	N/A	N/A	N/A	8.24	N/A	8.20	N/A	8.26 (0.06)
8A	8.34	N/A	N/A	N/A	N/A	N/A	8.30	N/A	8.20	N/A	8.28 (0.06)
8B	8.33	N/A	N/A	N/A	N/A	N/A	8.27	N/A	8.19	N/A	8.26 (0.06)
8C	8.34	N/A	N/A	N/A	N/A	N/A	8.28	N/A	8.17	N/A	8.26 (0.07)

9A	8.40	N/A	N/A	N/A	N/A	N/A	8.27	N/A	8.20	N/A	8.29 (0.08)
9B	8.40	N/A	N/A	N/A	N/A	N/A	8.30	N/A	8.26	N/A	8.32 (0.06)
9C	8.40	N/A	N/A	N/A	N/A	N/A	8.30	N/A	8.30	N/A	8.33 (0.05)
Mean per SP	8.36 (0.03)						8.26 (0.05)		8.22 (0.04)		

3.3.1.2 Chlorophyll *a* and Nutrients (Nearshore samples)

Mean nearshore CHL *a* was highly variable between sites (1.58 – 13.29 mg/m^3) but less variable between sampling periods (2.47 – 10.05mg/m^3) (Table 2). In order to meet the assumption of normality CHL *a* data were square root transformed. CHL *a* was significantly different between sites ($F_{7,63} = 6.502$, $p < 0.001$) with the follow up test finding that MB and LC had significantly higher CHL *a* concentrations compared to the other sites. Monthly differences in CHL *a* was also found to be significant ($F_{9,63} = 2.871$, $p = 0.007$) and attributed to higher CHL *a* in August than March and April.

Nearshore nitrate concentrations in the study sites fluctuated between 25.15 – 78.92 µg/L and 16.90 – 91.36 µg/L between months (Table 2). Data could not be normalised with transformation therefore nonparametric tests were used. Nitrate differed significantly between sites (Friedman's $X_2 = 30.582$, $p < 0.001$) and months (Kruskal Wallis $X^3_2 = 31.459$, $p < 0.001$). Significant differences among sites were caused by LC having higher nitrate concentrations compared to DR. Monthly differences were due to higher nitrate concentration in spring samples (March and April) than July and November.

Mean TP at nearshore sites varied spatially (10.83 – 31.31 µg/L) and temporally (8.75 – 27.05 µg/L) (Table 2). There was a significant difference found between sites ($F_{7,63} = 4.467$, $p < 0.001$) and months ($F_{9,63} = 2.946$, $p = 0.006$). Differences among sites were caused by the higher TP concentrations at MB. Monthly differences were caused by higher TP concentration in November compared to June and March.

Nearshore mean TSS differed among sites (1.35 – 11.11 mg/L) and months (2.38 – 11.40 mg/L) (Table 2). The data was not normally distributed therefore nonparametric tests were used. TSS was found to differ significantly among sites (Friedman's $X_2 = 31.167$, $p < 0.001$) mainly due to the higher TSS concentration in sites than SR. Monthly TSS did not significantly differ (Kruskal Wallis $X^3_2 = 14.157$, $p = 0.1168$).

3.3.1.3 Chlorophyll *a* and Nutrients (Open water samples)

Mean open water CHL *a* varied among sites (2.30 – 2.86 mg/m^3) and with months (1.49 – 4.25 mg/m^3). Differences among sites were not significant ($F_{7,23} = 0.09$, $p = 0.998$) but monthly were ($F_{2,23} = 17.62$, $p < 0.001$). This was due to the higher CHL *a* in August compared to June and October. Nitrate concentrations at the open water sites showed a similar pattern as that of CHL *a* by exhibiting a higher variability in the monthly samples (11.91 – 30.50 mg/L) than between sites (17.29 – 32.57 mg/L). Again differences were found to be not significant between sites ($F_{7,23} = 0.98$, $p = 0.481$) and significant between months ($F_{2,23} = 11.03$, $p = 0.001$). Monthly differences were caused mainly by the lower nitrate concentration in June. Mean TP concentrations fluctuated from 10.88 – 22.76 mg/L at sites and 12.41 – 22.26 mg/L monthly. These differences were not significant (sites $F_{7,23} = 0.42$, $p = 0.873$; months $F_{2,23} = 1.78$, $p = 0.204$). Mean TSS varied from 0.40 – 0.80 mg/L at sites and 0.36 – 1.26 mg/L between sampling periods. Sites differences were not significant ($F_{7,23} = 1.71$, $p = 0.185$). Monthly differences were significant ($F_{2,23} = 65.02$, $p < 0.001$) as a result of high TSS in August. Since there was no difference found between sites that means that depth had no effect on the above parameters.

Table 2. Nutrient and chlorophyll *a* data, mean values and standard deviation (in parenthesis) of environmental variables at nearshore and open water sampling sites during the 2015/2016 sampling period.

	Oct	Nov	Dec	Mar	Apr	May	Jun	Jul	Aug	Sept	Mean
CHL *a* (mg/m^3)											
Nearshore											
TR	1.56	7.63	2.26	2.28	4.80	11.22	3.83	4.52	4.09	2.59	4.48 (2.79)
LC	7.68	18.38	13.41	10.08	7.17	25.19	6.01	5.49	10.20	3.40	10.70 (6.33)
MB	11.80	15.24	3.71	3.16	5.66	8.57	33.29	8.51	31.44	11.50	13.29 (10.18)
AA	1.28	2.06	7.55	2.62	1.06	1.57	1.16	2.31	0.83	2.65	2.31(1.86)
AB	1.69	1.47	0.55	2.22	0.78	2.41	2.16	9.78	8.02	3.20	3.23 (2.96)
PO	1.00	0.00	2.65	0.00	0.98	6.77	4.89	33.41	18.82	2.26	7.08 (10.26)
SR	0.71	0.22	0.83	1.55	0.94	1.47	1.01	2.34	3.50	3.20	1.58 (1.04)
DR	0.75	4.27	0.78	0.82	0.90	1.94	4.28	14.66	20.42	4.93	5.37 (6.41)
Mean per SP	3.31 (3.87)	6.16 (6.61)	3.97 (4.16)	2.84 (2.90)	2.78 (2.47)	7.39 (7.56)	7.08 (10.05)	10.13 (9.62)	12.17 (9.83)	4.22 (2.86)	
Open water											
7A	3.06	N/A	N/A	N/A	N/A	N/A	1.57	N/A	2.27	N/A	2.30 (0.61)
7B	3.20	N/A	N/A	N/A	N/A	N/A	0.85	N/A	3.38	N/A	2.48 (1.15)
8A	1.47	N/A	N/A	N/A	N/A	N/A	1.59	N/A	4.55	N/A	2.53 (1.42)
8B	1.22	N/A	N/A	N/A	N/A	N/A	1.73	N/A	4.95	N/A	2.64 (1.65)
8C	0.91	N/A	N/A	N/A	N/A	N/A	1.59	N/A	6.09	N/A	2.86 (2.30)
9A	1.72	N/A	N/A	N/A	N/A	N/A	1.32	N/A	4.42	N/A	2.49 (1.38)
9B	2.16	N/A	N/A	N/A	N/A	N/A	1.81	N/A	3.81	N/A	2.59 (0.87)
9C	1.14	N/A	N/A	N/A	N/A	N/A	1.45	N/A	4.52	N/A	2.37 (1.53)
Mean per SP	1.86 (0.82)						1.49 (0.82)		4.25 (1.06)		
Nitrate (µg/L)											
Nearshore											
TR	70.24	32.19	28.26	59.44	40.29	32.44	37.10	18.52	42.26	35.14	39.59 (14.30)
LC	27.77	19.01	30.72	425.48	137.26	29.49	47.17	17.54	27.04	27.77	78.92 (120.23)
MB	26.05	32.19	41.77	142.66	76.62	44.47	38.82	18.03	28.26	30.96	47.98 (34.96)
AA	24.09	26.05	26.30	19.50	35.63	84.48	27.04	10.00	26.55	17.29	29.69 (19.38)
AB	17.54	26.30	17.05	10.00	60.91	26.05	35.87	24.83	17.29	24.83	26.07 (13.43)
PO	27.77	18.76	26.30	31.70	38.08	27.53	56.00	19.25	32.44	24.34	30.22 (10.24)
SR	25.32	17.05	10.00	24.83	71.96	28.26	26.55	17.05	19.75	17.54	25.83 (16.27)
DR	25.32	10.00	61.65	17.29	30.96	26.55	27.77	10.00	24.58	17.05	25.12 (14.01)
Mean per SP	30.51 (15.32)	22.69 (7.30)	30.26 (14.74)	91.36 (132.54)	61.47 (32.93)	37.41 (18.64)	37.04 (9.76)	16.90 (4.58)	27.27 (7.21)	24.36 (6.34)	
Open water											
7A	26.55	N/A	N/A	N/A	N/A	N/A	18.03	N/A	24.34	N/A	22.97 (3.61)
7B	24.58	N/A	N/A	N/A	N/A	N/A	10.00	N/A	24.09	N/A	19.56 (6.76)

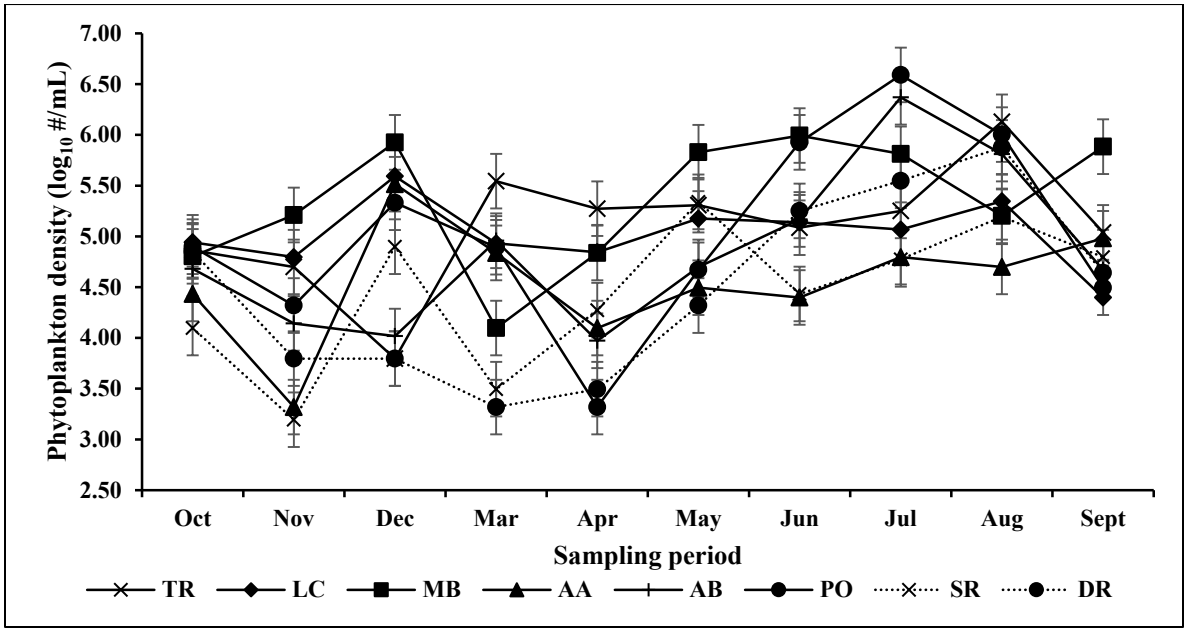
8A	25.81	N/A	N/A	N/A	N/A	N/A	17.29	N/A	17.29	N/A	20.13 (4.02)
8B	25.56	N/A	N/A	N/A	N/A	N/A	10.00	N/A	26.30	N/A	20.62 (7.52)
8C	61.90	N/A	N/A	N/A	N/A	N/A	10.00	N/A	25.81	N/A	32.57 (21.72)
9A	29.00	N/A	N/A	N/A	N/A	N/A	10.00	N/A	25.81	N/A	21.60 (8.31)
9B	24.09	N/A	N/A	N/A	N/A	N/A	10.00	N/A	17.78	N/A	17.29 (5.76)
9C	26.55	N/A	N/A	N/A	N/A	N/A	10.00	N/A	27.53	N/A	21.36 (8.04)
Mean per SP	30.50 (11.92)						11.91 (3.32)		23.62 (3.66)		
TP (µg/L)											
Nearshore											
TR	25.26	13.57	12.38	10.88	18.67	36.94	5.19	18.37	18.67	20.46	18.04 (8.27)
LC	12.38	15.07	10.88	15.37	18.07	18.07	0.00	15.97	18.07	18.97	14.28 (5.37)
MB	28.85	47.42	49.22	14.77	23.76	33.04	33.34	37.54	28.55	16.57	31.31 (10.90)
AA	29.75	9.68	24.36	9.38	6.99	25.86	7.28	6.99	0.70	14.17	13.52 (9.24)
AB	43.83	30.35	2.79	3.99	22.26	15.67	3.69	25.86	0.70	26.16	17.53 (13.79)
PO	18.07	48.92	10.58	7.88	25.86	11.48	1.89	23.16	12.68	11.18	17.17 (12.54)
SR	9.08	35.44	3.34	4.59	9.38	3.69	0.00	9.98	1.59	31.25	10.83 (11.73)
DR	21.66	15.97	4.84	3.09	8.18	1.89	25.26	6.09	9.68	16.57	11.32 (7.67)
Mean per SP	23.61 (10.30)	27.05 (14.68)	14.80 (14.53)	8.75 (4.44)	16.65 (6.99)	18.33 (12.03)	9.58 (11.79)	17.99 (10.01)	11.33 (9.50)	19.42 (6.11)	
Open water											
7A	15.07	N/A	N/A	N/A	N/A	N/A	37.53	N/A	6.99	N/A	19.86 (12.92)
7B	8.18	N/A	N/A	N/A	N/A	N/A	37.53	N/A	22.56	N/A	22.76 (11.98)
8A	5.79	N/A	N/A	N/A	N/A	N/A	13.57	N/A	13.28	N/A	10.88 (3.60)
8B	14.47	N/A	N/A	N/A	N/A	N/A	5.49	N/A	24.96	N/A	14.97 (7.96)
8C	15.67	N/A	N/A	N/A	N/A	N/A	9.68	N/A	33.64	N/A	19.67 (10.18)
9A	14.77	N/A	N/A	N/A	N/A	N/A	12.08	N/A	17.77	N/A	14.87 (2.32)
9B	11.48	N/A	N/A	N/A	N/A	N/A	28.85	N/A	24.06	N/A	21.46 (7.33)
9C	13.87	N/A	N/A	N/A	N/A	N/A	9.68	N/A	34.84	N/A	19.47 (11.01)
Mean per SP	12.41 (3.40)						19.30 (12.33)		22.26 (8.89)		
TSS (mg/L)											
Nearshore											
TR	2.63	3.79	1.23	17.00	14.47	15.25	4.61	5.72	44.28	2.11	11.11 (12.40)
LC	2.36	4.04	3.27	3.23	2.99	5.45	3.54	3.26	3.82	2.94	3.49 (0.79)
MB	3.64	8.69	4.85	1.80	3.02	13.15	24.33	11.24	10.58	26.50	10.78 (8.17)
AA	1.72	0.30	13.15	0.60	0.22	0.51	1.17	0.90	1.57	1.13	2.13 (3.71)
AB	4.82	0.35	0.26	0.30	0.72	1.15	1.97	24.54	11.18	3.63	4.89 (7.28)
PO	0.94	0.55	4.05	5.30	0.30	5.73	11.83	27.14	11.86	1.28	6.90 (7.88)
SR	0.46	0.38	0.89	0.29	0.54	4.18	1.64	2.05	1.55	1.54	1.35 (1.11)
DR	2.45	3.20	0.09	0.00	1.60	0.84	1.35	4.79	6.38	3.73	2.44 (1.98)
Mean per SP	2.38 (1.31)	2.66 (2.75)	3.47 (4.02)	3.57 (5.36)	2.98 (4.47)	5.78 (5.25)	6.31 (7.56)	9.95 (9.64)	11.40 (13.03)	5.36 (8.05)	

Open water											
7A	0.42	N/A	N/A	N/A	N/A	N/A	0.43	N/A	1.40	N/A	0.75 (0.46)
7B	0.38	N/A	N/A	N/A	N/A	N/A	0.51	N/A	1.36	N/A	0.75 (0.43)
8A	0.15	N/A	N/A	N/A	N/A	N/A	0.49	N/A	1.44	N/A	0.69 (0.54)
8B	0.38	N/A	N/A	N/A	N/A	N/A	0.51	N/A	1.24	N/A	0.71 (0.38)
8C	0.30	N/A	N/A	N/A	N/A	N/A	0.69	N/A	1.43	N/A	0.80 (0.47)
9A	0.31	N/A	N/A	N/A	N/A	N/A	0.58	N/A	1.31	N/A	0.73 (0.42)
9B	0.39	N/A	N/A	N/A	N/A	N/A	0.21	N/A	0.61	N/A	0.40 (0.17)
9C	0.53	N/A	N/A	N/A	N/A	N/A	0.60	N/A	1.25	N/A	0.79 (0.33)
Mean per SP	0.36 (0.10)						0.50 (0.13)		1.26 (0.25)		

3.3.1.4 Phytoplankton (Nearshore sites)

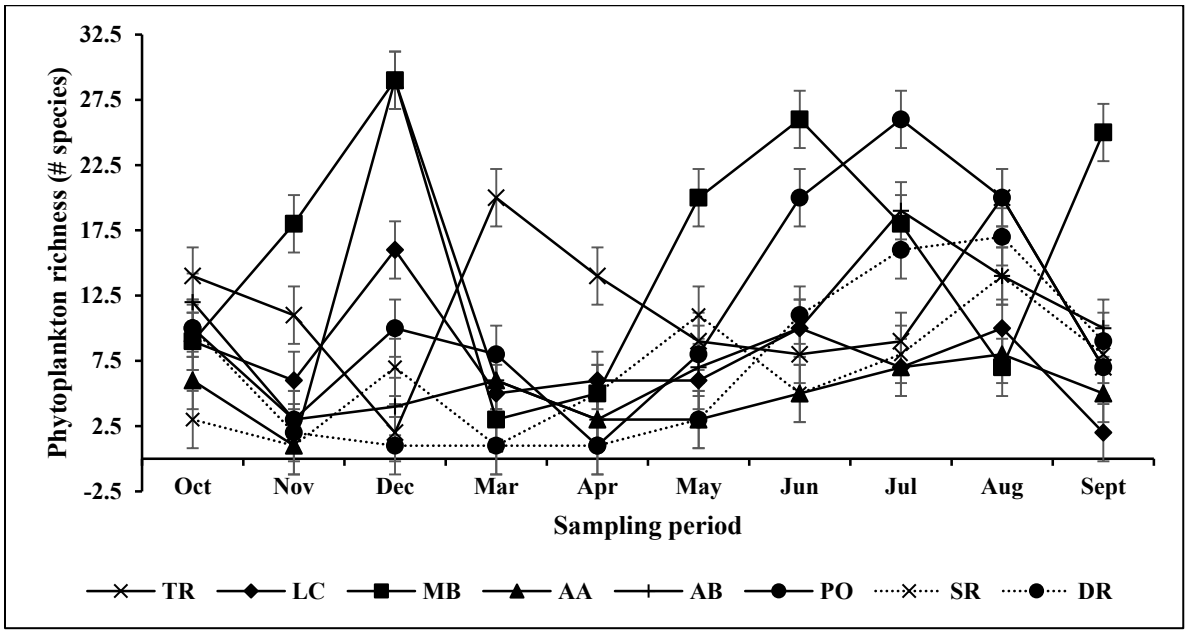
Mean phytoplankton density in nearshore sites differed greatly between sites ($6.39x10^7$ – $6.21\ x10^8$/L) and months ($4.00x10^7$ – $9.58x10^8$/L) (Figure 1). Phytoplankton density was log transformed before analysis to normalize the data. Density differed significantly due to MB having higher densities compared to AA and DR ($F_{7,63} = 3.402$, $p = 0.004$). Density also differed significantly among months ($F_{9,63} = 5.450$, $p < 0.001$) and this was due to seasonal differences in density with summer (July, August) > spring (March, April) > fall (November).

Figure 1. Log_{10} transformed phytoplankton density at nearshore sites and across the 2015/2016 sampling period. Error bars indicate standard deviation calculated from triplicate sample analysis.



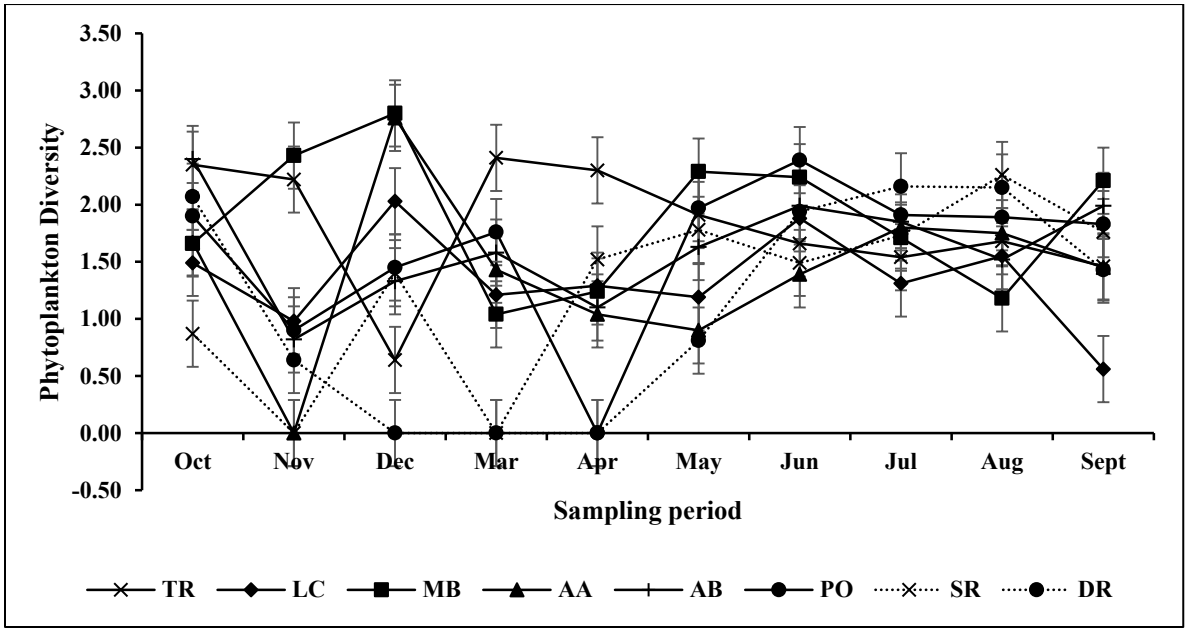
The mean nearshore phytoplankton richness varied across sites (6.3 – 16) and months (4.8 – 13.8) (Figure 2). These differences were found to be significantly different among sites (sites $F_{7,63} = 2.839$, $p = 0.012$) but not for months ($F_{9,63} = 2.374$, $p > 0.017$). Differences between sites was mainly caused by MB having greater richness compared to AA, SR and DR. Altogether there were 58 species of phytoplankton observed during the study (Appendix A). Out of this, 12 diatom species were observed in all sites (*Amphora ovalis, Cocconeis placentula*, *Cyclotella* sp., *Diatoma vulgaris*, *Fragilaria capucina*, *Fragilaria crotonensis*, *Gomphonema* sp., *Navicula gastrum*, *Navicula* sp., *Rhopolodia gibba*, *Synedra* sp. and *Synedra ulna*). Green algae was the second dominant phytoplankton group (12 species) with *Cosmarium* sp., *Scenedesmus quadricauda* and *Staurastrum gracile* found in greatest abundance. The cyanobacteria species observed were *Aphanocapsa* sp., *Chroococcus* sp., *Merismopedia glauca* and *Microcystis* sp.

Figure 2. Phytoplankton richness at nearshore sites and across the 2015/2016 sampling period. Error bars indicate standard deviation calculated from triplicate sample analysis.



Phytoplankton diversity did not appear to vary widely among nearshore sites (1.12 – 1.88) although it did with the sampling period (1.00 – 1.87) (Figure 3). Diversity did not differ significantly between sites ($F_{7,63} = 1.839$, $p = 0.095$). Monthly differences were also found to be not significant ($F_{9,63} = 2.132$, $p > 0.025$). Diversity equalled zero if there was only one species present in a sample.

Figure 3. Shannon Wiener phytoplankton diversity at nearshore sites and across the 2015/2016 sampling period. Error bars indicate standard deviation calculated from triplicate sample analysis.



3.3.1.5 Phytoplankton (Open water sites)

The phytoplankton community experienced minimal variation among the open water sites however it varied with season. Phytoplankton density ranged from $1.75 \times 10^7 – 4.88 \times 10^7$/L between sites and $1.10 \times 10^7 – 6.68 \times 10^7$/L between months (Figure 4). Density did not vary significantly among sites ($F_{7,23} = 0.64$, $p = 0.714$) but it did with season ($F_{2,23} = 17.73$, $p = 0.001$). This seasonal difference was caused by higher density in August compared to June and October. Spatially phytoplankton richness did not differ significantly (4.7 – 6.7; $F_{7,23} = 0.47$, $p = 0.838$) although it did seasonally (3.5 – 7.6; $F_{2,23} = 8.02$, $p = 0.005$). Richness differed as a result of higher species count in August (Figure 5). Phytoplankton diversity ranged from 0.77 – 2.01 among sites and 0.70 – 1.61 between months (Figure 6). The differences among sites were not significant ($F_{7,23} = 0.87$, $p = 0.511$) but with months the differences were significant ($F_{2,23} = 5.42$, $p = 0.018$). Depth had no significant impact on phytoplankton composition since there was no significant difference found between sampling sites. Similar to nearshore sites diatoms

dominated open water sites with 20 different species (Appendix B). *Cyclotella* sp., *Cymbella* sp. and *Navicula* sp. were found at all three open water sites. Green algae species such as *Chlamydomonas* sp., *Coelastrum* sp., *Cosmarium* sp. and *Scenedesmus quadricauda* were present. Two species of cyanobacteria *Chroococcus* sp. and *Microcystis* sp. were found in a limited number in open water sites.

Figure 4. Log_{10} transformed phytoplankton density at open water sites and across the 2015/2016 sampling period. Error bars indicate standard deviation calculated from triplicate sample analysis.

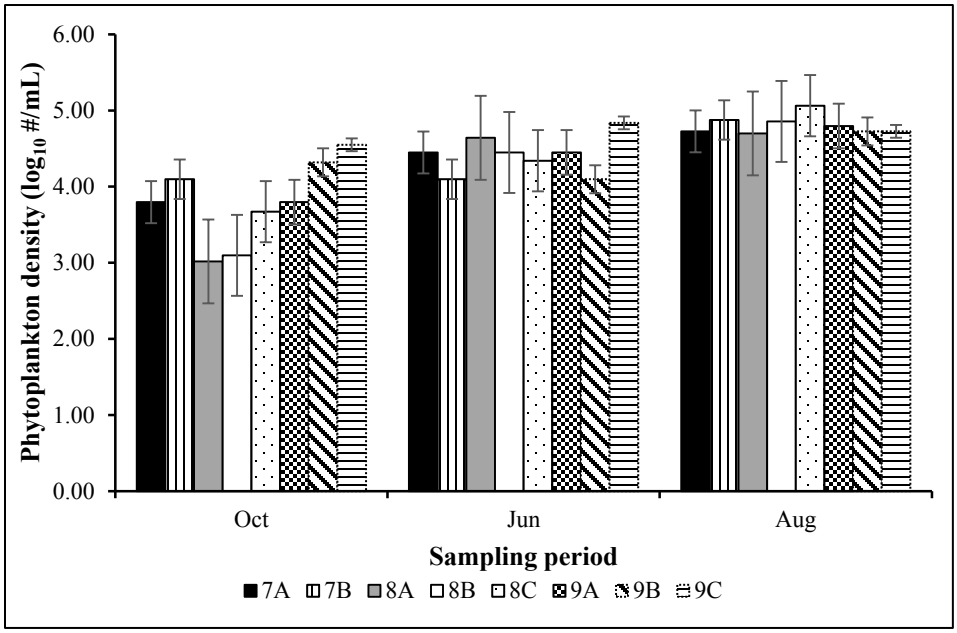


Figure 5. Phytoplankton richness at open water sites and across the 2015/2016 sampling period. Error bars indicate standard error.

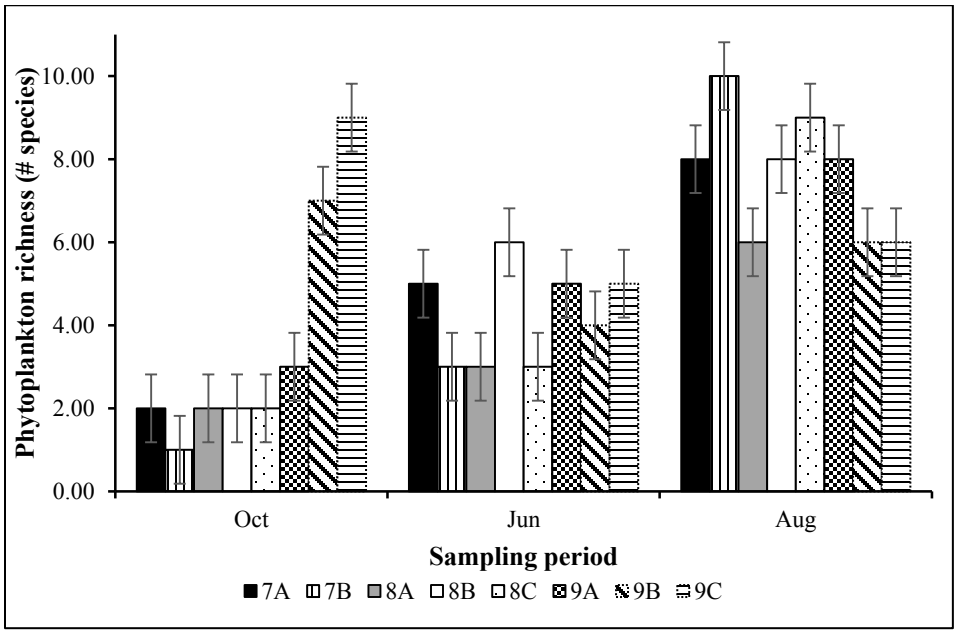
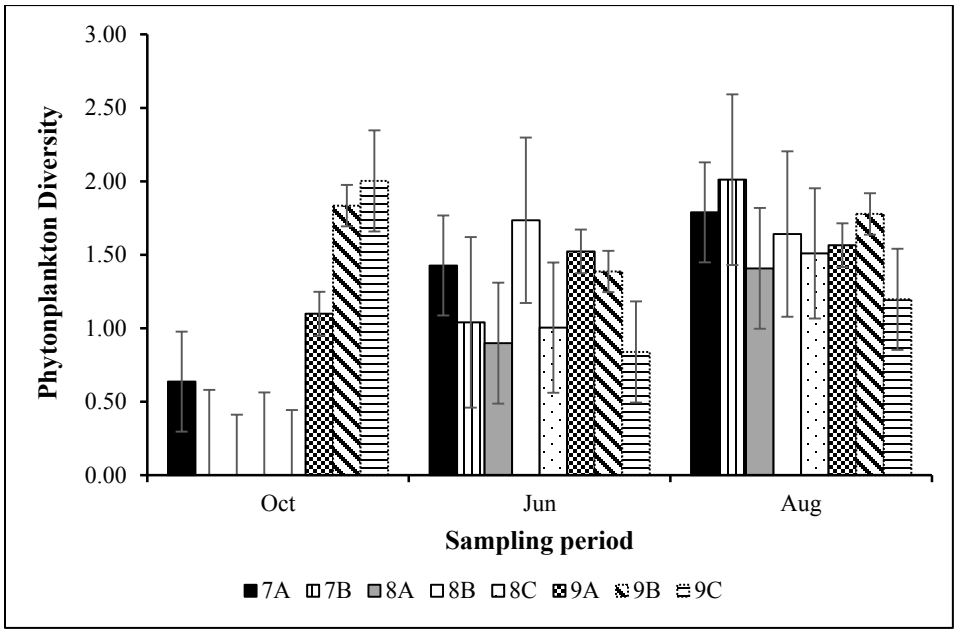


Figure 6. Shannon Wiener phytoplankton diversity at open water sites and across the 2015/2016 sampling period. Error bars indicate standard deviation calculated from triplicate sample analysis.



3.3.1.6 Differences between Nearshore and Open Water Sites

To determine differences between nearshore and open water sites data from the same sampling month were compared. This is because nearshore sites were sampled a total of 10 times whereas open water sites could only be sampled only three times. TEMP and DO did not differ significantly between nearshore and open water sites (Friedman's $X_2 = 14.919$, $p = 0.457$; $F_{15,30} = 1.686$, $p = 0.109$, respectively). Log transformed COND did vary significantly between sites ($F_{15,30} = 5.26$, $p < 0.001$); pH did not (Friedman's $X_2 = 28.80$, $p > 0.008$). Differences between COND were attributed to TR having much lower COND compared to all open water sites. pH differences was driven by nearshore sites variances.

Nitrate and TP concentration between the nearshore and open water sites did not show significant difference ($F_{15,30} = 1.783$, $p = 0.087$; $F_{15,30} = 0.820$, $p = 0.649$, respectively). CHL *a* concentration differed significantly ($F_{15,30} = 4.674$, $p < 0.001$) due to MB having much higher CHL *a* than all open water sites. The TSS concentrations differed significantly between the nearshore and open water sites ($F_{15,30} = 11.08$, $p < 0.001$). This difference was caused by TR, MB, AB and PO and to a lesser extent LC and DR having greater TSS concentrations than open water sites.

Phytoplankton communities showed significant variation between nearshore and open water sites. Phytoplankton density was found to be significantly varying between the two habitats ($F_{15,30} = 6.50$, $p < 0.001$). This difference being driven by the higher densities at PO and to some extent TR, MB, AB and DR compared to open water sites. Phytoplankton richness was significantly different between open water and nearshore habitats ($F_{15,30} = 3.94$, $p < 0.001$). This difference was due to the greater richness at PO compared to 7B, 8A, and 8C. Although both density and richness varied significantly among sites while diversity did not ($F_{15,30} = 1.723$, $p = 0.099$).

3.3.1.7 Between Lake Difference

The means for each of the environmental parameters monitored during this study are presented below separated into the respective lake they were sampled from (Table 3). When comparing means of the two lakes it is evident that some parameters varied little between lakes (TEMP, DO, COND and pH) while nutrient (CHL *a*, Nitrate, TP and TSS) and phytoplankton communities (density, richness and diversity) appeared to vary substantially. Out of 11 of the parameters measured 9 were found to differ significantly between sampling locations. Reviewing these differences closely revealed that differences between sites were actually driven predominantly by differences between lakes. For instance differences in DO was due to TR and MB having lower DO compared to all the sites in Lake Couchiching. This pattern continues for all parameters that were found to significantly differ with between the lakes contributing to at least 50% or more of the overall difference.

Table 3. Environmental variable averages for Lake Simcoe and Lake Couchiching sample sites.

	TEMP (°C)	DO (mg/L)	COND (µS/cm)	pH	CHL *a* (mg/m^3)	Nitrate (µg/L)	TP (µg/L)	TSS (mg/L)	Phyto Density (#/L)	Phyto Richness	Phyto Diversity
Lake Simcoe											
TR	13.8	10.02	299	8.05	4.48	39.59	18.04	11.11	$2.62x10^8$	11.4	1.82
LC	13.4	10.87	490	8.22	10.70	78.92	14.28	3.49	$1.35x10^8$	7.7	1.35
MB	13.7	9.91	510	7.78	13.29	47.98	31.31	10.78	$4.39x10^8$	16.0	1.88
AA	12.4	11.51	447	8.30	2.31	29.69	13.52	2.13	$7.02x10^7$	7.3	1.42
Mean	13.3	10.58	437	8.09	7.69	49.05	19.29	6.88	$2.27x10^8$	10.6	1.62
Lake Couchiching											
AB	12.9	11.81	447	8.26	3.23	26.07	17.53	4.89	$3.41x10^8$	8.8	1.62
PO	13.7	11.59	440	8.38	7.08	30.22	17.17	6.90	$6.22x10^8$	11.3	1.60
SR	13.6	11.79	436	8.32	1.58	25.83	10.83	1.35	$6.39x10^7$	6.3	1.28
DR	14.3	12.07	455	8.38	5.37	25.12	11.32	2.44	$1.44x10^8$	7.1	1.12
Mean	13.6	11.82	444	8.33	4.31	26.81	14.21	3.90	$2.93x10^8$	8.4	1.41

3.3.2 Zooplankton Community Dynamics

3.3.2.1 Composition

All nearshore species of zooplankton identified during the study are given in Table 4. Species are divided into their respective taxonomic group and identified to species or genus level. A total of 44 different species were identified; 5 Cyclopoid copepods, 4 Calanoid copepods, 1 Harpacticoid copepod, nauplii, 13 Cladocerans and 10 Rotifera. There were 9 species found at all sampling locations *Cyclopoid copepodid*, *Diacyclops thomasi*, *Leptodiaptomus* sp., nauplii, *Bosmina longirostris*, *Simocephalus serrulatus*, *Asplanchna* sp., *Keratella cochlearis* and *Polyartha* sp.

Table 4. List of all zooplankton species observed in the nearshore sites throughout the 2015/2016 sampling period. "X' indicates presence of the species at the site.

Taxa	Site							
	TR	LC	MB	AA	AB	PO	SR	DR
Cyclopoid Copepods								
Acanthocyclops sp.		X	X					
Acanthocyclops vernalis		X						
Cyclopoid copepodid	X	X	X	X	X	X	X	X
Diacyclops thomasi	X	X	X	X	X	X	X	X
Microcyclops varicans			X		X	X		
Calanoid Copepods								
Calanoid copepodid			X		X		X	X
Leptodiaptomus sp.	X	X	X	X	X	X	X	X
Epischura sp.					X			
Limnocalanus macrurus		X	X	X	X	X	X	X
Nauplii	X	X	X	X	X	X	X	X
Harpacticoid Copepod	X	X			X	X	X	X
Cladocerans								
Acroperus harpae	X		X	X	X	X	X	X
Bosmina longirostris	X	X	X	X	X	X	X	X
Ceriodaphnia sp.			X					
Chydorus sphaericus	X	X	X		X	X	X	X
Daphnia mendotae		X	X	X		X	X	X
Daphnia retrocurva				X				
Diaphanosoma birgei		X	X	X	X	X	X	X

Eurycercus spp.	X	X						X
Holopedium gibberum				X			X	
Leptodora kindtii				X				
Polyphemus pediculus					X		X	X
Sida crystalline						X		X
Simocephalus serrulatus	X	X	X	X	X	X	X	X
Rotifera								
Asplanchna sp.	X	X	X	X	X	X	X	X
Brachionus calyciflorus		X	X	X				
Filina sp.			X					
Kellicottia longispina					X	X	X	
Keratella cochlearis	X	X	X	X	X	X	X	X
Keratella tecta	X	X	X	X	X	X	X	
Lecane luna		X	X					
Monostyla lunaris	X	X		X				
Monostyla sp.			X					
Polyartha spp.	X	X	X	X	X	X	X	X

Table 5 lists all the species found at the open water sites. The total number of species identified was 26 consisting of 4 Cyclopoid copepods, 3 Calanoid copepods, 1 Harpacticoid copepod, nauplii, 8 Cladocerans and 9 Rotifera. There were only 7 species that occurred at all sites *Cyclopoid copepodid*, *Leptodiaptomus sp*., *Bosmina longirostris*, *Diaphanosoma birgei*, *Simocephalus serrulatus*, *Keratella cochlearis* and *Polyartha* sp.

Table 5. List of all zooplankton species identified in the open water sites throughout the three sampling periods. "X' indicates presence of the species at the site.

Taxa	Site							
	7A	7B	8A	8B	8C	9A	9B	9C
Cyclopoid Copepods								
Acanthocyclops sp.	X						X	X
Cyclopoid copepodid	X	X	X	X	X	X	X	X
Diacyclops thomasi	X	X		X	X			X
Microcyclops varicans	X	X		X	X	X	X	X
Calanoid Copepods								
Calanoid copepodid			X					
Leptodiaptomus sp.	X	X	X	X	X	X	X	X
Limnocalanus macrurus	X	X		X	X	X	X	X
Nauplii	X	X		X	X	X	X	X
Harpacticoid Copepod	X							
Cladocerans								
Acroperus harpae	X							
Bosmina longirostris	X	X	X	X	X	X	X	X
Chydorus sphaericus	X	X	X	X		X	X	X
Daphnia mendotae	X	X		X	X	X	X	X
Daphnia retrocurva	X					X		
Diaphanosoma birgei	X	X	X	X	X	X	X	X
Holopedium gibberum	X	X		X	X	X	X	
Simocephalus serrulatus	X	X	X	X	X	X	X	X
Rotifera								
Asplanchna sp.		X	X	X	X	X	X	X
Brachionus calyciflorus		X						
Filina sp.	X	X	X	X	X			
Kellicottia longispina		X		X	X	X	X	X
Keratella cochlearis	X	X	X	X	X	X	X	X
Lecane luna		X						
Monostyla lunaris			X		X	X	X	X
Polyartha spp.	X	X	X	X	X	X	X	X
Trichocerca pusilla		X						

Table 6 lists all the species identified at each sampling period for nearshore sites. There were 7 species that occurred in all 10 sampling months *Cyclopoid copepodid*, *Diacyclops thomasi*, *Leptodiaptomus* sp., nauplii, *Bosmina longirostris*, *Chydorus sphaericus* and *Polyartha*

sp. These same species were the same to occur at all sites with the exception of *Chydorus sphaericus*.

Table 6. List of all zooplankton species identified in the nearshore sites separated into sampling period. "X' indicates presence of the species at the site.

Taxa	Sampling Period									
	Oct	Nov	Dec	Mar	Apr	May	Jun	Jul	Aug	Sept
Cyclopoid Copepods										
Acanthocyclops sp.	X									
Acanthocyclops vernalis		X								
Cyclopoid copepodid	X	X	X	X	X	X	X	X	X	X
Diacyclops thomasi	X	X	X	X	X	X	X	X	X	X
Microcyclops varicans	X									
Calanoid Copepods										
Calanoid copepodid	X	X	X			X				
Leptodiaptomus sp.	X	X	X	X	X	X	X	X	X	X
Epischura sp.		X								
Limnocalanus macrurus	X									
Nauplii	X	X	X	X	X	X	X	X	X	X
Harpacticoid Copepod			X	X	X		X			X
Cladocerans										
Acroperus harpae						X	X	X	X	X
Bosmina longirostris	X	X	X	X	X	X	X	X	X	X
Ceriodaphnia sp.	X									
Chydorus sphaericus	X	X	X	X	X	X	X	X	X	X
Daphnia mendotae										X
Daphnia retrocurva										X
Diaphanosoma birgei								X	X	X
Eurycercus spp.				X	X			X	X	
Holopedium gibberum										X
Leptodora kindtii								X		
Polyphemus pediculus						X	X		X	
Sida crystalline	X						X			
Simocephalus serrulatus						X	X	X	X	X
Rotifera										
Asplanchna sp.	X	X	X	X	X	X	X		X	X
Brachionus calyciflorus		X	X	X	X	X	X			
Filina sp.			X							
Kellicottia longispina	X				X					
Keratella cochlearis	X	X	X	X	X	X	X		X	X

Keratella tecta			X	X	X					
Lecane luna	X									
Monostyla lunaris	X	X								
Monostyla sp.	X									
Polyartha spp.	X	X	X	X	X	X	X	X	X	X

Below in Table 7 all the species identified throughout the sampling periods for open water sites are presented. There were 4 species that occurred in all sampling periods *Diacyclops thomasi*, nauplii, *Bosmina longirostris*, and *Keratella cochlearis*.

Table 7. List of all zooplankton species identified in the nearshore sites separated into sampling period. "X' indicates presence of the species at the site.

Taxa	Sampling Period		
	Oct	Jun	Aug
Cyclopoid Copepods			
Acanthocyclops sp.	X		
Cyclopoid copepodid		X	X
Diacyclops thomasi	X	X	X
Microcyclops varicans	X		
Calanoid Copepods			
Calanoid copepodid			X
Leptodiaptomus sp.		X	X
Limnocalanus macrurus	X		
Nauplii	X	X	X
Harpacticoid Copepod		X	
Cladocerans			
Acroperus harpae		X	X
Bosmina longirostris	X	X	X
Chydorus sphaericus		X	X
Daphnia mendotae	X		X
Daphnia retrocurva			X
Diaphanosoma birgei			X
Holopedium gibberum			X
Simocephalus serrulatus		X	X
Rotifera			
Asplanchna sp.		X	
Brachionus calyciflorus	X		

Filina sp.		X	
Kellicottia longispina	X		
Keratella cochlearis	X	X	X
Lecane luna	X		
Monostyla lunaris	X		
Polyartha spp.		X	
Trichocerca pusilla	X		

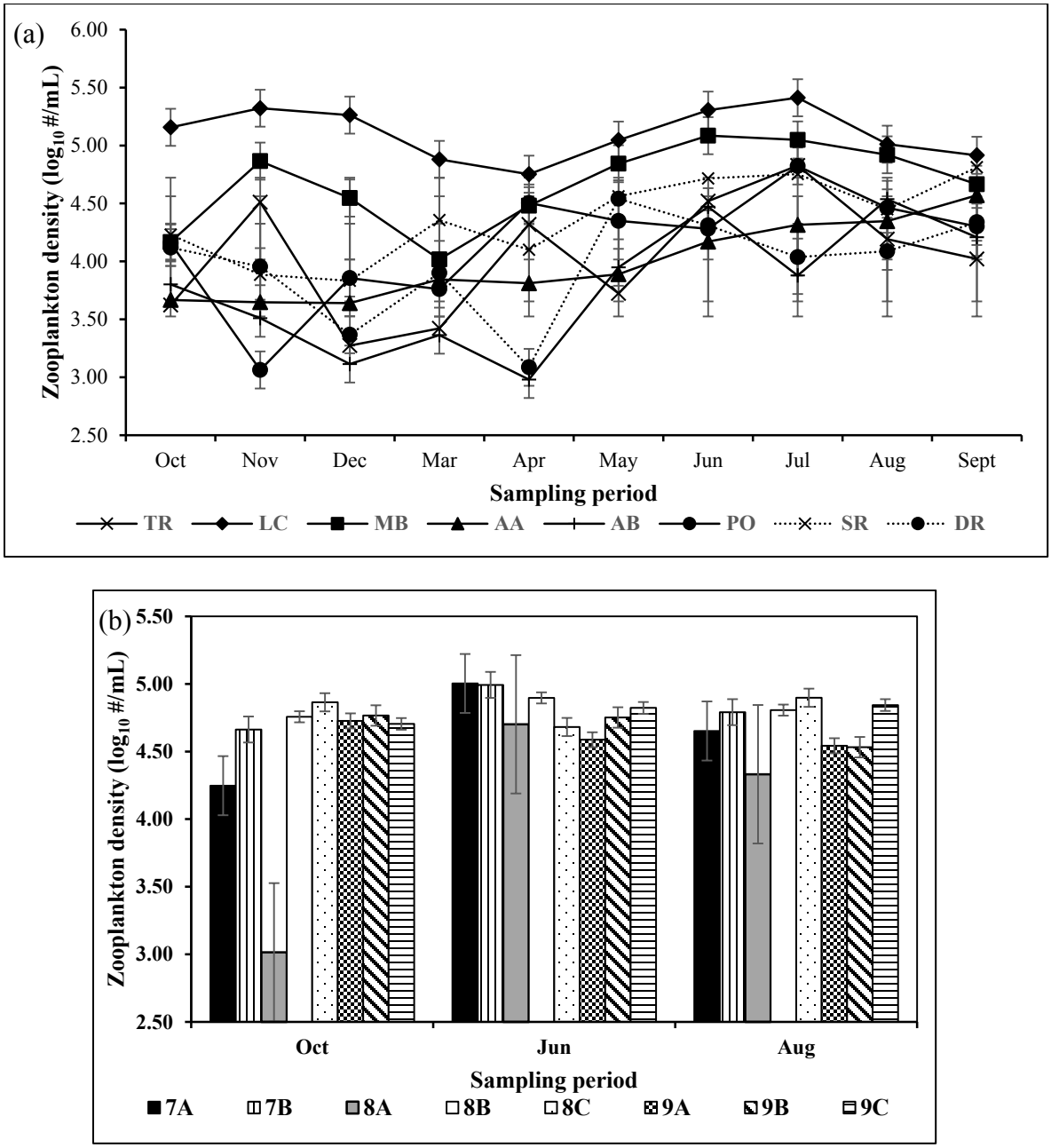
3.3.2.2 Density

Zooplankton density was determined at each site across all sampling periods. Figure 7 displays log transformed density for nearshore (a) and open water sites (b). Mean density ranged from $1.11 \times 10^7 - 1.43 \times 10^8$/L and $1.68 \times 10^7 - 7.49 \times 10^7$/L among nearshore sites and sampling periods, respectively. LC had the highest mean density and AB the lowest. Density was determined to be significantly different between sites ($F_{7,63} = 17.95$, $p < 0.001$) and months ($F_{9,63} = 6.30$, $p < 0.001$). Sampling period differences were mainly caused by seasonal differences with summer 2016 > fall 2015/2016 > spring 2016. Site differences were attributed to LC and MB with higher densities compared to rest of the sites.

Open water mean densities varied from $2.43 \times 10^7 - 6.87 \times 10^7$/L and $4.47 \times 10^7 - 6.72 \times 10^7$/L for sites and sampling periods, respectively. The highest mean density was at 7B and lowest at 8A. Unsurprisingly, density did not vary significantly among sites ($F_{7,14} = 1.75$, $p = 0.177$) which means that depth had no effect on zooplankton density. This is because in total there were 3 open water stations (7, 8, 9) and each letter (ie. A, B) signified a change in depth with A being 1 m from the surface and subsequent letters increasing in depth dependent on total depth. There was no significant difference in sampling period detected ($F_{2,14} = 2.67$, $p = 0.104$).

Density differences among the two different aquatic habitats were compared during the same sampling periods. Nearshore and open water zooplankton densities did vary significantly ($F_{15,30} = 7.648$, $p < 0.001$). LC had much higher density compared to all open water sites.

Figure 7. Log_{10} transformed zooplankton density at nearshore (a) and open water (b) sites and across the 2015/2016 sampling period. Error bars indicate standard deviation calculated from triplicate sample analysis.



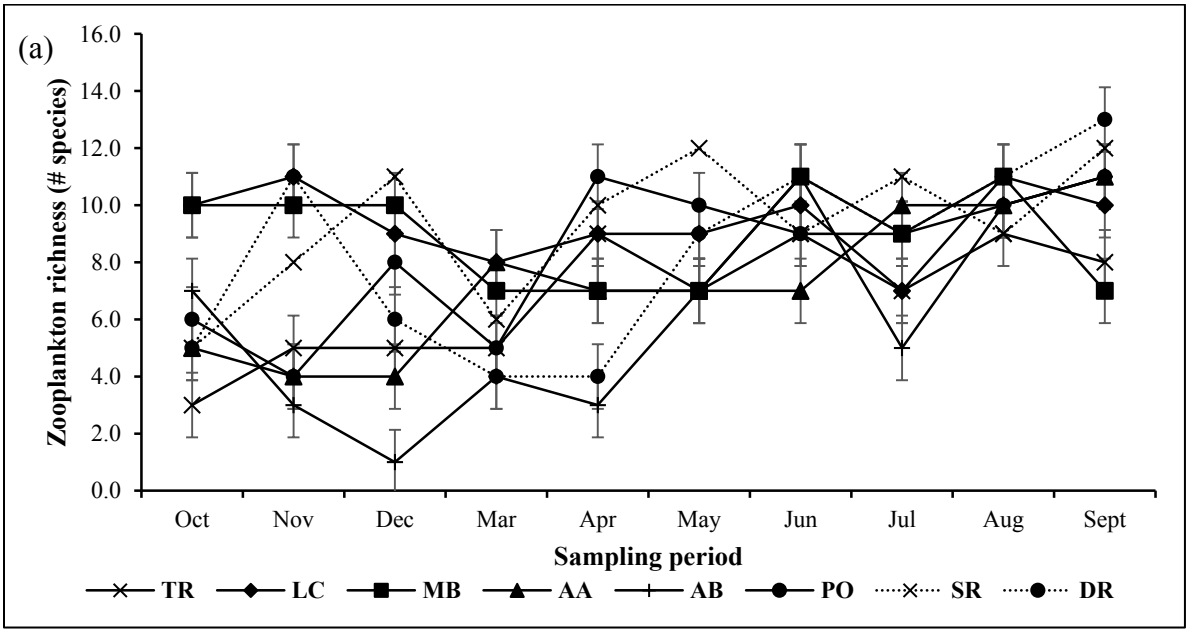
3.3.2.3 Richness

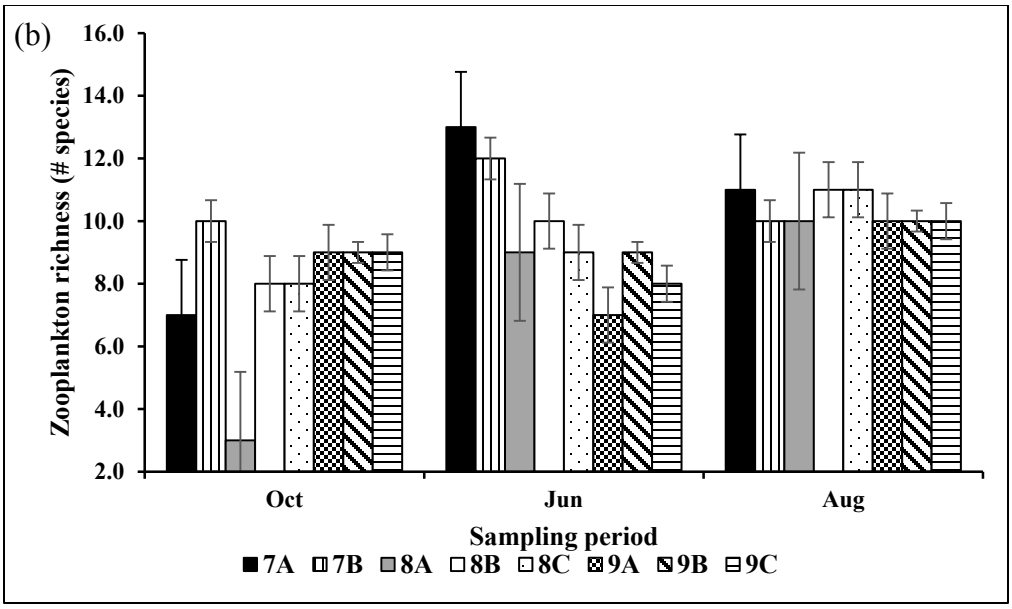
Zooplankton richness is presented in Figure 8 for nearshore (a) and open water sites (b) across all sampling periods. Mean species richness varied from 6.2 – 9.4 between sites and 5.9 – 10.4 between sampling periods. Similar to density, LC had the greater species richness and AB

had the lowest. Differences in richness between sites ($F_{7,63} = 3.29$, $p = 0.005$) and sampling periods ($F_{9,63} = 4.72$, $p < 0.001$) were significant. Site differences were attributed to lower richness at AB compared to LC and SR. Sampling period differences were a result of greater richness in SEPT compared to fall 2015 and spring 2016 and lower richness in MAR compared to summer 2017 richness.

Richness in open water sites varied moderately ranging from 7.3 – 10.7 between sites and 7.9 – 10.4 between sampling periods. Richness did not vary significantly among sites ($F_{7,15} = 1.105$, $p = 0.412$). Differences in richness between sampling periods was observed to be significantly different ($F_{2,15} = 4.57$, $p = 0.030$). This was caused by higher richness in AUG compared to OCT. Richness did not significantly vary between the two different habitat types ($F_{15,30} = 1.89$, $p = 0.067$).

Figure 8. Zooplankton richness at nearshore (a) and open water (b) sites and across the 2015/2016 sampling period. Error bars indicate standard deviation calculated from triplicate sample analysis.

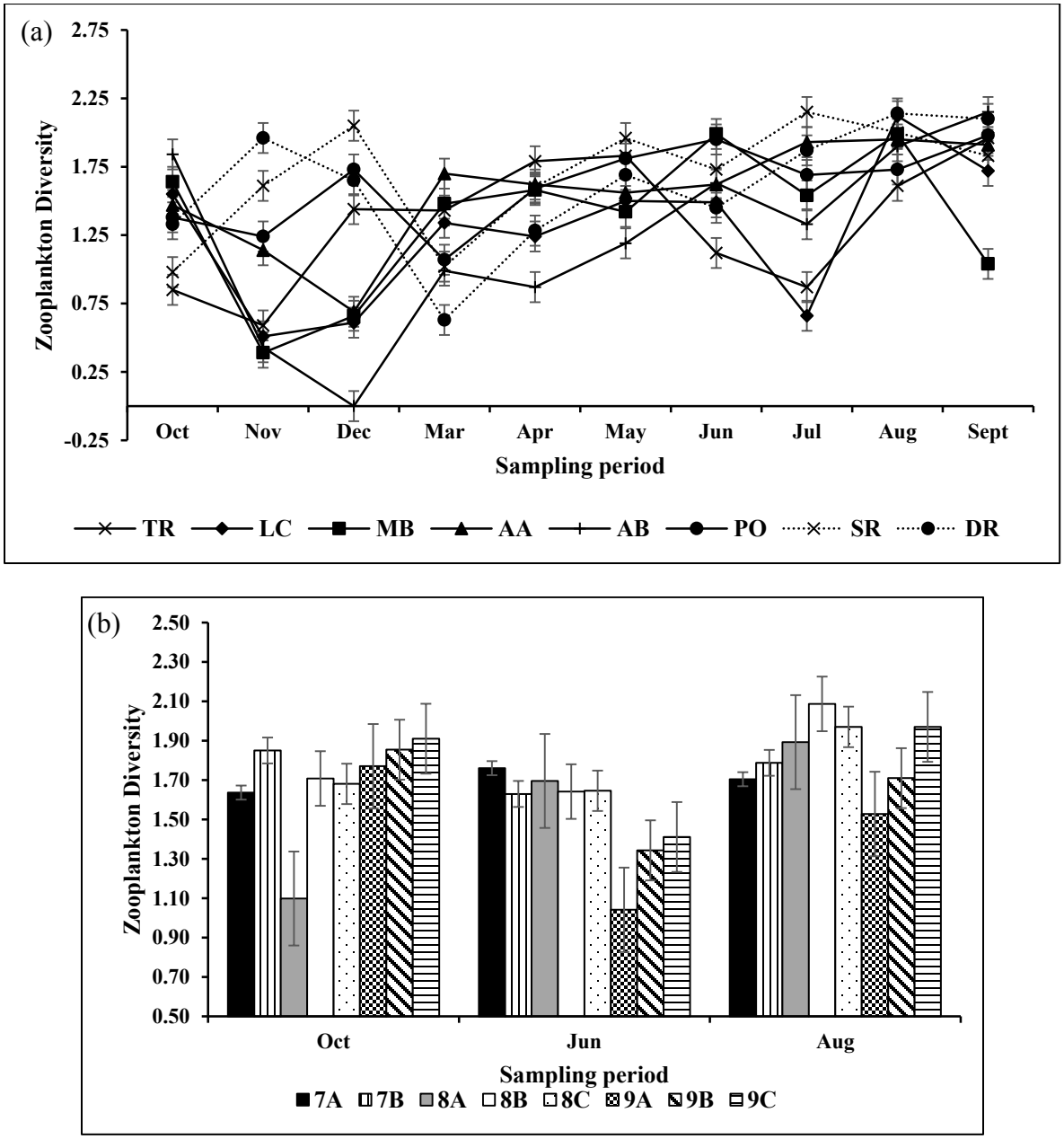




3.3.2.4 Species Diversity

Zooplankton diversity in nearshore (a) and open water (b) sites are presented in Figure 9. Diversity varied from 1.23 – 1.69 between nearshore sites and 0.94 – 1.93 between sampling periods. The differences in diversity between sites were not significant ($F_{7,63} = 1.86$, $p = 0.091$). However, diversity did vary significantly between sampling periods ($F_{9,63} = 4.92$, $p < 0.001$) due to high species diversity in August and low in November. Species diversity in open water sites varied more among sites (1.45 – 1.81) compared to sampling periods (1.52 – 1.83). Neither site or sampling period differences in diversity were significant (site $F_{7,15} = 0.85$, $p = 0.845$; sampling period $F_{2,15} = 3.49$, $p = 0.059$). Interestingly, diversity did not differ between nearshore and open water site ($F_{15,30} = 1.29$, $p = 0.269$).

Figure 9. Shannon Wiener diversity of zooplankton at nearshore (a) and open water (b) sites and across the 2015/2016 sampling period. Error bars indicate standard deviation calculated from triplicate sample analysis.

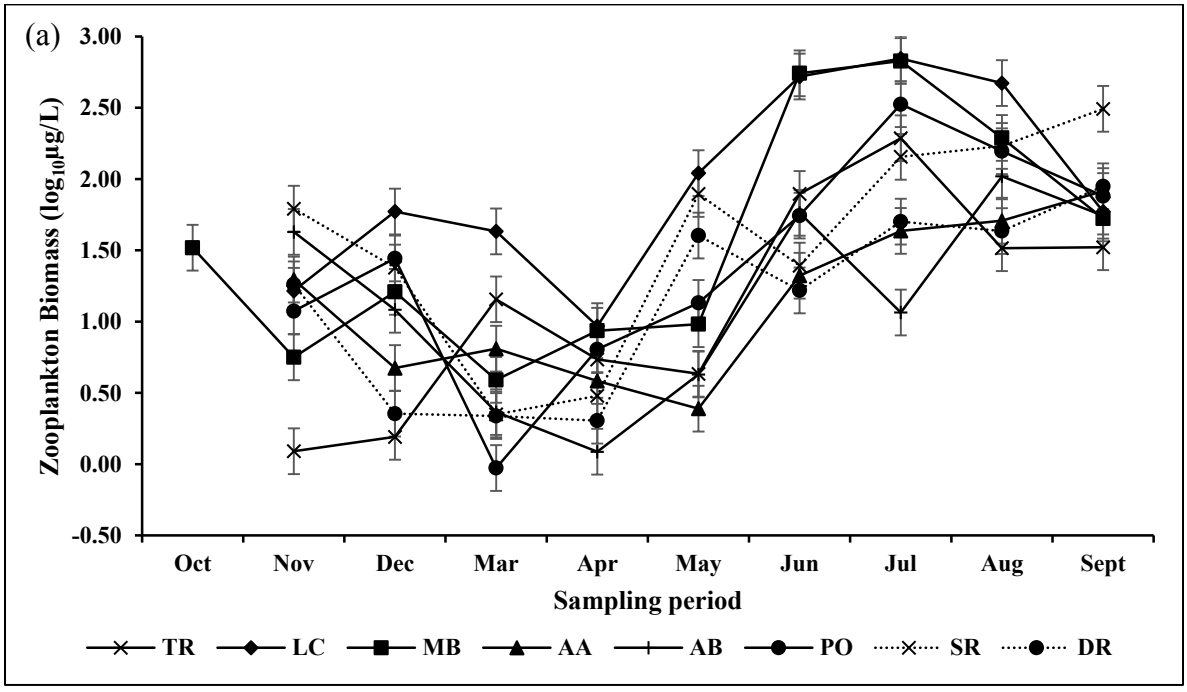


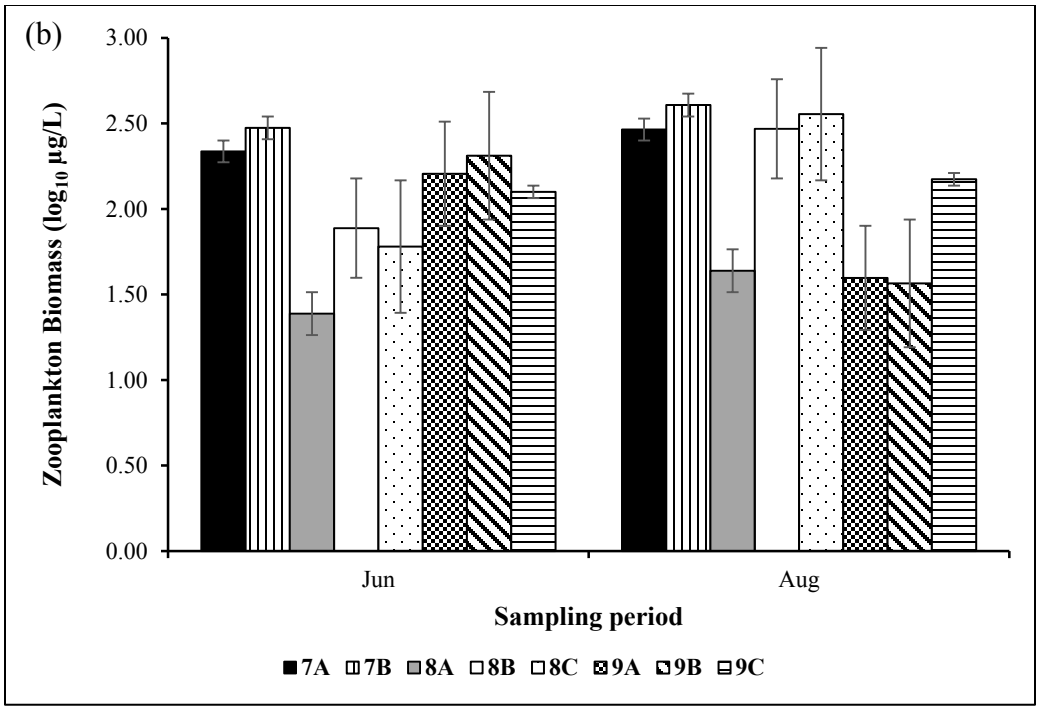
3.3.2.5 Biomass

Figure 10 shows zooplankton biomass at nearshore (a) and open water (b) sites. Mean biomass varied from 26.09 – 221.16 µg/L at nearshore sites and 4.98 – 268.53 µg/L between sampling periods. The analysis showed biomass was significantly different between nearshore

sites ($F_{7,63} = 4.32$, $p < 0.001$). This was due to the higher biomass at LC compared to TR, AA, AB and DR. The biomass also showed significant variation with the sampling period ($F_{8,63} = 14.87$, $p < 0.001$) with June, July, August and September having significantly greater biomass than fall 2015 and spring 2016. Biomass at open water sites ranged from 33.96 – 254.21 µg/L with sampling period biomass varying between 146.08 – 202.29 µg/L. Biomass did not vary significantly between sites (site $F_{7,15} = 1.60$, $p = 0.276$) or sampling periods ($F_{1,15} = 1.031$, $p = 0.344$). Although, biomass did differ significantly between nearshore and open water sites (site $F_{15,30} = 3.185$, $p = 0.016$) solely due to greater biomass at LC compared to 8A.

Figure 10. Zooplankton biomass at nearshore (a) and open water (b) sites and across the 2015/2016 sampling period. Error bars indicate standard deviation calculated from triplicate sample analysis.





3.3.2.6 Comparison between the Lakes

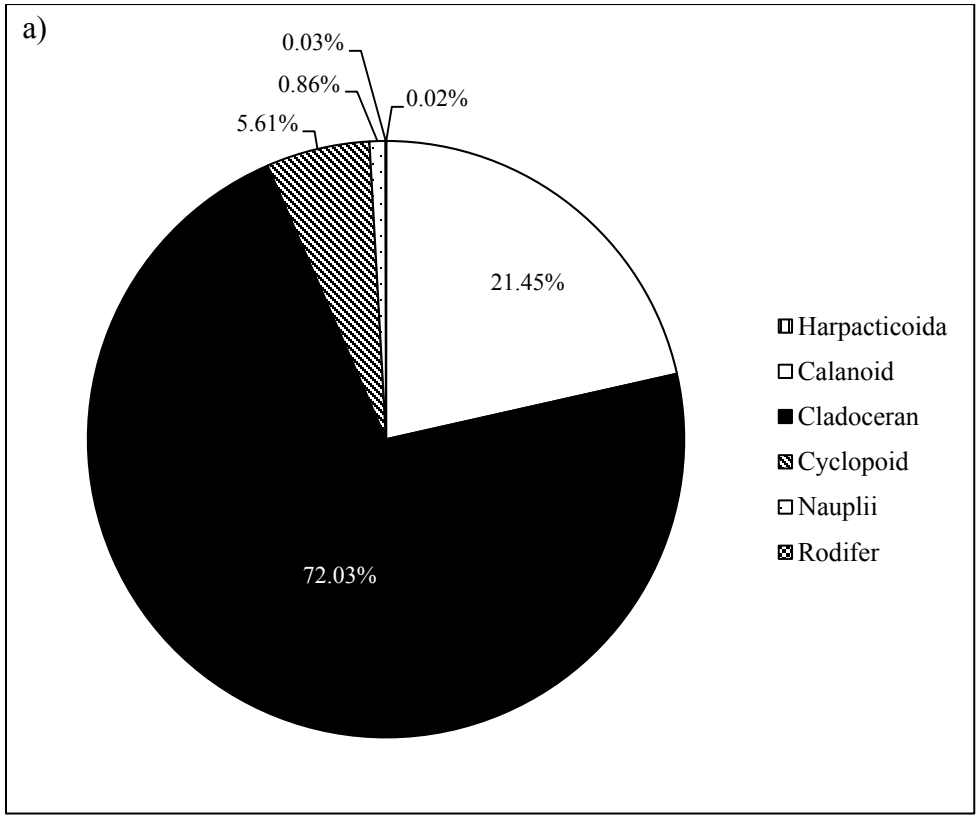
The mean zooplankton composition separated into the respective lake are presented in Table 8. It is evident that zooplankton density and biomass differed greatly between lakes while only small variance in species richness and density. Significant differences were found between sites for all parameters with the exception of species diversity. On closer examination of pairwise comparisons these differences were largely due to differences between lakes. LC and MB both had significantly greater densities compared to all of the Lake Couchiching sites. Furthermore, LC had greater richness and biomass compared to most Lake Couchiching sites. The differences between lakes contributed 42 – 75% to the overall difference between sampling locations.

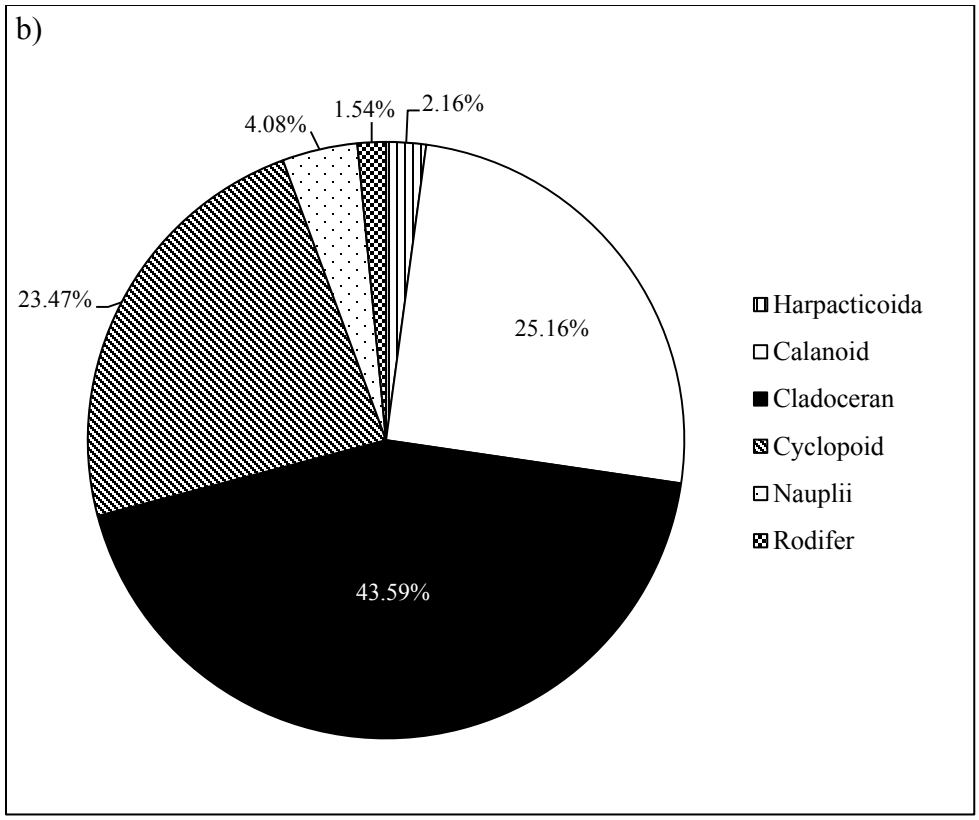
Table 8. Zooplankton composition averages for Lake Simcoe and Lake Couchiching sample sites.

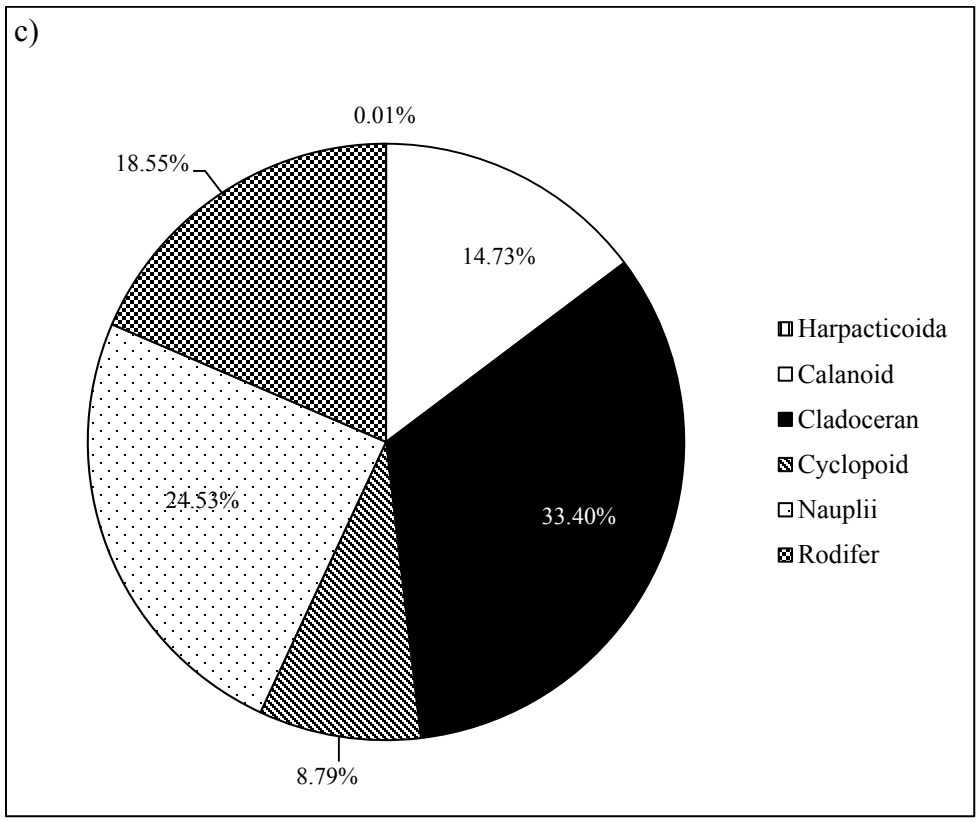
	Zooplankton Density (#/L)	**Zooplankton Richness**	**Zooplankton Diversity**	**Zooplankton biomass (µg/L)**
Lake Simcoe				
TR	$1.94x10^7$	6.7	1.35	40.52
LC	$1.43x10^8$	9.4	1.27	221.16
MB	$5.96x10^7$	8.9	1.37	154.73
AA	$1.30x10^7$	7.3	1.56	26.09
Mean	*$5.86 x10^7$*	*8.1*	*1.39*	*110.63*
Lake Couchiching				
AB	$1.11x10^7$	6.2	1.23	32.41
PO	$2.17x10^7$	8.3	1.58	75.92
SR	$3.05x10^7$	9.3	1.69	90.92
DR	$1.34x10^7$	8.3	1.61	29.31
Mean	*$1.92x10^7$*	*8.0*	*1.53*	*57.14*

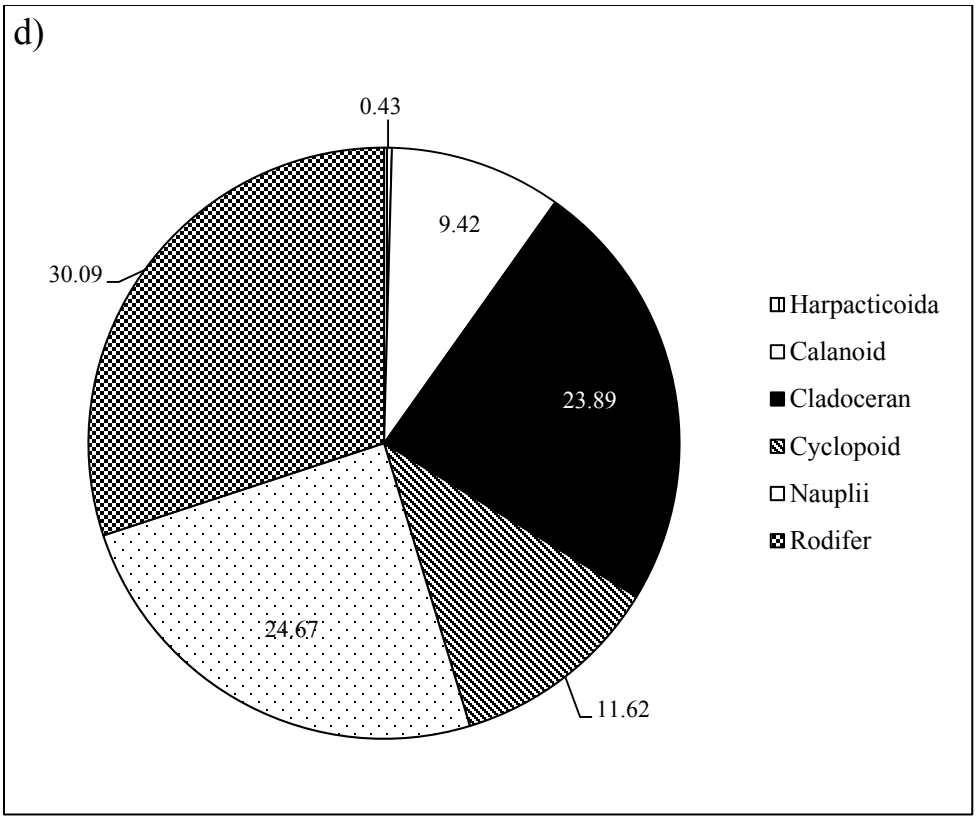
To gain a better understanding of which zooplankton groups dominated in each aquatic ecosystem percent composition with respect to density and biomass was calculated. In open water sites cladocerans dominated contributing 72.03% to species biomass with rotifer and harpacticoida species comprising the least (0.03 and 0.02%, respectively) (Figure 11, a). Similarly, in nearshore sites cladocerans registered the highest composition of biomass at 43.59% and rotifers the least at 1.54% (Figure 11, b). Cladocerans contributed the most to open water density composition at 33.40% and harpacticoida the least at 0.01% (Figure 11, c). In nearshore sites rotifers dominated density composition at 30.09% with harpacticoids and calanoids at only 0.43% and 9.42%, respectively (Figure 11, d).

Figure 11. Percent composition of zooplankton biomass in a) open water b) nearshore sites; zooplankton density composition in c) open water d) nearshore sites during the entire study period. Species are grouped into their respective order.



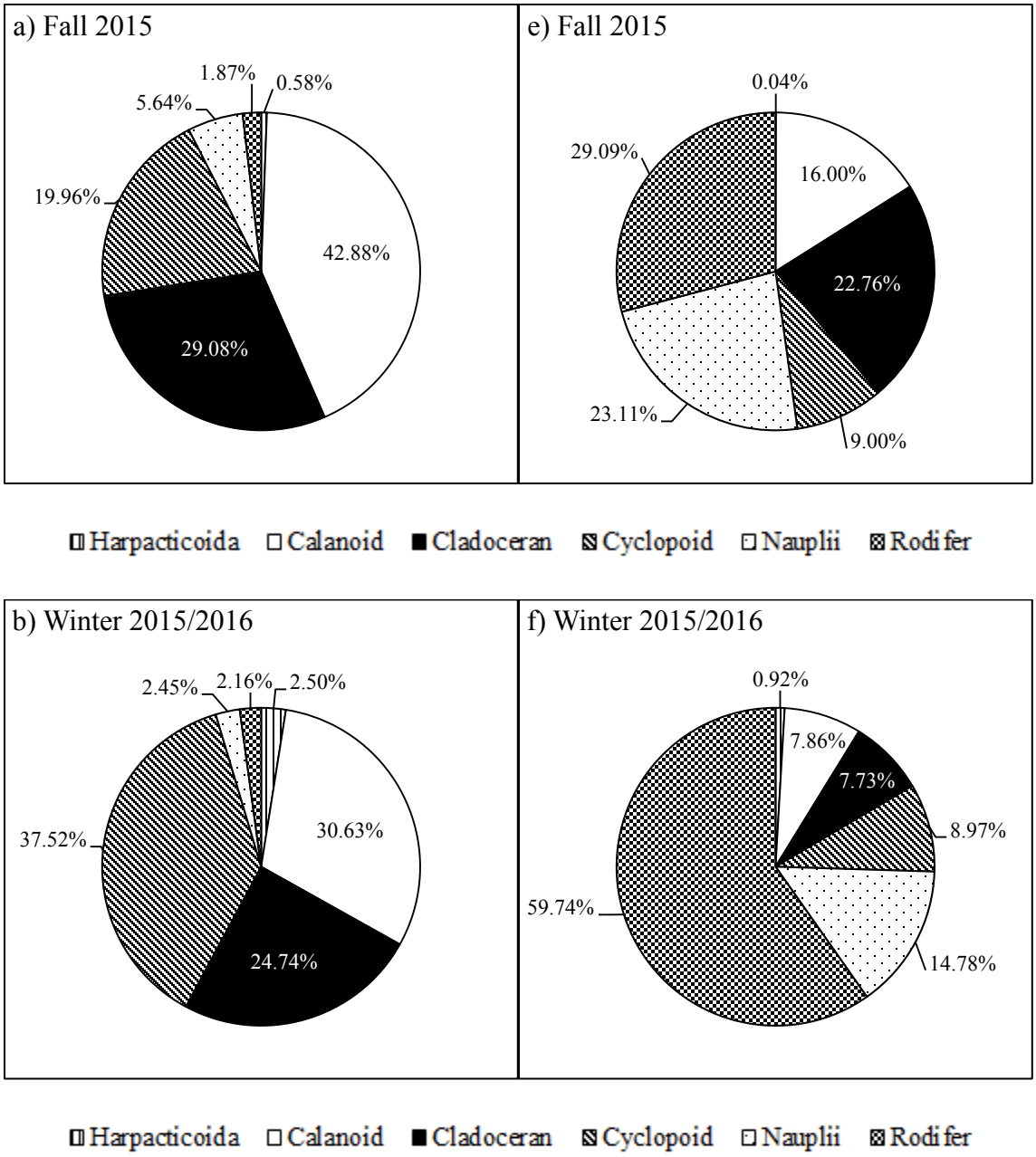

b)
2.16%
1.54%
4.08%
23.47%
25.16%
43.59%
Harpacticoida
Calanoid
Cladoceran
Cyclopoid
Nauplii
Rodifer

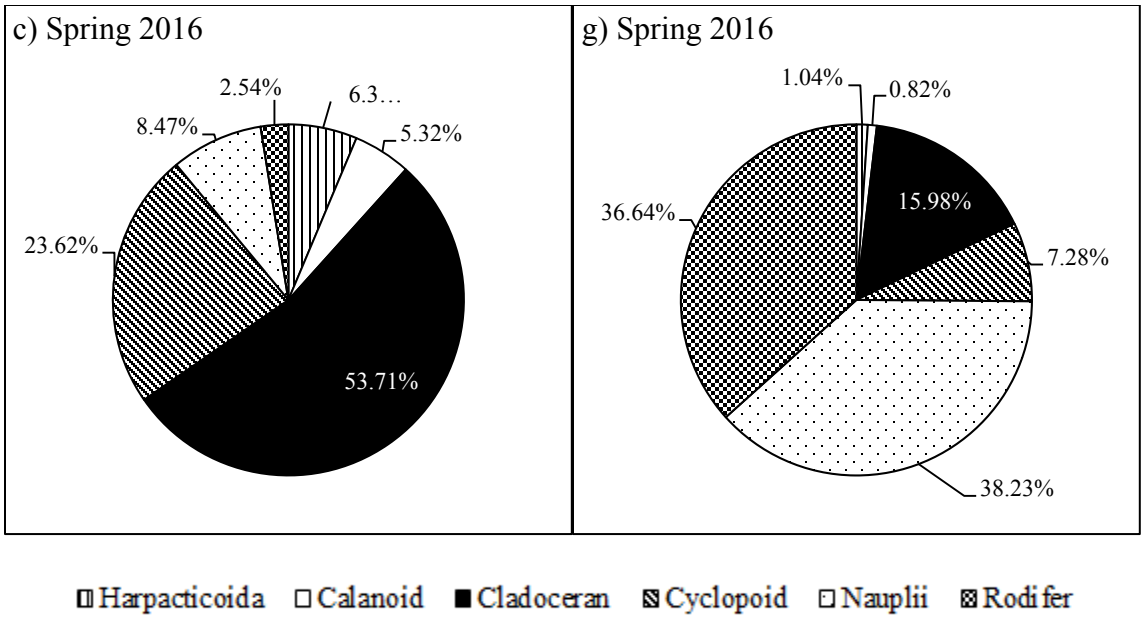

c)
0.01%
18.55%
14.73%
24.53%
33.40%
8.79%
Harpacticoida
Calanoid
Cladoceran
Cyclopoid
Nauplii
Rodifer

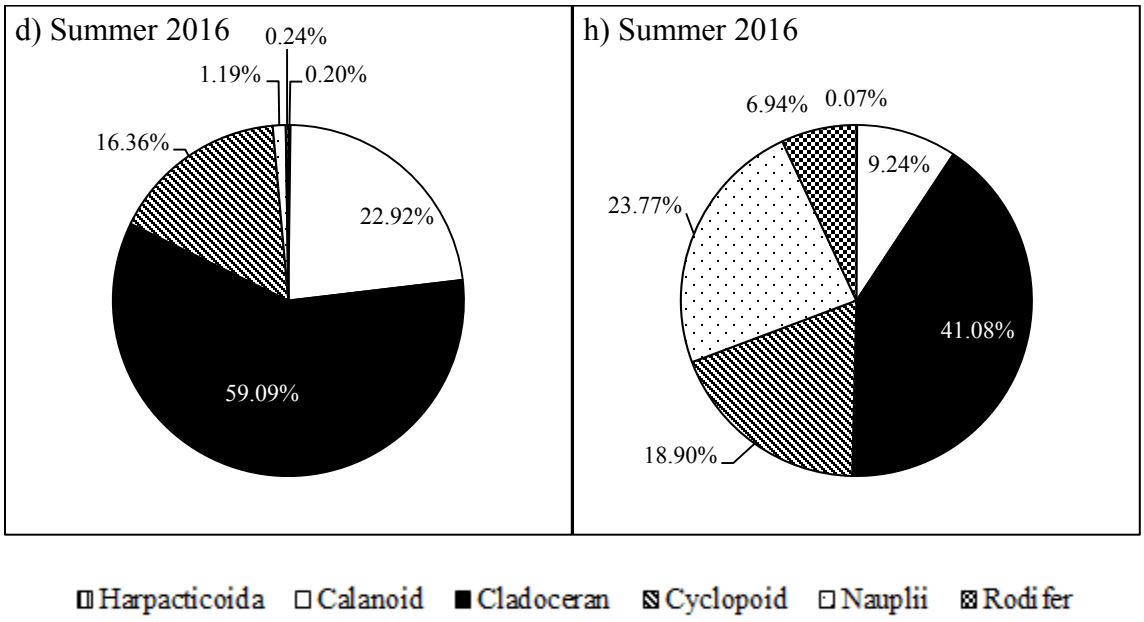


The seasonal differences in zooplankton biomass and density were determined at all nearshore sampling locations (Figure 12). In fall 2015/2016 the biomass of calanoids (42.88%) was the greatest while rotifers was the lowest (1.58%) (Figure 12, a). On the contrary, with respect to the zooplankton density, rotifers contributed the maximum (29.09%) and cyclopoids the minimum (9.00%) (Figure 12, e). Cyclopoids dominated biomass composition (37.52%) (Figure 12, b) in winter 2015/2016 while rotifers dominated density composition (59.74%) (Figure 12, f). In spring 2016 cladocerans comprised 53.71% of biomass composition and rotifers the lowest at 2.54% (Figure 12, c). Nauplii copepods had the greatest density composition (38.23%) and calanoid the lowest (0.82%) (Figure 12, g). Similarly, during the summer 2016 sampling period cladocerans had the highest biomass composition (59.09%) and rotifers the lowest (0.24%) (Figure 12, d). Cladocerans also dominated density composition at 41.08% with rotifers again being the lowest 6.94% (Figure 12, h).

Figure 12. Percent composition of nearshore zooplankton biomass in a) fall 2015 b) winter 2015/2016 c) spring 2016 d) summer 2016; nearshore zooplankton density composition in e) fall 2015 f) winter 2015/2016 g) spring 2016 h) summer 2016. Species are grouped into their respective order.







3.3.2.7 Influence of Environmental Variables on Species Composition: Multiple Regression Model

Multiple regression models were developed to determine the relationship between environmental variables and zooplankton composition (density, diversity, richness and biomass). An initial evaluation of predictor variables showed that CHL *a* and nitrate were not normally distributed and therefore they were square root and $\log_{10}$ transformed, respectively. Zooplankton

density and biomass had to be $\log_{10}$ transformed to meet the assumption of normality. There was evidence of covariation between predictor variables specifically phytoplankton diversity and richness; phytoplankton diversity and density. Phytoplankton density and richness were removed from the model, which resulted in all variables having variance inflation factors less than 2 as recommended by Zuur et al. (2010). The final model was significant ($F_{9,70} = 41.70$, $p < 0.001$) with TEMP, DO, COND, pH, CHL *a*, TSS, nitrate, TP, and phytoplankton diversity explaining 84.3% of variance in zooplankton density. Further analysis using partial regression plots determined that density increased significantly with CHL *a*, TSS and phytoplankton diversity (in order of increasing significance). Only 16.9% of variance in zooplankton diversity could be attributed to environmental variables and the overall model was not significant ($F_{9,70} = 1.59$, $p = 0.137$) although diversity decreased significantly with increasing DO (Table 9). Environmental variables explained 29.6% of variation in zooplankton species richness ($F_{9,70} = 3.26$, $p = 0.002$). Furthermore, richness decreased significantly with DO and increased significantly with CHL *a*. Lastly, the final model for biomass explained 55.4% of the variation ($F_{9,62} = 8.55$, $p < 0.001$) and similar to richness biomass significantly increased with CHL *a* and decreased with DO.

Table 9. Adjusted r –squared values for multiple regression analysis results between zooplankton composition and environmental variables. Significance indicated by p<0.05*, p<0.01** and p<0.001***. a and b signify log and square root transformation, respectively.

Environmental Variables	Zooplankton Density	Zooplankton Diversity	Zooplankton Richness	Zooplankton Biomass
TEMP	-0.012	-0.004	0.010	0.004
DO	0.006	0.041*	0.063*	0.334***
COND	-0.006	0.002	-0.004	-0.007
pH	0.022	0.001	-0.003	0.026
TP	0.016	0.005	-0.006	-0.014
Nitrate[a]	-0.011	-0.012	-0.010	0.009
TSS	0.272***	-0.010	0.012	0.001
CHL *a*[b]	0.178***	-0.005	0.081**	0.070*

Phyto density	-	-	-	-
Phyto richness	-	-	-	-
Phyto diversity	0.57***	-0.012	-0.011	-0.007

3.4 Discussion

3.4.1 Variation in Environmental Variables

Water temperature in nearshore and open water sampling locations were observed to follow natural seasonal changes with warmest temperatures in summer and coldest in winter explaining sampling period differences. There were only slight differences in temperature between sampling locations. DO also experienced seasonal variation, which was to be expected since DO concentrations are impacted by water temperature. The lowest DO concentrations were in August when water temperature was the highest and highest in December and March when water temperature was lower. DO differed between sampling locations which was expected due to variation in disturbances such as nutrient enrichment, recreational boating and shoreline alteration. MB and TR, both highly disturbed sites (refer to Table in Chp 2), had significantly lower DO compared to less disturbed sites. Almost all readings were above the recommended 7 mg/L set by the LSPP for the protection of natural coldwater fish recruitment. COND is influenced by dissolved salts and inorganic materials. Therefore, it is expected that readings would differ spatially due to natural variation in bottom sediment and geology and temporally due to changes in runoff occurrence and volume. This was found to be true as COND readings differed between sampling locations and periods. Again, more disturbed sites (LC and MB) had higher COND may be due to runoff containing suspended dissolved solids and inorganics. Interestingly, TR which is considered a highly disturbed site had significantly lower COND compared to all other sites. This could possibly be due to the nature of dissolved

particles (organic compounds such as benzene and toluene) as it is the main entrance to Lake Simcoe from TSW (EPA, 2012).

pH is impacted by natural sources such as precipitation, photosynthesis, respiration and decomposition and anthropogenic sources for example wastewater or mining discharge (Perlman, 2016a). Photosynthesis and respiration cause pH to fluctuate on a diel cycle by altering the amount of carbon dioxide in the water. Variation in pH at sampling locations was significant due to the lower pH values at MB. Sampling period also caused a significant difference in pH with lower amounts in spring and higher in summer. These differences were expected since natural and anthropogenic processes vary throughout the year. Most readings fell between 6.5 – 8.5 within the recommended guideline by MOECC (2016). Nutrient concentrations exhibited the hypothesized significant spatial and temporal variations. Nitrate concentrations were the greatest at LC, MB and TR (highly disturbed sites) and lowest at SR, DR and AB (least disturbed sites – SR moderately disturbed). Similarly, TP concentrations were highest at MB and TR and lowest at DR and SR. It is of interest to note that TP and nitrate experienced opposite seasonal trends with highest nitrate concentrations in March and April (spring) and lowest in July (summer) and November (fall); highest TP was in November (fall) while lowest in March (spring) and June (summer). It is expected that both concentrations would be lowest in summer because the area experienced an unusually hot summer with little precipitation. Nitrate concentrations could have been higher in spring because this is the time of year when farmers start applying fertilizers to crops and because nitrate is highly soluble any amounts that are not absorbed by plant roots is carried away by runoff or leaches into the soil (Liu et al., 2014). Fertilizer application would typically be greater in spring compared to fall when farmers are preparing their fields for the winter. It is possible that runoff containing leaf

debris or leaves entering waterways from nearby trees contributed to higher TP in the fall as autumn leaf litter has been found to increase nutrients in lakes and streams (Cowen & Lee, 1973; Kalinosky et al., 2014). All nitrate readings fell below the CCME recommended 13 mg/L for the protection of aquatic life with the highest reading being 0.43 mg/L at LC (CCME, 2003). To prevent the growth of nuisance algae the MOECC recommends TP concentrations of less than 20 µg/L (2016). Mean TP exceeded this limit for the months of October and November in nearshore sites and August for open water sites with mean concentrations of 23.6, 27.1 and 22.3 µg/L, respectively.

TSS varied significantly between sampling locations with highly disturbed sites MB, TR and moderately disturbed PO having the highest mean TSS. This was expected since all of these sites were noted to have very poor water clarity, in most cases 1 m depth was not visible.

Phytoplankton are the primary producers in aquatic ecosystems and as such they have been observed to change temporally and spatially influenced profoundly by nutrient concentrations and sunlight. The results from this study support this with phytoplankton density differing significantly between sampling periods and locations. PO, MB and AB had the highest densities and SR and AA had the lowest. High levels of CHL *a* are usually indicative of nutrient rich conditions with phytoplankton biomass increasing with eutrophication (Young & Jarjanazi, 2015; Nicholls & Dillon, 1978). The most disturbed sites, MB and LC, had the highest CHL *a* concentrations and less disturbed sites AA, AB and SR had the lowest concentrations. Surprisingly, diversity did not vary among sampling locations although MB and TR had the highest species diversity. Species richness was highest at MB followed by TR than PO and lowest at SR and DR. This is the opposite of what was expected because highly disturbed sites were thought to have the highest density and lowest species diversity and richness. Perhaps this

was a result of these sites having higher TP concentrations and therefore being able to support a greater diversity of species. These findings do support other studies with moderate enrichment resulting in increased phytoplankton biomass and diversity with representation from Bacillariophyceae (diatoms), Chlorophyta (green algae), Chrysophyceae, Dinophyta and Cynaobacteria groups throughout the growing season (Eloranta, 1986; Rosén, 1981; Sommer et al. 1986; Watson et al., 1997). The differences between sampling locations could possibly be driven more by natural variation in geomorphology and environmental variables and less so by anthropogenic influences.

3.4.2 Zooplankton Community Dynamics

Zooplankton composition along the TSW is an understudied topic. Data collected over the course of one year on the zooplankton population has yielded fascinating results. In total 44 species were observed in nearshore and 26 in open water sampling locations. Zooplankton composition exhibited significant temporal and spatial differences in nearshore sites. These results were expected because several studies have noted changes in zooplankton due to food concentrations and quality (phytoplankton composition) as well as temperature (Chang et al., 2014; McCauley & Murdoch, 1987; Welch & Jacoby, 2004) both of which were noted to vary with sampling period and location.

Zooplankton density and biomass were highest at the highly disturbed sites LC and MB and lowest at AA, AB and DR, moderately and least disturbed sites. This was expected since the highly disturbed sites had greater nutrient and CHL *a* (phytoplankton biomass) concentrations providing more grazing opportunities for zooplankton. Peak zooplankton biomass and density for open water sampling locations occurred at site DW1 where TP concentrations were consistently higher compared to DW2 and DW3 although differences were not significant. This

site is closer to Port of Orillia where it is likely greatly influenced by near shore activities. Furthermore, grazing by planktivorous fish in deeper sites may have led to lower zooplankton densities in these areas (SSEA, 2003). Density and biomass peaked in July for nearshore sites and in August and June, respectively for open water sites which corroborated results from other studies from other lakes (Chang et al., 2014; Pothovern & Fahnenstiel, 2015; Pothoven & Höök, 2014). Biomass was expected to peak at this time due to the presence of larger cladocerans species such as Diaptomidae sp. and *Bosmina longirostris*. Other species contributing to increased biomass include *Leptodiaptomus* sp., *Simocephalus serrulatus*, *Acroperus harpae* and *Diacyclops thomasi*. Species richness was greatest at LC, SR and MB and lowest at AA and AB. In contrast, species diversity was highest at SR and DR and lowest at AB and LC. The most disturbed sampling locations had consistently lower diversity. These results support the hypothesis that highly disturbed sampling locations would have lower diversity compared to less disturbed locations. Nutrient and food concentrations were optimal for supporting a vast number of species in highly disturbed sites although the abundance of species was not evenly distributed, this was indicated by the lower diversity. This could potentially be the result of only a few select species flourishing under the conditions at higher impacted sites even though species richness is greater. Diversity and richness did not vary significantly among open water sampling locations. Diversity displayed temporal trends with greatest values in August for both nearshore and open water sites. Similarly, richness was greatest in September for nearshore sites and August for open water sites.

Shifts in zooplankton taxonomic groups were observed as a direct result of seasonal changes. Zooplankton biomass consisted mainly of calanoids in the fall and cyclopoids in the winter than transitioned to a population dominated by cladocerans in the spring and summer.

Zooplankton density in contrast, had highest number of rotifers in the fall and winter months than calanoid nauplii in the spring with cladocerans taking over in the summer. Summer peaks of cladoceran are synchronous with other studies due to the increase in large bodied species (Chang et al., 2014; Pothovern & Fahnenstiel, 2015; Pothoven & Höök, 2014). Rotifers are relatively small (some can be < 50μm length) in comparison to cladocerans and copepods thus it makes sense that that they did not dominate biomass composition. This complemented research conducted in the Great Lakes on rotifer composition where they found that on average rotifers contributed 2 – 17% of total zooplankton biomass (Barberio & Warren, 2011; Stewart et al., 2010). It was interesting to note that rotifer species dominated total zooplankton density in fall and winter months. In a study conducted by Lavrentyev et al. (2014) they found that rotifers peaked in the shallow inner bay of Saginaw Bay, Lake Huron in fall increasing with chlorophyll *a*. Although, both phytoplankton and chlorophyll *a* were higher in summer compared to fall perhaps poor food quality influenced rotifer abundance. The presence of *Microcystis* in fall samples may have been beneficial to rotifers compared to crustacean zooplankton as the latter tend to not utilize this cyanobacteria as a food source (Branco et al., 2002).

In previous studies it has been established that environmental factors such as water chemistry, shoreline disturbance, and watershed land use impact zooplankton communities (Pinel-Alloul et al., 1990; Stemberger & Lazorchak, 1994; Patoine et al., 2000) although phytoplankton species composition determines the degree of species shift (Chang et al., 2014). A multiple regression analysis was undertaken to determine the relationship between environmental variables (water chemistry, nutrients and phytoplankton composition) and zooplankton composition (density, diversity, richness and biomass). It was determined that 84.3% of the variance in zooplankton density could be explained by environmental variables and

more specifically density was found to be significantly positively correlated with CHL a, TSS and phytoplankton diversity. Only 16.9% of zooplankton diversity variance could be attributed to environmental variables with diversity significantly decreasing with increasing DO. This low explanation of variance could be a result of the environmental variables used in the model. For instance variability in dissolved organic carbon is known to impact zooplankton richness and diversity (Shurin et al., 2010). Zooplankton richness variance was 29.6% explained by environmental variables and richness was positively correlated CHL *a* and negatively correlated with DO. Variance in biomass was 55.4% attributed to environmental variables increasing significantly with CHL *a* and decreasing significantly with DO. These results were to be expected because food availability measured by phytoplankton composition (density, richness, diversity and biomass (CHL *a*)) has been known to significantly influence zooplankton abundance (Chang et al., 2014; Patalas, 1972).

It was interesting to discover that increases in DO caused decreases in zooplankton richness, diversity and biomass. This is the opposite of what other studies have reported. Julies and Kaholongo (2013) found that zooplankton communities along the coast of Namibian were weakly positively correlated with DO and that DO indirectly influenced several biological and environmental factors that affect zooplankton composition. Another study conducted in a tropical lake discovered that certain zooplankton taxa were positively affected by DO (*Ceriodaphnia sp.*, *Mesocyclops sp.*, *Diaphanosoma sp.*, and rotifers) while others were negatively correlated (*Daphnia* spp. and *Thermocyclops sp.*) (Fetahi et al., 2011). Lastly, predation of calanoid copepods by large *Mnemiopsis leidyi* (warty comb jelly) appeared to have increased in low DO environments (Decker et al., 2004). Perhaps the negative correlation between DO and zooplankton richness, biomass and diversity is the result of a combination of

factors with DO indirectly influencing biological or environmental variables such as predation. DO concentrations were high enough to support coldwater fish species such as lake trout and lake whitefish which are known to feed on larger crustacean zooplankton (Young & Jarjanazi, 2015). Creel surveys conducted in Lake Simcoe have found that the latter species along with yellow perch, pumpkinseed and small and large mouth bass are commonly catch by anglers (Young & Jarjanazi, 2015). Therefore predation by these species could have contributed indirectly to the negative impact of DO on zooplankton richness, diversity and biomass in nearshore sites.

3.4.3 Differences between Nearshore and Open Water Sampling Locations

Nearshore and open water zooplankton communities differ as a result of varying abiotic and biotic factors within each habitat. Nearshore regions usually consist of various emergent and submergent aquatic plants, which can provide substrate for algae growth and habitat for zooplankton and fish. In contrast, open water areas provide less habitat and protection for zooplankton from predators. Typically nearshore TP, CHL *a*, and zooplankton density and biomass are distinctly higher in nearshore areas compared to the open water (Johannsson et al., 1991; Patalas, 1969). This has not been the case for the Great Lakes (Hall et al., 2003) and some smaller lakes (Mellina, 1995) colonized by invasive *Dreissena polymorpha* (zebra mussel). Differences in species assemblages have been found between nearshore and open water sites even if biomass and density did not differ significantly (Pothovern & Fahnenstiel, 2015; Nicholls & Tudorancea, 2001). This was substantiated by the findings of this study with zooplankton density composition being dominated by cladoceran species in open water locations and rotifer species in nearshore locations. Although, biomass composition was predominately cladocerans in both environments.

Of the 11 environmental variables monitored only 5 were found to vary between habitat type. Differences were attributed to lower COND at TR, higher CHL *a* at MB and higher TSS at TR, MB and PO compared to open water locations. Phytoplankton density and richness was significantly greater at PO compared to open water sites. Zooplankton density and biomass were both found to significantly vary with greater results in LC in comparison to open water sites. These results were expected given what other studies have reported with nearshore areas having greater CHL *a*, zooplankton density and biomass (Johannsson et al., 1991; Patalas, 1969). If only nearshore Lake Couchiching sites are compared with open water sites even less difference is present which is reasonable to anticipate since Lake Couchiching is a relatively small and shallow lake with no thermal stratification so one would not expect too much variance in environmental variables or biological communities. Evidently, the open water sampling locations in this study appeared to be impacted more temporally than seasonally where the nearshore sampling sites are heavily influenced by both.

3.4.4 Lake Simcoe and Lake Couchiching Differences

The eight nearshore sampling locations in this study were chosen based on their proximity to the TSW and accessibility. This resulted in four sampling locations in Lake Simcoe (TR, LC, MB and AA) and four in Lake Couchiching (AB, PO, SR and DR). It was interesting to find that of the eleven environmental variables sampled nine were found to significantly vary between sampling locations. Upon further scrutiny at least 50% of these differences were caused by inter lake variability. Between lake differences in environmental variables (DO, pH and COND) were due to lower DO at TR and MB, lower pH at MB and lower COND at MB. Similarly, nutrient differences between lakes was attributed to higher nitrate concentrations at LC, higher TP at MB, and greater TSS at TR. Phytoplankton composition varied between lakes

as a result of greater biomass at MB and LC and higher density at PO. Inter lake variability accounted for a minimum of 42% of differences between zooplankton composition. Differences between lakes was caused by higher densities at LC and MB as well higher richness and biomass at LC. These results support the initial evaluation of sampling locations based on anthropogenic disturbances and subsequent scale ranking (see Chapter 2 Table 1). Higher nutrient concentrations along with greater primary and secondary productivity were thought to be found in more disturbed sites (TR, LC and MB) with lower DO, pH and higher COND compared to less disturbed sites (AA, AB, SR and DR). This research also supports other studies stating that Lake Simcoe is a mesotrophic lake (Palmer et al., 2011; Young & Jarjanazi, 2015) and that Lake Couchiching is an oligotrophic lake (SSEA, 2005).

3.5 Conclusion

The main objective of this chapter was to provide insight into zooplankton community dynamics in the TSW. The hypothesis that zooplankton dynamics would vary due to spatial, temporal and anthropogenic disturbance differences was supported by this research findings. Nearshore regions of the TSW are experiencing temporal and spatial variance in biological and environmental variables attributed to natural and anthropogenic disturbances. TEMP, DO, COND, pH, nitrate, TP and phytoplankton composition (biomass, density, richness and diversity) all exhibited temporal differences indicative of seasonal dynamics as a result of varying water temperatures, precipitation, run off and other factors which influence temperate lakes. Furthermore, these variables were found to vary significantly spatially with highly disturbed sites showing signs of poor water quality indicated by low DO, higher nutrient and CHL *a* concentrations compared to less impacted sites. The zooplankton community experienced changes temporally and spatially in nearshore sites. Zooplankton biomass and density were

greatest at highly disturbed sites supported by greater nutrient and CHL *a* (phytoplankton biomass) concentrations compared to less disturbed sites. Biomass peaked in early summer with the appearance of large bodied cladocerans and copepods. Environmental variables examined in this study explained the greatest variance in zooplankton density followed by biomass, richness and diversity. Zooplankton density, richness and biomass were found to be positively correlated with CHL *a* while richness, diversity and biomass were negatively correlated with DO. Nearshore sampling locations exhibited greater CHL *a*, TSS, phytoplankton density and richness but lower zooplankton density and biomass compared to open water sites. Open water sampling locations appeared to be impacted more temporally than spatially where nearshore sites were heavily influenced by both. Lake Simcoe nearshore sites experienced greater nutrient concentrations, TSS, phytoplankton composition (biomass, diversity and richness), zooplankton composition (density, biomass, and richness) and lower DO in comparison to Lake Couchiching sites. This study provides useful information on the zooplankton community along the TSW and determined that this community responds to fluctuations in temporal, spatial and environmental variables within this area.

Chapter 4 Zooplankton Community Composition as Indicators of Water Quality in Nearshore Regions of the Trent Seven Waterway

4.1 Introduction

Biological indicators of water quality are used to monitor environmental changes, inform decision makers about the changes early and, provide early detection of potential ecological shifts (Siddig et al., 2016). Zooplankton and phytoplankton species assemblage can act as useful biological indicators of water quality because they have short life cycles, and respond quickly to environmental changes. Therefore standing crop and species composition most likely indicate the water quality where they were sampled from (APHA, 2005). It is recommended that indicator species are interpreted with simultaneously collected physiochemical and biological variables to limit error in deciphering water quality (APHA, 2005). The zooplankton community provides a pivotal link between understanding top down regulators (fish) and bottom up dynamics (nutrients and phytoplankton) of water quality indicators (Jeppensen et al., 2011). Monitoring just fish or phytoplankton communities would not be sufficient in obtaining the same information derived from zooplankton on trophic interactions; fish monitoring would have to include young of the year and invertebrate predators which is very costly compared to methodologies involving zooplankton (Jeppensen et al., 2011).

Chapter 1 briefly outlined zooplankton species used as water quality indicators. Their use as indicators in different regions will be discussed in more detail. In Brazil, studies have reported using rotifer and cladocerans populations to monitor the presence of the cyanobacteria *Microcystis aeruginosa* in the water column (Branco et al., 2002). *Microcystis aeruginosa* inhibits cladoceran population growth, filtering rates and body size providing favourable conditions for rotifer species such as *Euchlanis dilatata* and *Brachionus calyciflorus*. These

species of rotifers were also found to inhabit lakes with greater temperature, chlorophyll *a*, bacterial content and lower water transparency. Interestingly, smaller cladocerans were noted to feed on *Microcystis aeruginosa* in the Funil Reservoir (R.J., Brazil). The decline in cladoceran biomass and zooplankton production during summer blooms of *Microcystis aeruginosa* corroborated findings in lakes from Europe, North America and South Africa (Branco et al., 2002).

Restoration efforts in eight ponds in Spain resulted in changes to the zooplankton community. Water quality in the ponds was observed to improve with increased dissolved oxygen and decreased chlorophyll *a* (Anton-Pardo et al., 2013). This was further supported by the rapid recovery of zooplankton richness and diversity with changes found after only a year. The community transitioned from the one dominated by copepods (mostly nauplii) before restoration to another with greater richness, co-dominated by nauplii and rotifers and significantly greater cladoceran abundance (Anton-Pardo et al., 2013). The higher abundance of submerged macrophytes in restored ponds was believed to be the main contributor of recovering rotifer and cladoceran species because they increase habitat heterogeneity and dissolved oxygen, provide shelter from predators and act as a food source (Duggan, 2001; Kuczynska-Kippen, 2001).

In 2011, several scientists argued the importance of keeping zooplankton in the ecological quality assessment of lakes as part of the European Water Framework Directive (WFD) (Jeppensen et al., 2011). They argued based on zooplanktons position in the food web that they are crucial indicators of top down and bottom up controls in ecosystems and that it would be costly to get the same information from only fish monitoring. Furthermore, they present findings from several regions explaining their importance as indicators. For example,

zooplankton communities in Danish and Estonian lakes experience changes to richness and structure due to exposure to eutrophic gradients (Jeppensen et al., 2000). Species richness decreased while biomass increased with increasing total phosphorous. Changes in zooplankton dynamics can influence phytoplankton communities with mean zoo:phyto ratios decreasing in summer attributed to higher total phosphorous and increasing water clarity (Jeppensen et al., 2000). In 81 shallow European lakes representing all climatic zones the zooplankton biomass and total phosphorous possess the same relationship and therefore are considered to be indicators of climate change (Gyllström et al., 2011). Fish biomass and fish:zoo ratios increased from cold to warm lakes while zoo:chlorophyll *a* ratios decreased. This translates to increased predation of zooplankton in warmer lakes thereby reducing grazing pressure on phytoplankton. Ultimately, bottom up controls (nutrients) were found to act as the most prominent predictor of zooplankton biomass but climate influenced top down controls of standing zooplankton biomass and composition (Gyllström et al., 2011). In another study by Jeppensen et al., (2005) examined impacts on zooplankton community in lakes recovering from eutrophication. They observed that small cladoceran biomass decreased in summer and fall and the proportion of *Daphnia* to cladoceran biomass increased (Jeppensen et al., 2005) and therefore it was recommended that zooplankton be added as one of the biological quality elements part of the European WFD to properly monitor ecological statuses of lakes in Europe (Jeppensen et al., 2011).

Pinto-Coehlo et al. (2005) studied the influence of trophic status on crustacean zooplankton communities in lakes and reservoirs in temperate and sub-trophic regions found in Ontario, Alberta, Quebec, Florida and Brazil. They discovered that species richness was greatest in temperate oligotrophic lakes but eutrophic conditions supported greater density. Cladocerans and cyclopoids favoured eutrophic environments while calanoids preferred temperate

oligotrophic lakes. Total phosphorous was a better predictor of biomass compared to chlorophyll *a* in all these study locations (Pinto-Coehlo et al., 2005).

Zooplankton community structure can be an indicator of land use, environmental variables and aquatic vegetation as reported by Dodson et al. (2005) in a study conducted in Wisconsin, US. Reference sites were characterized by greater zooplankton richness and aquatic vegetation. In contrast, agricultural sites maintaining a large buffer of vegetation (>30m) had significantly more zooplankton species sites than sparely vegetated sites. A non-metric multidimensional scaling ordination indicated that there was a single community among differing land use types with only slight variation caused by aquatic vegetation and hydrologic source (Dodson et al., 2005).

Another study on northeastern shallow lakes in North America investigated the use of paleolimnological data to assess cladoceran community responses to changing land use and total phosphorous concentrations. Albert el al. (2010) determined that total phosphorous was a significant predictor of subfossil cladoceran populations. Chydorid diversity was significantly negatively correlated with total phosphorous as well as proportion of disturbed land. Changes in land use especially those that impact phosphorous loading can have deleterious impacts on cladocerans resulting in reduced biodiversity.

There have been several studies completed in Canada on the use of zooplankton as indicators. One completed by Gélinas and Pinel-Alloul (2008) examined the impact of residential and land cover disturbances in watersheds on zooplankton communities in Canadian shield lakes. Research conducted in other regions found that zooplankton communities were sensitive to large-scale disturbances from agriculture (Stemberger & Lazorchak, 1994; Dodson et al., 2005/2007), forest and logging, and wildfire (Patoine et al., 2000/2002). Total phosphorous

was the primary factor connecting residential disturbance to increased biomass of small bodied cladocerans (*Bosmina* sp., *Ceriodaphnia* sp., and *Diaphansoma* sp.) and *Daphnia* spp. (*Daphnia mendotae*, *Daphnia dubia* and *Daphnia ambigua*) (Gélinas & Pinel-Alloul, 2008). Additionally, residential disturbances and environmental factors explained 42% and 57%, respectively, differences in crustacean zooplankton communities among lakes studied. Evidently, anthropogenic interferences play a pivotal role in influencing zooplankton communities.

Due to the vast amount of mining in Sudbury over the last century this area has been significant in investigating the impact of acidification and metal concentrations on aquatic biota. MacIssac et al. (1986) studied the recovery of rotifer populations in Swan Lake an acidic metal contaminated lake near Sudbury, ON. New legislation to decrease emissions for smelting operations greatly influenced lakes across the region. Swan Lake showed signs of recovery with increasing pH and decreasing metal concentrations over the course of 8 years. The decrease in acidophile *Keratella taurocephla* and significant increase in densities of other rotifer species (*Polyartha* spp., *Chromogaster ovalis*, and *Trichocerca similis)* further cemented the increase in water quality (MacIssac et al., 1986). Keller and Yan (1991) investigated crustacean zooplankton populations in eight acidic and metal contaminated lakes in the same area from 1973-1986. These lakes also exhibited signs of improvement with decreasing acidity and metal concentrations. Overall, a considerable increase in species richness was found in lakes with favourable pH and lower metal concentrations due to the arrival of new species and increase in existing species. Average annual richness positively correlated with pH and negatively correlated with aluminium, copper and nickel concentrations (Keller & Yan, 1991). The rate and extent to which species recovered differed between lakes possibly as a result of initial degree of

impact, extent of water quality improvement, available recolonization time and dispersal ability of new species (Keller & Yan, 1991).

Lake Winnipeg, the largest naturally eutrophic lake in the Canadian prairies, proved to be an excellent region to monitor changes in crustacean zooplankton as a result of changing environmental parameters (Hann et al., 2017). Cultural eutrophication in the lake has intensified and this has been mainly attributed to climate induced flooding, surface water warming and increasing agricultural pressures (Schindler et al., 2012). Changes in climate over the last two decades has resulted in amplified nutrient loading causing greater abundance of phytoplankton (Kling et al., 2011) and increased the occurrence, duration and magnitude of cyanobacteria blooms (McCullough et al., 2012). Ultimately, this has caused an elevation in baseline productivity substantiated by greater lake-wide abundances of zooplankton. Shifts in the zooplankton composition signify environmental changes. Abundance of *Leptodiaptomus minutus* has declined over the last decade in the North basin with increasing dominance by *Leptodiaptomus ashlandi* synchronous of water bodies dominated by cyanobacteria (Kling et al., 2011). Additionally, the Red Flood of 1997 delivered high concentrations of sediments and organic debris and in the following year the community was dominated by cyclopoids which are more readily available to utilize bacteria and organic particles as food (Hann et al., 2017).

Mimouni et al (2015) examined biodiversity of zooplankton in 18 urban waterbodies across Montreal. These lakes were typically characterized by rotifers and cladocerans most likely caused by differences in life history traits. Rotifer and cladocerans reproduce by asexual parthenogenesis (can perform sexual reproduction as well) with rapid generation times therefore they can quickly colonize new habitats compared to copepods that only reproduce sexually with small number of male species (Balcer et al., 1984; Welch & Jacoby, 2004). Medium to large

sized cladocerans (*Ceriodaphnia* sp., *Daphnia* spp., etc.) increased as a result of winter draining, low algal biomass, small waterbody size and absence of fish (not always). Small rotifers such as *Keratella* sp., *Polyarthra spp.*p., *Euchlanis* spp., and *Plationus patulus* increased in regions with greater algal biomass (particularly green and brown algae) and presence of fish. Furthermore, shallow communities were depicted by *Lecane* sp. and deeper areas by *Bosminidae* spp. and *Diaphanosoma* sp.

Several studies thus indicated zooplankton can be used as indicators of natural and anthropogenic environmental change across the world. This chapter concentrates on the second objective of determining the effectiveness of zooplankton community dynamics as water quality indicators in the TSW. The overall question being *Are there certain species of zooplankton that could be used as biological indicators of water quality in this area*?

Certain species of zooplankton have been found to be good indicators of water quality in freshwater ecosystems. *Limnocalanus macrurus* and *Senecella calanoides* are indicators of oligotrophic conditions therefore are mainly found in high dissolved oxygen, colder waters (Gannon & Stemberger, 1978). Paleolimnological studies have found shifts from the oligotrophic *Bosmina longispina* to more eutrophic species *Bosmina longirostris* indicative of eutrophic transitions in lakes. Changes in proportions of crustacean groups can also be useful indicators. For instance, cladocerans and cyclopoid copepods are typically more plentiful in eutrophic waters compared to calanoid copepods (Gannon & Stemberger, 1978). It is expected that there will be several species or community dynamics that will be useful indicators in the TSW with nutrient rich species being found more abundant in highly disturbed sites and less sensitive species in less disturbed sites.

4.2 Methods

4.2.1 Study Location

A detailed general methodology is provided in Chapter 2. In brief, this study was conducted at eleven sampling sites located in northern Lake Simcoe and throughout Lake Couchiching (Figure 1). The sites are as follows: Talbot River (TR), Gamebridge, ON (44°28'21.0216, 079°10'08.6772"); Lagoon City (LC), Brechin, ON (44°32'57.6960", 079°13'05.2860"); McPhee Bay (MB), Orillia, ON (44°35'01.7160", 079°18'35.8128"); Atherley Narrows A (AA) (44°36'02.1816", 079°22'16.5216") and Atherley Narrows B (AB) (44°36'21.7440", 079°22'12.0216"), Orillia, ON; Port of Orillia (PO), Orillia, ON (44°36'44.0280", 079°24'43.3260"); Severn River (SR), Washago, ON (44°44'54.2364", 079°20'34.3191"); Dock rd (DR), Washago, ON (44°42'56.7684", 079°20'30.7648"); and Deepwater 1 (DW1) (44°37'01.5024", 079°24'22.8609"), Deepwater 2 (DW2) (44°37'09.8652", 079°24'04.3453") and Deepwater 3 (DW3) (44°37'21.9864", 079°22'46.0962").

4.2.2 Zooplankton Analysis

Altogether 126 zooplankton samples (100 nearshore and 26 open water) over the one-year ice free sampling period. Zooplankton were counted and identified using several identification keys (Haney et al., 2013; Witty, 2004). The community was described by determining density, diversity, richness and biomass. Triplicate samples were collected at one station per sampling period to understand the repeatability of sampling episodes.

4.2.3 Environmental Variables

Environmental variables monitored in this study include DO, TEMP, COND, pH, phytoplankton and nutrients (CHL *a*, TSS, TP and nitrate). TEMP, DO, pH and COND were measured in-situ.

4.2.4 Statistics

Canonical ordination techniques were used to determine the relationship between zooplankton species presence and environmental variables. This analysis indicates whether or not zooplankton composition would be a good indicator of water quality in the TSW. Redundancy Analysis (RDA), a constrained linear canonical ordination technique (Van der Wollenberg, 1977), was performed in CANOCO 4.56 software. The model was run with only the environmental variables causing significant influence on species presence.

Multiple regression was also employed to assess how zooplankton richness and diversity correlated to environmental variables in varying levels of disturbed sites. For a more in depth description of the multiple regression refer chapter 2.

4.3 Results

4.3.1 All Sampling Locations

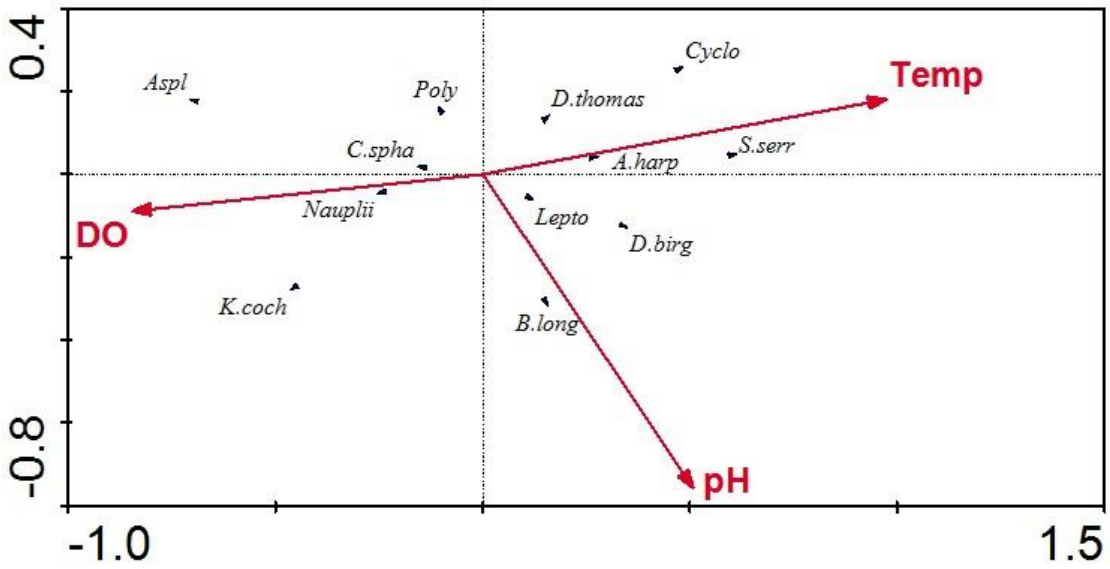
To gain a better understanding of the ability of the zooplankton community to act as a biological indicator, RDA was performed between environmental variables and zooplankton distribution across all sampling locations. Table 1 lists zooplankton species and their abbreviations used in RDA bi-plots. Eigenvalues were 0.173 and 0.033 for the first and second axis, respectively. Axis 1 explained 15.6% of the variance in species distribution while axis 2 explained a further 3.0% (a combined total of 18.6%). The environmental variables, which most significantly explained variation in zooplankton distribution, were TEMP, pH and DO in decreasing order (Figure 1, Table 2). RDA biplots are analyzed by comparing the direction of environmental arrows to the position of species. Environmental variables are positively correlated to species if the arrow for an environmental variable arrow points in a similar direction as the species and negatively if it points the opposite direction (Leps & Smilauer,

2003). *Acroperus harpae*, *Simocephalus serrulatus*, cyclopoid copepodid and *Diacyclops thomasi* were all positively correlated with TEMP and negatively correlated with DO. Nauplii and *Chydorus sphaericus* were positively correlated with DO and negatively correlated with TEMP. *Bosmina longirostris* was strongly correlated with pH while *Polyarthra spp*. and *Chydorus sphaericus* were negatively correlated.

Table 1. Zooplankton species name and abbreviation used in RDA bi-plots.

Taxa	Abbreviation	Taxa	Abbreviation
Acanthocyclops sp	Acan	*Harpacticoida*	Harp
Acanthocyclops vernalis	A.vern	*Holopedium gibberum*	H.gibb
Acroperus harpae	A.harp	*Kellicottia longispina*	K.long
Asplanchna sp	Aspl	*Keratella cochlearis*	K.coch
Bosmina longirostris	B.long	*Keratella tecta*	K.tecta
Brachionus calyciflorus	B.caly	*Lecane luna*	L.luna
Calanoid copepodid	Calan	*Leptodiaptomus sp*	Lepto
Ceriodaphnia sp	Cerio	*Leptodora kindtii*	L.kind
Chydorus sphaericus	C.spha	*Limnocalanus macrurus*	L.macr
Cyclopoid copepodid	Cyclo	*Microcyclops varicans*	M.varians
Daphnia mendotae	D.mend	*Monostyla lunaris*	M.luna
Daphnia retrocurva	D.retro	*Monostyla sp*	Mons
Diacyclops thomasi	D.thomas	Nauplii	Nauplii
Diaphanosoma birgei	D. birg	*Polyarthra spp.*	Poly
Epischura sp	Epis	*Polyphemus pediculus*	P.pedi
Eurycercus spp.	Eury	*Sida crystalline*	S.crys
Filinia sp	Filinia	*Simocephalus serrulatus*	S.serr

Figure 4. RDA bi-plot of zooplankton species distribution at all sites with environmental variables. Only variables having a significant effect on axis 1 and 2 are shown. The first two canonical axes explained 15.6% and 3.0% variance, respectively.



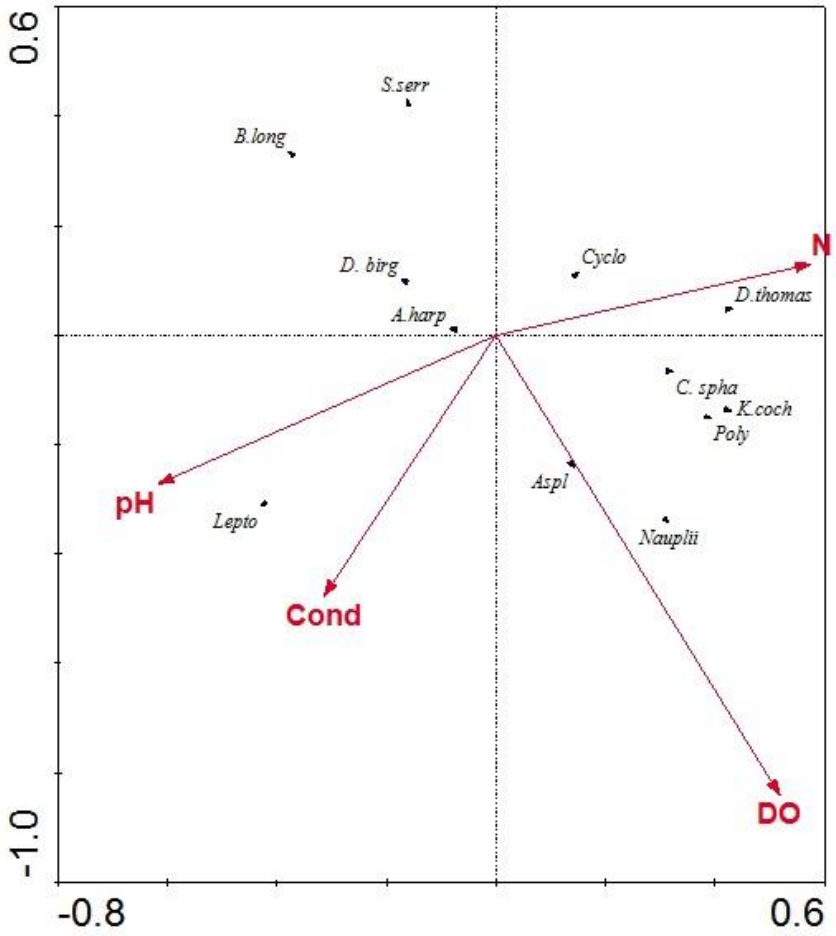
RDA axis 1 explained 12.5% and axis 2 a further 6.9% (19.4% total) of variation in zooplankton biomass at all sampling locations. Eigenvalues were 0.125 and 0.070 for axis 1 and 2, respectively. DO, pH, COND and N had a significantly explanatory effect on biomass variation (Figure 2, Table 2). Specifically, *Asplancha* sp. and nauplii biomass were found to positively correlate with DO while biomass of *Acroperus harpae*, *Bosmina longirostris*, *Diaphanosoma birgei* and *Simocephalus serrulatus* were negatively correlated. *Diacyclops thomasi*, cyclopoids, *Keratella cochlearis* and *Polyarthra spp*. biomass were positively correlated with N and *Leptodiaptomus* sp. biomass was negatively correlated. *Leptodiaptomus* sp. biomass positively correlated with COND and pH with cyclopoid copepodid biomass negatively correlated.

Table 2. Weighted correlation matrix values for corresponding environmental variables in RDA analysis of species biomass in all sites, least disturbed sites (DR), moderately disturbed sites (AA, AB, SR) and highly disturbed sites (TR, LC, MB, PO). Significance indicated by $p<0.05$* and $p<0.01$**.

Variable	SPEC AXIS 1	SPEC AXIS 2
All Sites		
TEMP	-0.28	0.43
DO	0.29	-0.49**
pH	-0.44**	-0.08
COND	-0.24**	-0.24
CHL *a*	-0.12	0.08
N	0.41*	0.01
TP	-0.10	-0.21
TSS	-0.06	0.18
Phyto density	-0.20	-0.03
Phyto richness	-0.19	0.07
Phyto diversity	-0.06	0.06
Highly Disturbed Sites		
TEMP	-0.56	-0.27
DO	0.60**	0.44
pH	-0.37*	0.25
COND	-0.10	0.46
CHL *a*	-0.24	0.18
N	0.43	-0.15
TP	-0.18	0.47*
TSS	-0.24	-0.10
Phyto density	-0.28	0.20
Phyto richness	-0.32	0.11
Phyto diversity	-0.11	0.07
Moderately Disturbed Sites		
TEMP	0.01	0.54
DO	0.19	-0.60*
pH	-0.14	0.40
COND	-0.10	0.01
CHL *a*	-0.34	-0.02
N	0.63**	-0.06
TP	-0.19	-0.33
TSS	-0.23	-0.01
Phyto density	-0.18	-0.04
Phyto richness	-0.22	0.11
Phyto diversity	0.02	0.018

Least Disturbed Sites		
TEMP	0.56	-0.38
DO	-0.40	0.73
pH	0.72*	0.02
COND	-0.31	-0.33
CHL *a*	0.38	-0.46
N	-0.55	-0.12
TP	0.69	-0.27
TSS	0.44	-0.58
Phyto density	0.30	-0.41
Phyto richness	0.66	-0.41
Phyto diversity	0.73	-0.48

Figure 2. RDA bi-plot of zooplankton biomass at all sites with environmental variables. Only variables having a significant effect on axis 1 and 2 are shown. The first two canonical axes explained 12.5% and 6.9% variance, respectively.



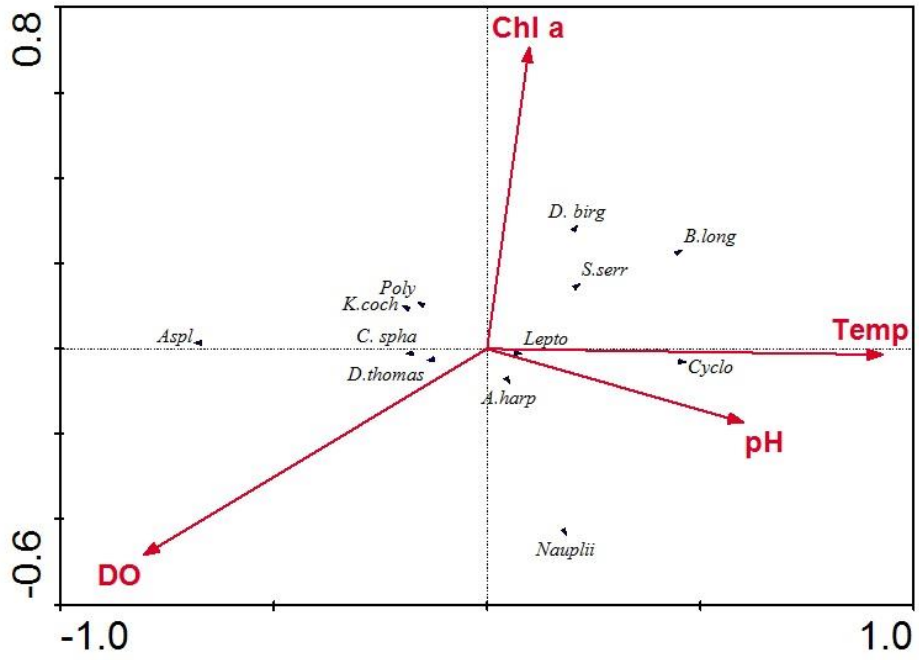
Environmental variables accounted for 21.4% (axis 1 16.7% and axis 2 4.7%) variation in zooplankton density at all sampling locations as found with RDA. Eigenvalues were 0.167 and 0.047. Of all the environmental variables measured TEMP, DO, pH and CHL *a* were found to have a significant impact on zooplankton density (Figure 3, Table 3). DO positively correlated with *Diacyclops thomasi* density and negatively correlated with densities of *Leptodiaptomus* sp., *Bosmina longirostris*, *Diaphanosoma birgei* and *Simocephalus serrulatus*. CHL *a* was found to positively correlate with densities of *Diaphanosoma birgei* and *Simocephalus serrulatus*. Densities of cyclopoid copepodid and *Leptodiaptomus* sp. positively correlated with TEMP; *Diacyclops thomasi*, *Chydorus sphaericus*, *Asplanchna* sp., *Keratella cochlearis* and *Polyarthra spp.* densities were negatively correlated with TEMP. *Acroperus harpae* and cyclopoid copepodid densities positively correlated with pH and *Chydorus sphaericus*, *Keratella cochlearis* and *Polyarthra spp*. densities were negatively correlated.

Table 3. Weighted correlation matrix values for corresponding environmental variables in RDA analysis of species density in all sites, least disturbed sites (DR), moderately disturbed sites (AA, AB, SR) and highly disturbed sites (TR, LC, MB, PO). Significance indicated by $p<0.05$* and $p<0.01$**.

Variable	SPEC AXIS 1	SPEC AXIS 2
All Sites		
TEMP	0.70**	0.03
DO	-0.60**	-0.24
pH	0.46*	0.00
COND	-0.31	0.14
CHL *a*	0.05	0.31**
N	-0.18	-0.11
TP	-0.07	0.13
TSS	0.16	0.18
Phyto density	0.17	0.24
Phyto richness	0.07	0.28
Phyto diversity	0.16	0.05
Highly Disturbed Sites		
TEMP	0.74**	0.14

DO	-0.68*	-0.03
pH	0.47*	-0.24
COND	-0.38	-0.18
CHL *a*	0.12	0.01
N	-0.21	0.38
TP	-0.09	-0.15
TSS	0.26	0.26
Phyto density	0.24	-0.14
Phyto richness	0.11	-0.07
Phyto diversity	0.02	-0.07
Moderately Disturbed Sites		
TEMP	-0.69**	-0.22
DO	0.60	0.39
pH	-0.38	-0.18
COND	0.44	-0.17
CHL *a*	0.09	-0.23
N	-0.21	0.48*
TP	0.05	-0.15
TSS	-0.02	-0.19
Phyto density	-0.08	-0.21
Phyto richness	0.01	-0.13
Phyto diversity	-0.34	-0.03
Least Disturbed Sites		
TEMP	0.28	-0.34
DO	-0.45	0.59*
pH	-0.08	-0.42
COND	0.29	-0.07
CHL *a*	0.57	0.38
N	0.08	0.20
TP	-0.38	-0.64
TSS	0.52	-0.57
Phyto density	0.43	-0.34
Phyto richness	0.37	-0.60
Phyto diversity	0.11	-0.75

Figure 3. RDA bi-plot of zooplankton density at all sites with environmental variables. Only variables having a significant effect on axis 1 and 2 are shown. The first two canonical axes explained 16.7% and 4.7% variance, respectively.



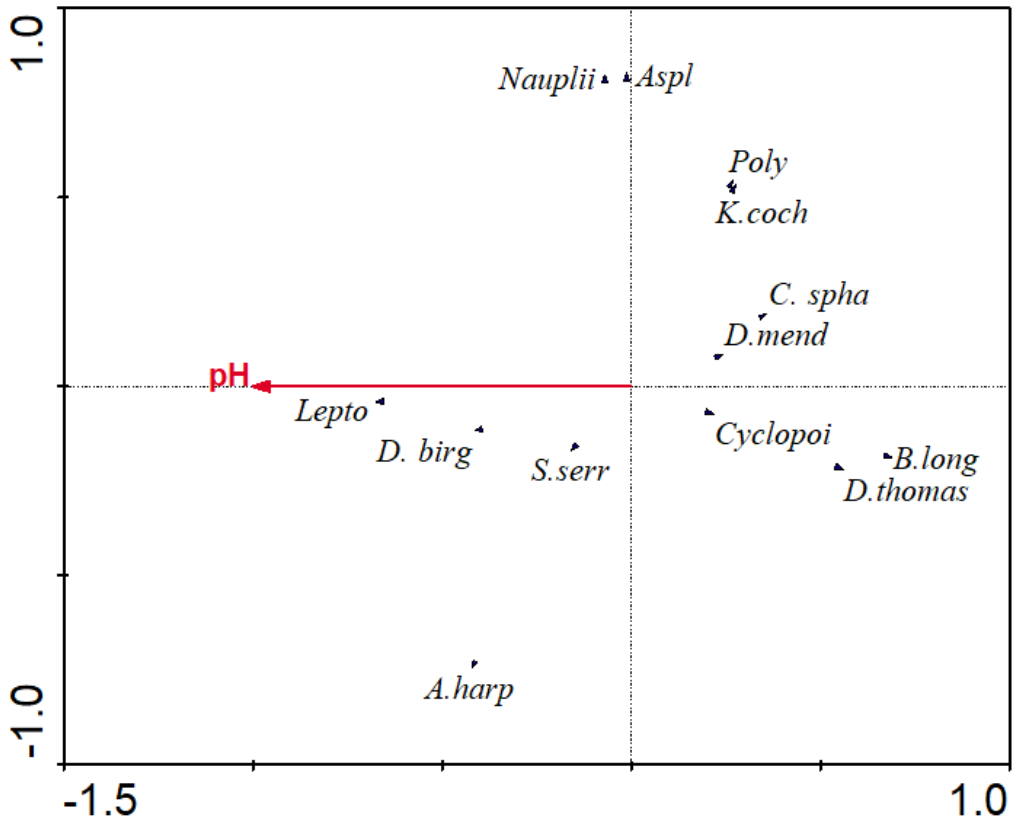
In Chapter 2 sampling locations were ranked based on their exposure to various anthropogenic disturbances including boating, public access, shoreline alteration and urban runoff. The least disturbed sampling location was determined to be DR. Moderately disturbed sites were AA, AB and SR. TR, LC, MB and PO were all ranked highly disturbed. Ordination analysis was performed on each of these groups to determine if zooplankton species found in varying degrees of disturbed sites responded differently to environmental variables. Species richness and diversity were not suited for ordination analysis since they are calculated for the entire sample and not on a species level and as such were analyzed utilizing multiple regression.

4.3.2 Least Disturbed Sampling Locations

4.3.2.1 Biomass

Eigenvalues for the RDA were 0.345 and 0.253 for axis 1 and 2, respectively. RDA axis 1 explained 34.5% and axis 2 a further 25.3% (59.8% total) of variance in biomass the least disturbed site (DR). pH was the only environmental variable that influenced biomass at DR (Figure 4). pH was positively correlated with biomass of *Diaphanosoma birgei* and *Leptodiaptomus* sp. Biomass of *Bosmina longirostris* and *Diacyclops thomasi* were negatively correlated with pH. Cladoceran species such as *Bosmina longirostris* and *Chydorus sphaericus* dominated the biomass composition at the least disturbed sites. Calanoid species *Leptodiaptomus* sp. and calanoid copepodid were the second highest contributor of biomass with rotifer species being the least contributor. *Acroperus harpae* and *Bosmina longirostris* on an average had the highest biomass at 8.86 and 6.94 µg/L, respectively.

Figure 4. RDA bi-plot of zooplankton biomass at the least disturbed site (DR) with environmental variables. Only variables having a significant effect on axis 1 and 2 are shown. The first two canonical axes explained 34.5% and 25.3% variance, respectively.



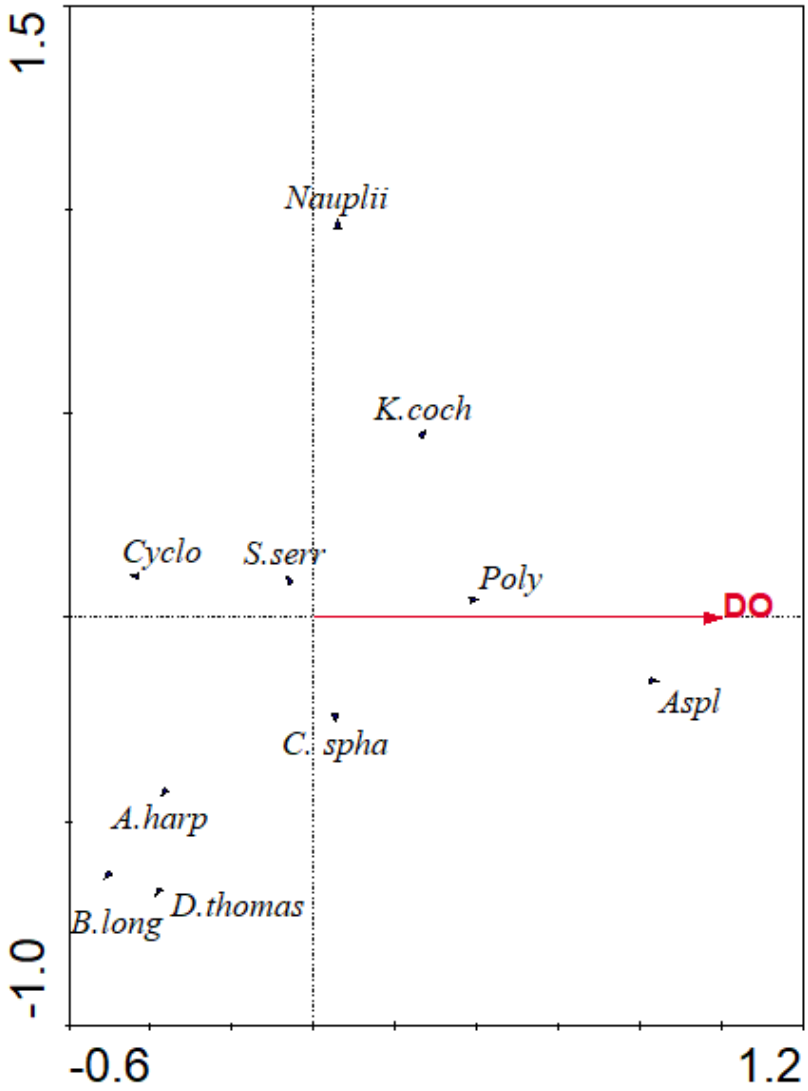
4.3.2.2 Density

RDA results displayed Eigenvalues of 0.362 and 0.230. Variance in density at the least disturbed site (DR) was explained 36.2% by axis 1 and 23.0% by axis 2. Furthermore, DO had a significant impact on density variance (Figure 5). DO positively correlated with densities of *Asplanchna* sp. and *Polyarthra spp.*; it correlated negatively with Cyclopoid copepodid and *Bosmina longirostris* densities. Nauplii dominated the density composition followed by rotifer species (*Keratella cochlearis*, *Asplanchna* sp., *Polyarthra spp.*, *Brachionus calyciflorus*, *Kellicottia longispina*, *Monostyla* sp.). Cyclopoids were the least contributor of density. Both

nauplii and *Bosmina longirostris* had the highest species density at $3.82x10^3$ and

$1.91x10^3$individuals/L, respectively.

Figure 5. RDA bi-plot of zooplankton density at the least disturbed site (DR) with environmental variables. Only variables having a significant effect on axis 1 and 2 are shown. The first two canonical axes explained 16.1% and 10.4% variance, respectively.



4.3.2.3 Richness

The multiple regression analysis found that 37.1% variance in zooplankton richness in the least disturbed site (DR) could be explained by environmental variables ($F_{4,5} = 0.734$, $p = 0.60$). The final model was not significant.

4.3.2.4 Diversity

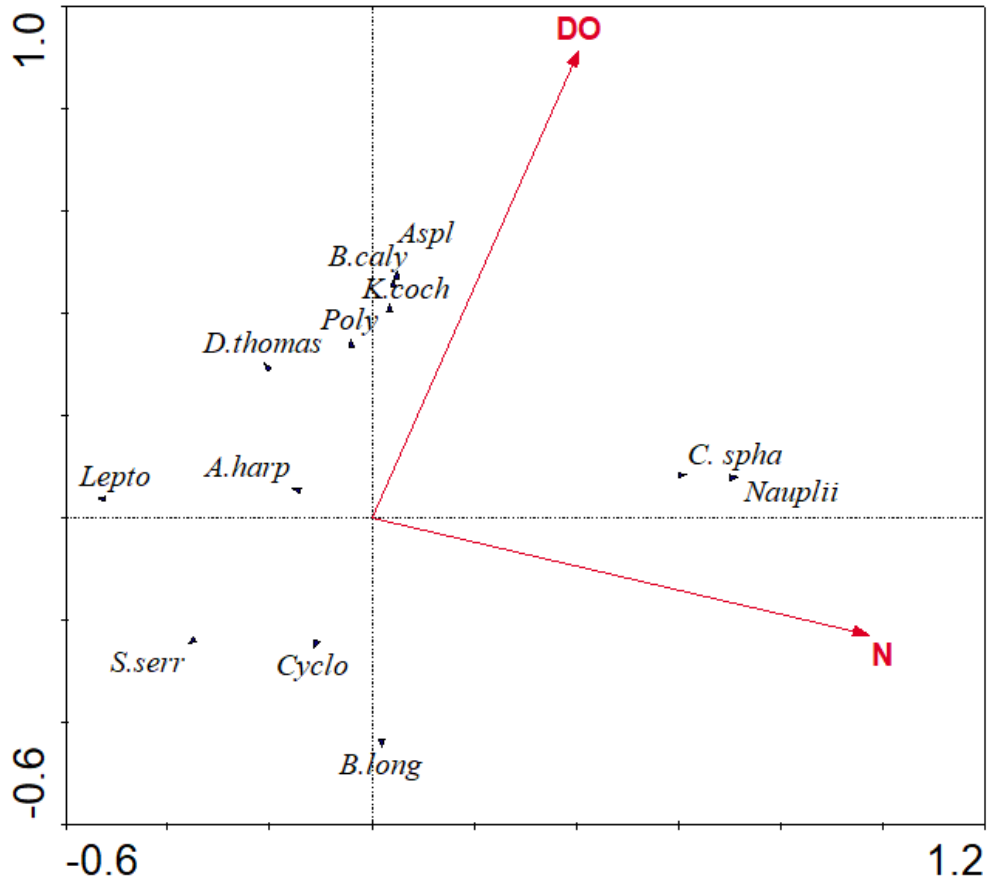
53.6% variance in diversity was explained by environmental variables found with a multiple regression analysis ($F_{4,5} = 1.445$, $p = 0.34$). The final model was not significant.

4.3.3 Moderately Disturbed Sampling Locations

4.3.3.1 Biomass

RDA explained 33.9% (23.1% axis 1 and 10.8% axis 2) of variance in biomass at moderately disturbed sites (AA, AB, SR). Eigenvalues were 0.231 and 0.108 for axis 1 and 2, respectively. DO and N significantly impacted biomass (Figure 6). *Bosmina longirostris*, Cyclopoid copepodid, *Simocephalus serrulatus* biomass all negatively correlated with DO. *Brachionus calyciflorus, Keratella cochlearis*, *Polyarthra* spp. and *Asplanchna sp*. biomass postively correlated with DO. *Leptodiaptomus* sp. was negatively correlated with N while *Chydorus sphaericus* and Nauplii were postively correlated with N. *Leptodiaptomus* sp. and *Bosmina longirostris* had the greatest average species biomass at 21.5 and 11.3 µg/L, respectively.

Figure 6. RDA bi-plot of zooplankton biomass at moderately disturbed sites (AA, AB, SR) with environmental variables. Only variables having a significant effect on axis 1 and 2 are shown. The first two canonical axes explained 23.1% and 10.8% variance, respectively.

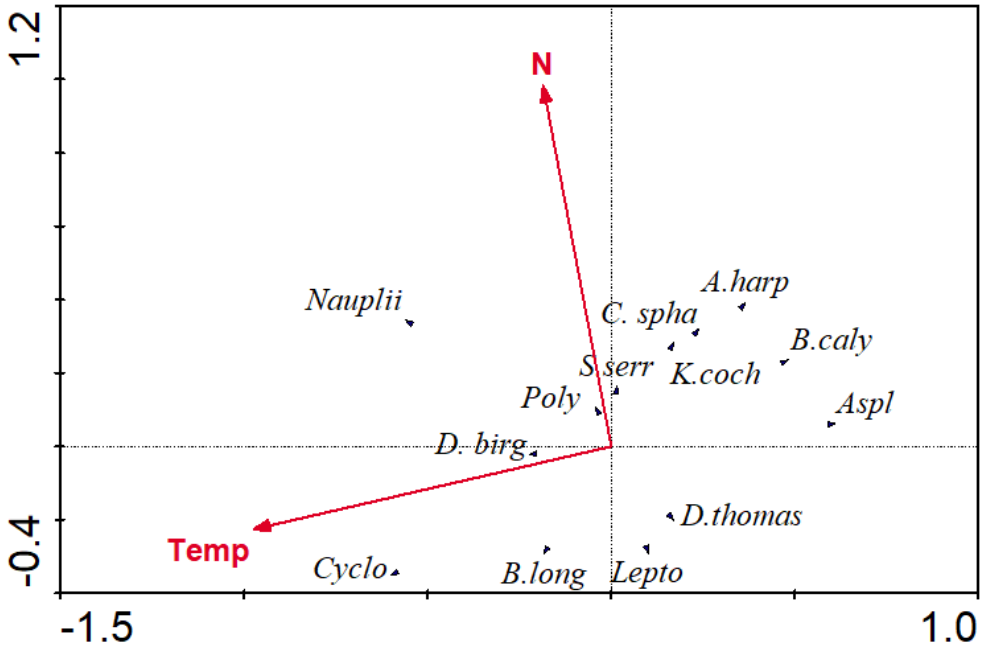


4.3.3.2 Density

Eigenvalues for the RDA were 0.254 and 0.156 for axis 1 and axis 2, respectively. Axis 1 explained 25.4% of variance in density at moderately disturbed sites where axis 2 explained a further 15.6%. Density was significantly influenced by Temp and N (Figure 7). Density of Cyclopoid copepodids and *Bosmina longirostris* was positively correlated with TEMP; *Acroperus harpae*, *Asplanchna* sp., *Chydorus sphaericus* and *Brachionus calyciflorus* were negatively correlated. N negatively correlated with *Bosmina longirostris* and *Leptodiaptomus*

sp. densities. Juvenile copepods contributed the greatest to species density and cyclopoids the least. Nauplii and *Leptodiaptomus* sp. had the highest average species density at $5.49x10^3$ and $2.47x10^3$ individuals/L, respectively.

Figure 7. RDA bi-plot of zooplankton density at moderately disturbed sites (AA, AB, SR) with environmental variables. Only variables having a significant effect on axis 1 and 2 are shown. The first two canonical axes explained 24.5% and 15.6% variance, respectively.



4.3.3.4 Richness

Multiple regression analysis showed 43.6% of species richness could be attributed to environmental variables ($F_{7,22} = 2.43$, $p = 0.053$). However, the final model was found to be not significant.

4.3.3.4 Diversity

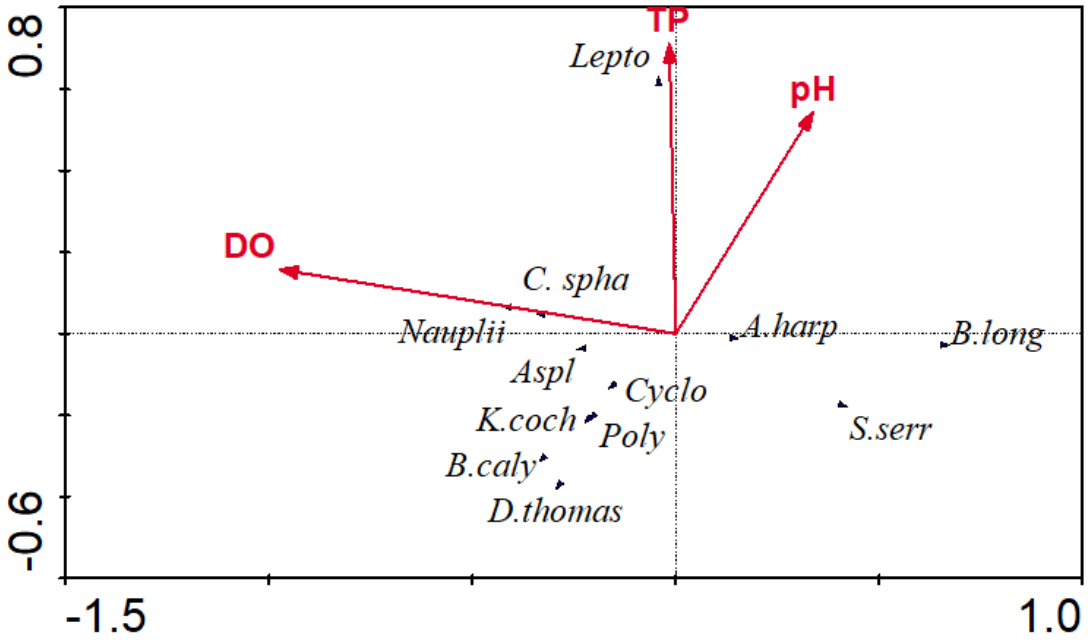
After performing the multiple regression analysis species diversity could be explained 36.7% by environmental variables ($F_{6,23} = 2.22$, $p = 0.08$). The final model was not found to be significant.

4.3.4 Highly Disturbed Sampling Locations

4.3.4.1 Biomass

Eigenvalues were 0.185 and 0.136 for axis 1 and 2, respectively. RDA explained 32.1% (18.5% axis 1 and 13.6% axis 2) of variance in zooplankton biomass at highly disturbed sites (TR, LC, MB, PO). DO, TP and pH were the only variables to significantly influence biomass in highly disturbed sites. *Leptodiaptomus* sp. was positively correlated with TP; *Diacyclops thomasi* was negatively correlated. *Bosmina longirostris* biomass negatively correlated with DO while *Chydorus sphaericus* positively correlated. pH was negatively correlated with *Diacyclops thomasi* and *Brachionus calyciflorus.* *Bosmina longirostris* had the highest biomass with an average of 43.60 µg/L followed by *Simocephalus serrulatus* with 33.57 µg/L.

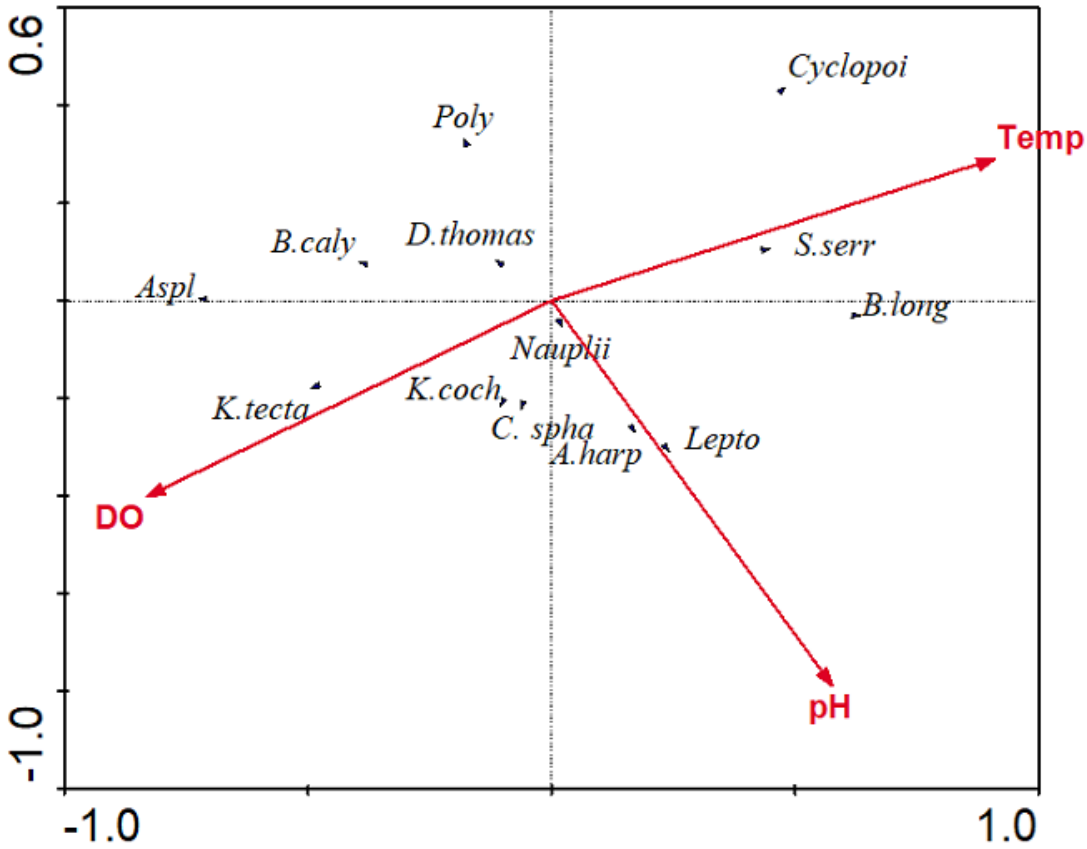
Figure 8. RDA bi-plot of zooplankton biomass at highly disturbed sites (TR, LC, MB, PO) with environmental variables. Only variables having a significant effect on axis 1 and 2 are shown. The first two canonical axes explained 18.5% and 13.6% variance, respectively.



4.3.4.2 Density

RDA axis 1 explained 24.6% of variance in density at highly disturbed sites while axis 2 explained an additional 7.3% (total 32.0%) (Figure 9). Eigenvalues for axis 1 and 2 were 0.246 and 0.073, respectively. DO, TP and pH all significantly impacted biomass (Figure 9). *Leptodiaptomus* sp. and *Acroperus harpae* biomass was positively correlated with pH and *Polyarthra* spp. negatively correlated. DO and Cyclopoid copepodids, *Bosmina longirostris* and *Simocephalus serrulatus* biomass was positively correlated with TEMP and negatively correlated with DO. *Keratella tecta*, *Keratella cochlearis* and *Asplancha* sp. positively correlated with DO and negatively with TEMP. *Bosmina longirostris* also had the greatest species density (1.55×10^4 individuals/L) followed by *Asplanchna* sp. (1.45×10^4 individuals/L).

Figure 9. RDA bi-plot of zooplankton density at highly disturbed sites (TR, LC, MB, PO) with environmental variables. Only variables having a significant effect on axis 1 and 2 are shown. The first two canonical axes explained 24.6% and 7.3% variance, respectively.



4.3.4.3 Richness

Variance in species richness could 39.51% be explained by environmental variables after performing a multiple regression analysis ($F_{8,31} = 2.53$, $p = 0.03$). DO, COND, pH, TP, N, CHL *a*, TSS and phytoplankton diversity were included in the final model but only CHL *a* was found to be significantly positively correlated with richness.

4.3.4.4 Diversity

A multiple regression analysis found that environmental variables at highly disturbed sites explained 21.7% of variance in diversity ($F_{8,31} = 1.08$, $p = 0.41$). The final model was not significant.

4.4 Discussion

4.4.1 All Sampling Locations

Ordination analysis was utilized to get a better understanding of how zooplankton dynamics varied due to environmental variables at a species level and if certain species could be used as biological indicators. The initial ordination analysis conducted on species distribution (measured as presence absence of species) at all sampling locations found TEMP, pH and DO influencing species presence, although only a small percentage of variance was explained by environmental variables (18.6%). Results for zooplankton density and biomass variation at all sampling locations were similarly explained by environmental variables at 21.4% and 19.6%, respectively. The same occurred when running the ordination analyses on zooplankton density and biomass in least, moderately and highly disturbed sites with variance explained ranging from 26.5 – 35.5%. These results are not surprising because other studies have used ordination to detect species level changes including non-metric multidimensional scaling (Nicholls & Tudorancea, 2001), principal components analysis (Anas et al., 2013) and canonical correspondence analysis (Attayde & Bozelli, 1998) and have yielded similar results. The low level of variation explained by ordination reflects other biological interactions occurring that were not captured by the data set. This may include species specific interactions including predation by planktivorus fish. Additionally, there may have been other factors related to anthropogenic stressors not measured in the study that could have explained more variance in

species composition. The presence and absence of vegetation altered by land use was found to significantly impact zooplankton taxon in 73 shallow lakes in the United States (Dodson et al., 2005). This is something that could be addressed with future monitoring and research.

4.4.2 Zooplankton Use as a Biological Indicator

Species biomass in least, moderately and highly disturbed sampling locations was found to differ although not significantly. *Bosmina longirostris* dominated species biomass at all sampling locations. *Bosmina longirostris* is a known indicator of eutrophic conditions and is tolerant of highly degraded environments (Halser, 1947; Lougheed & Chow, 2002; Minder, 1938; Nicholls & Tudorancae, 2001). The ordination analysis supported this as well with *Bosmina longirostris* preferring warmer, low oxygenated and higher CHL *a* conditions (Figure 2 & 3) indicative of degraded water quality. Even though *Bosmina longirostris* dominated at all sampling locations it is interesting to note that its biomass in highly disturbed sites was 3.8 and 6.3 times greater than moderately and least disturbed sites, respectively. Furthermore, biomass in highly disturbed sites was greater than least disturbed sites, which is to be expected since nutrient (TP, N, and TSS) and primary productivity (CHL *a* and phytoplankton density) were highest in these areas. Ejsmount-Karabin & Karabin (2013) reported the same conclusion that zooplankton biomass correlated with high trophic status of lakes in Poland. Additionally, these results support findings from other research (Gannon & Stemberger, 1978; Jeppensen et al., 2000; Pinto-Coehlo et al., 2005) and suggests that zooplankton biomass can be used as a biological indicator for detecting environmental disturbances.

Zooplankton density was found to significantly differ between least, moderately and highly disturbed sites. Not surprisingly, highly disturbed sites had greater density compared to least and moderately disturbed sites. Hann et al. (2017) had similar findings in Lake Winnipeg

with elevations in zooplankton abundance as a direct result of increased nutrient loading. Nauplii dominated species density in all sampling locations and this was similar to the findings by Young & Jarjanazi (2015) in Lake Simcoe sites. *Bosmina longirostris* was the second dominant species in least and highly disturbed sampling locations. As previously mentioned these species is known to tolerate degraded water quality conditions (Attayde et al., 1998; Lougheed & Chow, 2002) thus it was not surprising that it was in high abundance in highly disturbed sites where nutrient concentration is high and DO is low. Additionally, Loughreed & Chow identified that environmental variables (TSS, TP, CHL *a*, and presence of submergent vegetation) explained zooplankton abundance, which supports the present studies' findings. Species density was found to be a good biological indicator in these sampling locations.

Species richness could not be explained by environmental variables in least and moderately disturbed sites. It was found to be postively correlated with CHL *a* in highly disturbed sites. Species richness did not differ significantly between varying degrees of disturbances with an average richness of 8.3, 7.6 and 8.3 for least, moderately and highly disturbed sites, respectively. These findings conflicted with other studies where species richness was found to be lower in impacted areas (Attayde et al., 1998; Dodson et al., 2007).

Species diversity was not influenced by environmental variables at any of the sampling locations. Additionally, diversity did not differ between sampling locations. These findings conflict with other reports where diversity was found to be greater in the least impacted sites and lower in the highly impact sites (Attayde et al., 1998; Dodson et al., 2007). Similarly, Loughreed & Chow (2002) found that zooplankton species richness and diversity could not be significantly explained by environmental variables in Great Lakes wetlands experiencing degraded to pristine water quality. Instead they created a wetland zooplankton index based on trends from a partial

principal components analysis which ended up explaining more of the variation in environmental variables. Furthermore, zooplankton composition predicted wetland quality gradients with plant associated species dominating high quality wetlands and pollutant tolerant species dominating degraded wetlands (Loughreed & Chow, 2002). The current study was not conducted in wetlands although the information may apply to nearshore areas of the TSW as all sites sampled with the exception of AA contained many species of submergent plants such as *Ceratophyllum demersum* (coontail) , invasive *Myriophyllum spicatum* (Eurasian watermilfoil), and *Elodea Canadensis* (Canada waterweed).

Overall, the species biomass and density proved to be good biological indicators in sampling locations exposed to varying degrees on anthropogenic influence. Both density and biomass were significantly greater in highly disturbed sites. Subsequently, the dominance of eutrophic species indicators (*Bosmina longirostris*) in highly impacted sites supports the hypothesis that certain species could be used as indicators of environmental health. Although these species are ubiquitous and can tolerate a wide range of environmental conditions and therefore should be assessed alongside species biomass and density to provide a better understanding of water quality. Ordination analysis found that *Bosmina longirostris* responded to environmental conditions and preferred degraded water quality conditions. This species would be the best suited to act as an indicator species in the TSW.

4.5 Conclusion

The second objective of this study was to determine the effectiveness of the zooplankton community as water quality indicators in this area. Species biomass and density could be used as biological indicators in the TSW. The hypothesis that highly disturbed sites would have greater biomass and density compared to least disturbed sites could be accepted. Zooplankton biomass

and density were greatest at highly disturbed sites supported by greater nutrient and primary productivity (CHL *a* and phytoplankton density) compared to less disturbed sites. Furthermore, the dominance of highly tolerant species supports the usefulness of zooplankton as indicators of anthropogenic influence in the TSW. Species richness and diversity were less useful indicators for these particular sites possibly suggesting that the communities are similar among these sites. The TSW and specifically the nearshore regions of Lake Simcoe and Lake Couchiching experience intensive anthropogenic pressures including runoff from urban and agricultural areas, natural shoreline alteration and recreational activities such as boating. This research found that zooplankton dynamics in varying degrees of anthropogenically disturbed sampling locations correlated the most with dissolved oxygen and nutrient conditions and varied expectedly with increases from human related stressors. Continuous monitoring of zooplankton composition would be a cost effective strategy to measuring water quality in the TSW overtime.

Chapter 5 Conclusion

Indicators of water quality provide cost effective and rapid detection of aquatic ecosystem degradation. Zooplankton can be used as water quality indicators because they reproduce quickly with short life cycles, respond rapidly to changing environmental conditions and can be resistant or sensitive to nutrient enrichment, pollutants and other environmental variables (APHA, 2005; Gannon & Stemberger, 1978). Therefore, deteriorating water quality conditions due to anthropogenic disturbances could theoretically be indicated by changes in zooplankton community composition. Data collected from eight nearshore sites across Lake Simcoe and Lake Couchiching found that the zooplankton community responded predictably to water quality impairments through shifts in species density, biomass, richness and diversity. Further supporting the assumption that zooplankton are suitable indicators of water quality and subsequently anthropogenic influence in nearshore regions.

To determine if water quality and zooplankton composition varied spatially and temporally, data were collected from eight different nearshore sites in Lake Simcoe and Lake Couchiching exposed to varying degrees of anthropogenic disturbance. The study was repeated ten times to capture seasonally differences. The overall objectives were to provide baseline information on the zooplankton community in the TSW and to determine the effectiveness of zooplankton community to act as an indicator of water quality in the nearshore region. Environmental variables were measured as a proxy of water quality to determine if variance in these variables were indicative of anthropogenic disturbances affecting the nearshore region. Zooplankton data was collected to provide baseline information of the community in nearshore regions of the TSW and to analyze species density, biomass, richness and diversity variance spatially and temporally.

Environmental variables and zooplankton community composition were expected to vary spatially and temporally. It was hypothesized that varying degrees of anthropogenic disturbances would influence water quality and zooplankton dynamics in nearshore regions. Least disturbed sites (DR, AA and AB) will have the greatest water quality, species richness and diversity but lowest species density and biomass. Most disturbed sites (TR, LC, MB) will have the lowest water quality, species richness and diversity and highest biomass and density. Water quality at moderately disturbed sites (PO, SR) would fall somewhere in between.

Results from this study demonstrated that nearshore regions of the TSW experience a range of environmental conditions, varying from mesotrophic (TN = 25.12 µg/L, TP = 10.83 µg/L, CHL *a* = 1.58 mg/m^3) to mesoeutrophic conditions (TN = 78.92 µg/L, TP = 31.31µg/L, CHL *a* = 13.29 mg/m^3). Additionally, environmental variables exhibited significant variation spatially and temporally and water quality reflected the degree of anthropogenic disturbance. The highly disturbed sites (TR, LC, MB) experienced higher nutrient concentrations and conductivity with lower dissolved oxygen concentrations characteristic of degraded water quality. The least disturbed sites (DR, AA, AB) had greater water quality with higher dissolved oxygen and lower nutrient concentrations. Moderately disturbed sites (PO, SR) experienced intermediate water quality. Zooplankton density, biomass, richness and diversity differed temporally and all except diversity varied spatially.

The second objective of this study was to determine the effectiveness of the zooplankton community to act as an indicator of water quality. The hypothesis that indicator species will have a relationship with environmental variables in nearshore regions. Variation in species level dynamics was marginally explained by environmental variables suggesting that the community consists of similar species in nearshore regions and that perhaps individual species are impacted

by unmeasured factors or react differently to environmental conditions at each site. Immature copepods dominated density in all sampling locations; *Bosmina longirostris* dominated biomass in all sampling locations. The latter species is an indicator of eutrophic status and can tolerate degraded environmental conditions (Attayde et al., 1998; Lougheed & Chow, 2002). This species was present in least and moderately disturbed locations but in lower abundance. *Bosmina longirostris* is well suited to be an indicator of water quality in the TSW. Further monitoring of the zooplankton community and additional factors would help clarify species level changes due to environmental variables and potentially provide additionally species that could act as indicators of water quality. Zooplankton density and biomass responded as hypothesized to varying anthropogenic disturbances. Highly disturbed sites had higher biomass and density. Least disturbed sites had lowest biomass and density. Biomass and density at moderately disturbed sites fell in the middle. Overall, there was some evidence of species specific differences between varing degrees of anthropogenic disturbances; zooplankton biomass and density proved to be good indicators of anthropogenic influence for these sites.

The TSW is a heavily used route by boaters as it is the only channel connecting Lake Ontario to Georgian Bay. Furthermore, with boat traffic and increases in population around Lake Simcoe it will be crucial to support naturalized shorelines and other projects that increase biodiversity and water quality in nearshore regions. Nearshore areas are vital to fish because they provide spawning areas and juvenile planktivorous fish forage zooplankton in these areas. With this in mind conservation efforts should focus on monitoring and protecting these habitats to prevent further water quality impairment. Loughreed & Chow (2002) developed a wetland zooplankton index (WZI) to assess wetland quality in the Laurentian Great Lakes. The development and use of a similar index for nearshore regions of the TSW would greatly benefit

water quality monitoring and ecosystem management in this area. This tool would provide an easy way for conservation authorities, researchers, citizen scientists, and policy makers to monitor water quality and adjust conservation efforts as needed. Addtionally, freshwater ecosystems provide many services to humans such as drinking water, food, transportation and recreation. Therefore, it is essential that government officials and general public understand the importance of maintaining good water quality to preserve ecological services as well as human related benefits. Future research should focus on identifing the impact of localized stressors such as salinity on zooplankton community dynamics.

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Appendix A Phytoplankton species presence at nearshore sites during 2015/2016 sampling

Nearshore	TR	LC	MB	AA	AB	PO	SR	DR
Diatoms								
Achnanthes inflate		X						
Achnanthidium minutissimum	X			X		X	X	X
Amphora ovalis	X	X	X	X	X	X	X	X
Asterionella Formosa		X				X	X	
Cocconeis pendiculus	X	X		X	X	X		
Cocconeis placentula	X	X	X	X	X	X	X	X
Cyclotella sp.	X	X	X	X	X	X	X	X
Cymatopleura eliptica			X					
Cymatopleura solea			X	X		X		
Cymbella lancelota				X	X	X		
Cymbella sp.	X	X	X	X		X	X	X
Cymbella tumida				X		X		
Diatoma sp.	X		X		X		X	X
Diatoma tenuis		X	X	X		X	X	X
Diatoma vulgaris	X	X	X	X	X	X	X	X
Entomoneis paludosa			X					
Entomoneis sp.			X					
Epithemia sorex		X	X	X	X	X	X	X
Epithemia sp.	X		X		X	X		
Epithemia turgida			X	X	X	X	X	
Fragilaria capucina	X	X	X	X	X	X	X	X
Fragilaria crotonensis	X	X	X	X	X	X	X	X
Fragilaria sp.	X		X	X	X	X		
Gomphoneisis sp.	X			X				
Gomphonema sp.	X	X	X	X	X	X	X	X
Gomphonema truncatum	X				X	X		
Gomphonema turgidum			X					
Gyrosigma sp.	X	X	X					
Hippodontas sp.	X		X					
Melosira varians	X	X	X					
Meridion circulare			X					
Meridion sp.			X		X	X		
Navicula gastrum	X	X	X	X	X	X	X	X
Navicula lanceolate	X					X	X	
Navicula sp.	X	X	X	X	X	X	X	X
Nedium affine			X		X			

Nitzschia acicularis			X	X				
Nitzschia brevissima						X		
Nitzschia palea	X	X	X	X	X	X		
Nitzschia sigmoidea	X	X	X	X				
Nitzschia sp.			X			X		
Pinnularia sp.	X		X	X		X		
Placoneis gastrum	X	X	X			X		
Placoneis sp.				X	X			
Rhoicospheria cuvvata	X	X	X		X			
Rhopolodia gibba	X	X	X	X	X	X	X	X
Sellaphora pupula			X					
Sellaphora sp.	X							
Staurosira construens			X	X				
Staurosirella pinnata	X		X		X	X		
Stephandiscus sp.					X			
Surirella ovalis	X		X					
Synedra acus		X	X	X		X	X	X
Synedra capitata			X			X		X
Synedra sp.	X	X	X	X	X	X	X	X
Synedra ulna	X	X	X	X	X	X	X	X
Tabellaria sp.	X		X		X		X	X
Tabuleria sp.	X		X			X	X	
Green algae								
Chlamydomonas sp.	X	X						
Closterium sp.				X				
Coelastrum spp.		X		X	X	X	X	X
Coleochaete spp.		X						X
Cosmarium sp.	X	X	X	X	X	X	X	X
Dictyosphaerium pulchellum							X	X
Kirchneriella sp.						X		
Pediastrum sp.		X						
Pediastrum tetras				X		X		
Scendesmus sp.		X	X		X	X		
Scenedesmus quadricauda	X	X	X	X	X	X	X	X
Staurastrum gracile		X	X	X	X	X	X	X
Cyanobacteria								
Aphanocapsa sp.						X	X	X
Chroococcus sp.			X				X	X
Merismopedia glauca					X			X
Microcystis sp.			X		X			X

Other								
Actinosphaerium sp.		X						

Appendix B Phytoplankton species presence at openwater sites during 2015/2016 sampling

Openwater	7A	7B	8A	8B	8C	9A	9B	9C
Diatoms								
Achnanthidium minutissimum							X	
Amphora ovalis				X	X			
Cocconeis placentula		X		X			X	X
Cyclotella sp.	X	X	X	X	X	X	X	
Cymbella sp.	X	X	X	X	X	X	X	X
Diatoma sp.						X		
Diatoma tenuis				X				
Diatoma vulgaris								X
Epithemia sorex	X	X		X			X	X
Epithemia turquida		X	X				X	X
Fragilaria crotonensis	X	X	X	X	X		X	X
Fragilaria sp.				X				
Gomphonema sp.				X				
Navicula gastrum				X		X	X	
Navicula sp.	X	X	X	X	X	X	X	X
Pinnularia sp.	X							
Rhopolodia gibba				X				X
Synedra acus	X		X	X			X	
Synedra sp.	X		X	X	X	X	X	
Synedra ulna				X		X	X	X
Green algae								
Chlamydomonas sp.					X	X	X	X
Coelastrum sp.		X	X	X	X	X	X	X
Cosmarium sp.		X	X			X		
Merismopedia glauca	X		X	X	X			
Scenedesmus quadricauda		X	X	X	X	X		
Scenedesmus sp.						X		
Staurastrum gracile	X		X	X				
Protozoa								
Arcella sp.	X							
Cyanobacteria								
Chroococcus sp.					X			
Microcystis sp.			X	X				