Periphyton community dynamics in varying natural environments: A study from Northern Lake Simcoe.

A thesis presented to

The Faculty of Graduate Studies

of

Lakehead University

by

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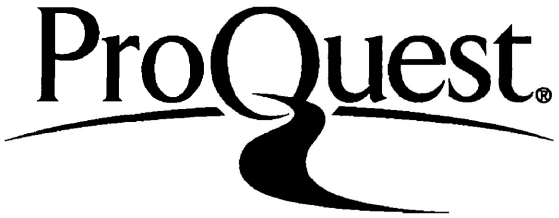
In partial fulfillment of requirements

for the degree of

Master of Science in Biology

September 3, 2014



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Abstract

Globally, freshwater ecosystems are constantly under the threat of various biological and chemical stressors. In Canada, millions of dollars are spent on the rehabilitation process of these water bodies every year. Maintenance of healthy water systems is important for their conservation and survival of human kind. This study examines the use of periphyton as a tool for monitoring water quality by examining the dynamics (biofilm thickness, species density, species richness, species diversity, and biomass) in periphyton communities in lentic environments. Previous research validates the successful use of periphyton in lotic environments.

This multi-proxy (Bacillariophyceae, Chlorophyceae, Cyanophyceae, and protozoa) study investigated the colonization pattern of periphyton on inert glass slides (10 X 3 X 0.1cm) suspended in the littoral zone of 3 sampling locations in northern Lake Simcoe, to a maximum period of 30 days (per sampling period) with intermittent sampling. The study was repeated four times in different seasons during 2011-2013. The retrieved slides were observed under a microscope for taxonomic composition of periphyton communities, species density, and biofilm thickness. The hypotheses tested were (1) periphyton community dynamics vary with season and location, (2) species diversity decreases as a result of increase in nutrient concentration, (3) diatom abundance and species composition will increase in spring and fall seasons as a result of lake turnover processes.

Results indicated that there was significant variation in the periphyton colonization pattern with seasons, locations and with the duration of slide exposure. The overall periphyton growth (biofilm thickness, biomass and species density) exhibited an increase during the early phase (rmAnova $p<0.05$ between days of exposure); a climax during the mid phase (rmAnova $p<0.05$ between days of exposure); a sloughing-off period, and an increase in growth towards the late growth phase (rmAnova $p<0.05$ between days of exposure). The highest species density (site LC: $7.91_{\log 10}$) was observed during summer (rmAnova $p<0.05$ between seasons) when a decrease in diversity in Bacillariophyceae was observed. However, Bacillariophyceae abundance and diversity increased during spring and fall sampling periods as a result of lake turnover processes and the availability of nutrients. Overall species diversity did not decrease when the total phosphorus concentration increased in the water column. This is mainly because of the increased diversity in Chlorophyceae, Cyanophyceae and protozoa.

The periphyton community varied with the environmental stressors such as variations in conductivity and nutrient concentrations. Thus, the mature periphyton community composition and their dynamics demonstrated that they can be used as an indicator of water quality changes in this study area.

Lay summary

The mission statement of Lakehead University's Department of Biology is "Faculty and students in the Department of Biology are bound together by a common interest in explaining the diversity of life, the fit between form and function, and the distribution and abundance of organisms." The current study focuses on the dynamics of periphyton, which is a multidimensional matrix composed of an initial layer of bacteria on a substrate followed by the attachment of algae, protozoa, and various invertebrates. This study contributes to one of the central research themes outlined in the mission statement, the relationships between life forms and their environmental functions. The study advances our understanding of various biotic and abiotic factors influencing periphyton growth in near-shore lake environments. Understanding the succession of periphyton communities and their environmental niches is a valuable tool in assessing water quality as periphyton members are able to rapidly respond to physical and chemical stressors. Three major research questions were investigated. 1. What are the effects of seasonality and nutrient concentrations on periphyton? 2. How periphyton growth patterns relate to water quality? 3. Can the periphyton composition represent the nutrient availability of each sampling site? Results showed that the species composition varied considerably between sites and sampling periods and that nutrient concentration influenced the presence or absence of certain species. This study provides a baseline dataset for periphyton and water quality measurements in northern Lake Simcoe. Furthermore, it may be useful to the development of a periphyton based water quality index for northern Lake Simcoe which will assist scientists and policy makers in their efforts towards more efficient water resources management.

Acknowledgments.

I have had enormous support from many colleagues, friends and family during this portion of my academic journey. First and foremost I want to thank my beautiful daughters for putting up with my "just give me 10 more minutes to write" which really meant 30 minutes. Love you girls with all my heart. Always remember that you have choices and options no matter what your life situation is. I thank my parents and my sister for their continuous unconditional love and support in all aspects of my life. There is no doubt that without your support I could have accomplished this. To my good friend, Florin, who is my greatest critic and supporter who laughed with me, wiped away my tears, and kept me moving forward, thank you.

To my supervisors, Dr. Sreekumari Kurissery and Dr. Nandakumar Kanavillil, I thank you so much for making this part of my academic journey a reality and initiating access to graduate studies at the Orillia campus of Lakehead. You are true trailblazers and I have grown so much personally and professionally as a result of your expertise, leadership and guidance. Thank you!

Thank you so much to my LU lab family, Vicki, Steve, Carly, Diane, Thamara and Carolyn. Vicki, I thank you all for your support and having the patience to view my presentations (sometimes multiple times), listen to my ideas and give me thoughtful feedback. Steve a special thank you for all of your reading and multiple edits during the writing phase of my thesis. A big thank you to you all!

I have said before that "it takes a village to raise a graduate student" and indeed it did. Thank you to everyone at Lakehead for their constant support, friendly smiles, hugs and coffee when needed. I will carry the memory of my Lakehead family wherever I go.

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Chapter 1: Introduction

The latin translation for periphyton is "attached plants". Periphyton plays a dominant role in natural biofilm development which is dominated by phototrophic algae, but also includes heterotrophic organisms including bacteria, protozoa, and some invertebrates (Wetzel 1983; Zippel et al., 2007; Azim et al., 2010; Wu et al., 2011). It can be found in all aquatic environments such as lakes, rivers and streams, as well as brackish and salt water environments, although species composition varies within and between the environments.

Periphyton contributes to the primary productivity of an environment and therefore acts as a key food source to larger organisms within the aquatic ecosystem. Periphyton communities actively take part in environmental processes such as nutrient cycles and precipitation of pollutants. Water quality is defined as "the condition of the water, including chemical, physical, and biological characteristics, usually with respect to its suitability for a particular purpose such as drinking or swimming (NOAA 2014). Periphyton can act as an important tool to monitor water quality changes in aquatic environments as it is composed of a variety of microorganisms that are sensitive to these changes (Wetzel 1983; Azim et al., 2010).

The population dynamics of periphyton is often related to the phytoplankton community, as phytoplankton contributes propagules to periphyton (Peterson et al., 1996; Sekar et al., 1998; Bellinger & Sigee., 2010). The typical growth of a periphyton community begins with bacterial and debris attachment to a bare (or disturbed) substratum surface. The growth regime generally includes three phases; an early phase, mid phase and late phase (Figure 1). Depending on the environmental conditions, a periphyton community takes different durations to attain maturity wherein autogenic sloughing off and reattachment of periphyton are common (Stevenson et al., 1996; Sekar et al., 2004; Bellinger & Sigee 2010; Kanavillil et al., 2012).

The abundance and species composition of periphyton community vary during each of these developmental phases. For example, the diatom succession begins with the attachment of a highly populated species from the water column. At this time the light intensity may be high (Peterson et al., 1996; Sekar et al., 1998; Bellinger & Sigee 2010). These early colonizers generally possess a rapid reproduction strategy and therefore colonize the substratum quickly (Sekar et al., 2004; Bellinger & Sigee 2010; Kanavillil et al., 2013). The diatom species arriving at the mid-successional phase generally possess a morphological advantage of a longer mucilaginous stalk with which they grow vertically from the basal attachment to the substratum (e.g. *Cymbella* spp.). This will help them obtain higher levels of irradiance (Bellinger & Sigee 2010). The diatom species arriving at the late successional phase are generally highly motile with special morphological features such as keels (i.e *Nitzschia* sp) and are able to maintain a high growth rate at low irradiance level (Sekar et al., 2004; Bellinger & Sigee 2010; Kanavillil & Kurissery, 2013).

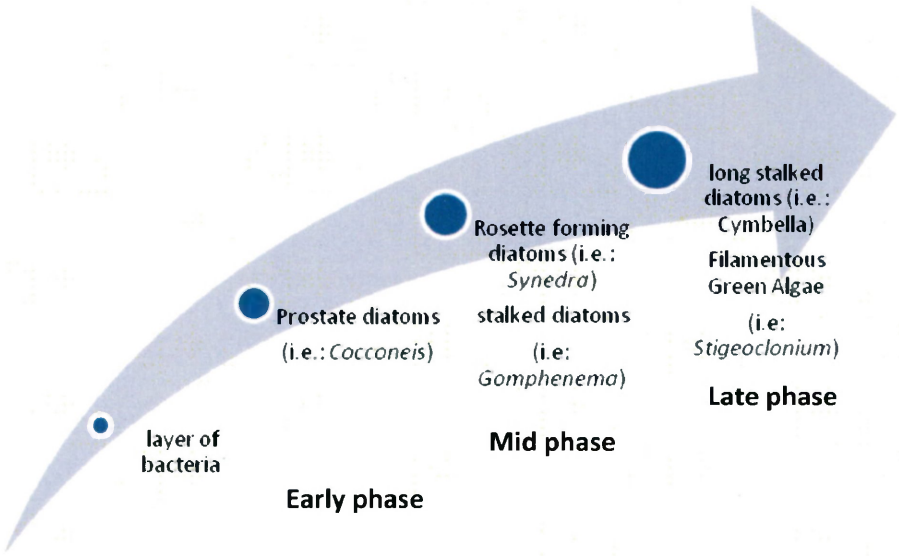


Figure 1. Development of natural biofilm (Sekar et al., 2004; Bellinger & Sigee 2010).

In addition, the periphyton community also responds to variations in abiotic and biotic environmental factors from within the periphyton community and the water column. This is because organisms in the periphyton community possess distinct ecological preferences and tolerances for different environmental factors (Bellinger & Sigee, 2010).

Abiotic factors:

The major abiotic factors affecting the periphyton community includes temperature, light, nutrient concentrations and hydraulic conditions of the water column such as flow rate.

Temperature

The requirement of thermal energy to carry out physiological processes such as enzymatic catalytic conversions is well known for all organisms (Larned 2010). Temperature is an important physical factor in the periphyton growth (Butterwick et al., 2005). Optimum growth temperatures may range from 10-30°C, however, individual species may have varied tolerance levels within this range. For this reason, periphyton species composition and abundance would reflect the temperature changes (Butterwick et al., 2005; Larned (2010). However, temperature alone may not be a limiting factor in periphyton growth (De Nicola et al., 2003; Liboriussen & Jeppesen 2006).

Light

The relative abundance of a periphyton community is determined by the availability of sunlight for the organisms in the community (Masseret et al., 1998). Some of the dominant members of the periphyton community belong to Bacillariophyceae as well as Chlorophyceae which are photoautotrophs. Therefore, light availability is one of the most influential variables that determine the growth and composition of periphyton communities due to the variation in the

optimal irradiance ranges for different groups of periphyton communities. Photoautotrophic periphyton under heavily shaded habitat undergoes physiological changes that maximize their photosynthetic efficacy at lower light levels (Hill 1996; Stevenson et al., 1996)

The physical structure of a biofilm, for example biofilm thickness can influence the light penetration. Community members respond to light availability resulting in taxonomic shifts within the periphyton community (Dodds 1992). The taxonomic shifts may include organisms capable of performing metabolic activities at lower light intensity dominate the light reduced area within the periphyton community (Hillebrand et al., 2000). Shifts also include the presence of longer stalked diatoms and Chlorophyceae, in addition to a higher abundance of cyanobacteria in the periphyton community (Hillebrand et al., 2000; Liboriussen et al., 2006).

Nutrients

The nutrient acquisition in the periphyton community occurs internally (from nutrient cycles such as decomposition of waste products) and externally from sources such as surrounding water, substrate and sediment. Numerous studies have been performed on nutrient gradients and the resulting shifts in taxonomic compositions, specifically, the relative abundance of different species of periphyton (Liboriussen & Jeppesen, 2006; Vis et al., 2008; Ferragut & de Campos Bicudo, 2010; Schneider et al., 2011). Schneider et al (2011) observed that minor taxonomic shifts within periphyton communities occurred with small changes in total phosphorus (TP) concentrations (between 5-10μg/L) and major shifts with large changes (between concentrations of 10-30μg/L). Vis et al. (2008) indicated that the relative abundance of Cyanophyceae increased with exposure to urban wastewater with high nutrient concentration. Winter and Duthie (2000) observed similar results in their study of agricultural and non-agricultural study localities along two Southern Ontario waterways, Laurel and Carrol Creeks.

They determined that there was a difference in algal dominance when these sites were compared. It was concluded that run off from the agricultural field into the stream affected the overall growth of benthic algae especially with respect to the percentage cover of algae on the rocky surfaces and their taxonomic composition (Winter & Duthie, 2000).

Nutrient cycling within the periphyton community is thought to be sustainable for short periods of time (Mulholland et al., 1996; Mulholland & Webster 2010). Studies suggest that nutrient recycling within the periphyton community was responsible for 10-70% of the phosphorus (P) uptake with a daily P turnover rate of <15% per day (Mulholland et al., 1996; Mulholland & Webster 2010). This process would seem to benefit the periphyton community as a response to a reduced nutrient input from external sources.

Hydraulic Conditions

The effects of flow velocity, floods and spate events on periphyton community dynamics have been widely studied for many years, mostly in lotic environments (Peterson 1986; Stevenson 1990; Iwaniec et al., 2006; Gottlieb et al., 2006; Wiklund et al., 2010; Izagirre et al., 2009; Larned 2010). These studies excluded lentic systems and the role that hydraulic conditions play, such as wave turbulence, micro/macro currents, and water level changes (Larned 2010).

Biotic Influences

The periphyton community represents organisms belonging to several trophic levels. These members such as bacteria, micro algae, and protozoa engage in inter-specific interactions (Burgmer et al., 2010). Large invertebrate grazers use periphyton communities as habitat and also contribute to inter-specific interactions between trophic levels via grazing. Thus, predation and grazing play an important role in determining periphyton species composition.

Fitter & Hildebrand (2009) and Burgmer et al (2010) have observed that the entire assemblage of the periphyton community responded to the presence or absence of grazing meiofauna. Invertebrate grazers such as insect larvae, crustaceans and snails leave obvious tracks upon the non selective ingestion of the periphyton and therefore can produce a heavy grazing impact on periphyton community (Burgmer et al., 2010).

Meiofauna grazers, such as nematodes, can cause both positive and negative impacts on periphyton communities (Madji et al., 2011). The negative impacts include grazing and non selective disturbance within the periphyton community (Madji 2011), while the positive impacts include increased turnover rates of oxygen within the community. In addition, the grazing benefits microbial metabolism by creating increased light penetration as a result of tunnelling and disturbance (Madji et al., 2011; Mathieu 2007).

Phytoplankton

Phytoplankton are considered as one of the propagule pools for the periphyton community and therefore it influences immigration and emigration rates of periphyton members (Bellinger & Sigee 2010). The dynamic relationship between phytoplankton and periphyton is dependent on the drivers of light and nutrients. At high levels of nutrients, phytoplankton can proliferate at high rate and as a result, increases water turbidity causing a shading effect on periphyton by reducing light availability (Azim et al., 2010). However, Azim et al (2010) also found that at high rates of nutrient loading, periphyton communities experienced increased growth rates and as a result increased productivity within the community. This will result in increased rates of breakages and dislodging thereby contributing to the turbidity of the water column (Azim et al., 2010).

Thus, ecologically phytoplankton and periphyton communities are seen as separate communities which function independently, but have a strong interaction between each other in terms of resource availability. However, according to Vadeboncoeur et al (2002), Azim et al (2010), and Bellinger & Sigee (2010) the periphyton communities contribute slightly more than half to the lentic ecosystem productivity as a whole.

Periphyton as a tool for bio-monitoring

There are many characteristics of periphyton that can be used as water quality indicators of nutrients. These include variations in biomass (Ash Free Dry Weight or Dry Weight), taxonomic composition, species diversity, chlorophyll *a* and species succession. Many researchers have contributed to the validity of using periphyton as a bioindicator through quantification of these characteristics that periphyton provides (Table 1). Hillebrand and Sommer (2000) found that algal species diversity (Shannon Diversity) responded more sensitively to cultural eutrophication than other measures such as evenness and therefore is a better measure of eutrophication in a particular area. Many studies have used multi-proxy approaches by employing more than one parameter, such as Chlorophyceae, Bacillariophyceae, Cyanophyceae, to determine the validity of using periphyton as a reliable indicator of water quality, whereas, others studied only one group, such as Bacillariophyceae, protozoa or Chlorophyceae. The list demonstrates that there is a limited amount of research on periphyton from lentic aquatic systems (Table 1). It can be noted that diatoms are often the main focus of the study as opposed to other members of periphyton which may be due to the already developed trophic diatom indices over the last few years (Kelly et al., 1995; Rott et al., 1999; Potapova and Charles., 2003; Lavoie et al., 2006). The trophic diatom indices were developed as a rapid assessment tool for monitoring

changes within an aquatic ecosystem. Diatoms are ideal to use as there are typically the most dominant group within a periphyton community.

Table 1: Summary of studies that used periphyton as a monitoring tool of water quality based on nutrient measurements.

Author	Biomass	Chl *a*	Taxonomic Composition (including species diversity)	Diatoms or Periphyton (D or P)	Lakes or Rivers /Streams
Carrick et al., 1988			X	P	L
Delong & Brusvren, 1992		X		P	S
Kelly et al., 1995			X	D	R
Kwandrans, 1998			X	D	R
Vis et al., 1998	X	X		P	R
Masseret et al., 1998	X	X	X	P	R
Chessman et al., 1999			X	D	R
Carpenter & Waite., 2000			X	P	S
Winter & Duthie, 2000		X		D	S
Winter & Duthie, 2000			X	D	S
Hill et al., 2000	X	X	X	P	S
King et al., 2000		X	X	P	L
Lewis et al., 2001	X	X		P	Marine
Kumulaynen, 2002	X	X	X	P	R
Blinn & Herbst 2003			X	P	S
Kitner & Poulickova, 2003			X	D	L
Lemmen, 2003	X			P	Marine
Catteano et al., 2004			X	D	L
Poulickova et al., 2004			X	D	L
Komulaynen, 2004	X	X	X	P	R
Lavoie, 2004	X	X	X	D	R
Gaiser et al., 2006	X	X	X	D	Everglades
Potapova & Charles, 2007			X	D	R
Kelly et al., 2008			X	D	L
Lambert et al., 2008	X	X		P	L
Reavie et al., 2010			X	P	R
Delgado et al., 2010	X	X	X	D	R
Cardinale, 2011		X	X	P	S
Komulainen & Slastina 2012	X	X	X	P	R
Schneider et al., 2012			X	P	S
Smucker & Vis 2013		X	X	D	S
Schneider et al., 2013			X	P	S

Previous studies have shown that algae are capable of responding quickly and predictably to a wide range of pollutants (McCormick & Cairns 1994; Pan et al., 1996; Potapova et al., 2003). Historically, the mention of freshwater organisms as water quality indicators was done by Kolenati in 1848 and again by Cohn in 1853 (Liebmann 1962: Bellinger & Sigee 2010). Both these studies determined that the composition of freshwater organisms was different in polluted and non-polluted areas. Benthic algal communities have also been reported to be useful indicators of water quality (Dixit et al., 1992). More recent studies have evaluated the response of algae, specifically planktonic diatoms, to the input of newly identified water pollutants such as triclosan, atrizine and road salt run off (Nietch et al., 2013; Prosser et al., 2013; Cook & Francoeur 2013) in addition to the response of periphyton production after the biological invasion of *Dreissenid* mussels (Ozersky et al., 2013).

The use of periphyton as a successful indicator of phosphorus (P) has been reported by Gaiser et al (2006) while studying in the Florida Everglades aquatic ecosystem. They found that the species composition of periphyton community closely represented the total phosphorus (TP) concentration in Everglades through the observation of the dominance shift of taxa in relation to the TP measurements. However, the need of marsh specific approach in determining periphyton species composition based water quality index has been suggested by many workers as there is a possibility of variation caused by local environment as many periphyton species will be common within a specific geographic area (Potapova & Charles 2002; Gaiser et al., 2006; Reavie 2010). However, the species dominance will be directed by TP values at specific sites. While studying in wetland ecosystems, Lane & Brown (2007) and Reavie (2010) found that epiphytic diatoms were more responsive to human disturbances than phytoplankton. The significance of local and geographical scales while comparing phytoplankton and periphyton as water quality indicators

has been stressed by Potapova & Charles (2002) and Reavie (2010). Periphyton is currently being used as a bio-assessment tool of water quality in the European Union, the Environmental Protection Agency, USA and the Ministry of the Environment in New Zealand. All of these protocols focus on stream bio-assessments. The role of a bio-assessment tool is to rapidly determine the health of a water body at a moment in time.

Past periphyton studies

Periphyton has been a topic of study for over several decades. Early studies included the impacts of abiotic parameters and the seasonal variation of periphyton growth in streams (Brown 1908; Eddy 1925) (Figure 2). This was followed by more comprehensive studies which included the N-fixation of cyanobacteria (Allison & Morris 1930) (Figure 2). By the mid to late 20th century studies on algal response to abiotic factors such as the water flow velocity, light and substrate have appeared (Huntsman 1948; McConnell & Sigler 1959; McIntire 1966; Siver 1977; Horner & Welch 1981) (Figure 2).

The breadth, depth and complexity of periphyton studies have advanced greatly over time due to technological advances such as the introduction of the Scanning Electron Microscope, Molecular Biology techniques, etc. More recent studies (Table 2) include the examination of molecular finger printing of microalgae (Szabo et al., 2008); the allelopathic control of cyanobacteria blooms by periphyton biofilms (Wu et al., 2011); the taxonomic distinctness of algae (Leira., 2009); the effects of biological invasive species on periphyton (Cecala et al., 2008; Ozersky et al., 2013; Stevic 2013); the effect of multiple stressors on periphyton (Rotter et al., 2013); and the relationship dynamics between periphyton and phytoplankton assemblages (Zebek 2013, Mihaljevic., et al 2013).

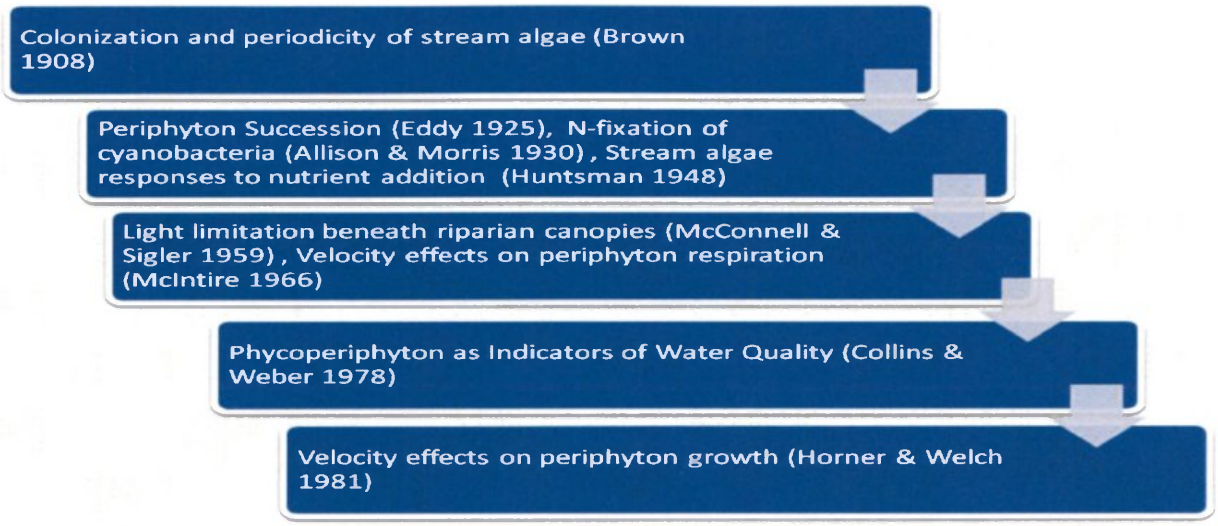


Figure 2: The evolution of periphyton studies (Larned 2010).

Table 2: Specific areas of periphyton research and the references.

Area of study of Periphyton	Author/ year
Effects of light/riparian zone shade on periphyton growth	Burgmer et al., 2010; Porter- Goff, 2010; Bellinger & Sigee 2010.
Invertebrate grazing/ herbivory effects	Rosemond et al., 1993; Burgmer et al., 2010
Taxonomic distinctness	Leira et al., 2009
Effect of substrata	Siver, 1977; Catteneo & Amireault 1992; Rodriguez 1993; Lowe et al., 1996; Sabater et al., 1998; Danilova & Ekelund, 2001; Pizarro et al., 2002; Potapova & Charles, 2005.
Use as a water quality indicator	Carrick et al., 1988; Delong & Brusvren 1992; Kelly et al., 1995; Kwandrans et al., 1998; Vis et al., 1998; Masseret et al., 1998; Chessman et al., 1999; Carpenter & Waite 2000; Winter & Duthie 2000; Hill et al., 2000; King et al., 2000; Lewis et al., 2001; Komulaynen, 2002; Kitner & Poulickova 2003; Lemmen, 2003; Catteano et al., 2004; Poulickova et al., 2004; Komulaynen, 2004; Lavoie 2004; Gaiser et al., 2006; Potapova & Charles 2007; Kelly et al., 2008; Lambert et al., 2008; Reavie et al., 2010; Delgado et al., 2010; Cardinale, 2011; Smucker & Vis 2013
Use of periphyton in aquaculture as alternative food source for fish	Azim et al., 2005
Colonization of organisms in the periphyton community (Community growth dynamics)	Szabo et al., 2008; Sekar et al., 1998; O'Toole et al., 2000; Sekar et al., 2004; Taniwaki et al., 2013.
Periphyton responses to anthropogenic toxic inputs (i.e gas spill, herbicides, triclosan, atrizine, road salt,	Shortreed & Stockner, 1983; Kosinski, 1984; Falasco et al., 2009; Larras 2013; Nietch et al., 2013; Prosser et al., 2013; Cook & Francoeur 2013.
Allelopathic control of cyanobacteria blooms by periphyton biofilms	Wu et al., 2011
Effects of the biological invasive *Dreissena polymorpha* on periphyton	Cecala et al., 2008; Ozersky et al., 2013; Stevic 2013
Multi stressor influences on periphyton	Rotter et al., 2013
Inter-relationship of periphyton and phytoplankton assemblages	Zebek, 2013; Mihaljevic et al., 2013

Knowledge Gap & Research Rationale

There is a limited amount of research on the use of periphyton as a water quality indicator in lentic environments as well as studies on colonization, succession and autogenic processes occurring within periphyton communities (see Tables 1 & 2). However, there is a copious amount of literature available on periphyton from lotic environments. Studies that used algae as a water quality monitoring tool mostly collected phytoplankton samples from the pelagic zone of a lake or that occurred on deep water sediment samples (Poulickova et al., 2004; Liboriussen & Jeppeson 2006). This opens up the need for studies on periphyton community collected from the near shore areas to be used as a tool to monitor water quality of the lentic systems. Moreover, periphyton growing in the fringe areas is exposed directly to land originated effluents and therefore is considered as one of the best suitable communities to understand the water quality changes of lentic water systems.

In a lentic system, periphyton acts both as a source of primary production as well as a connecting link between near-shore and pelagic areas since they contribute to the phytoplankton population (by breakage/ sloughing-off) though this is a topic of debate (Moss et al., 1981). The hydrodynamics of a water body help the distribution of land originated nutrients along the shoreline towards the deeper benthic zones and into pelagic zones (Macintyre & Melack, 1995). However, in addition to the hydrodynamic distribution of nutrients, organisms with high motility often assist in the distribution of nutrients by moving littoral matter from the near shore areas to the pelagic zone (Hampton et al., 2011). Many remediation and restoration strategies for lakes occur in the littoral zone through the use of vegetation, and the use of periphyton as a bio-

monitoring tools by identifying and quantifying members of the community. This could contribute greatly to the sustainability of the ecosystems by providing early detection of changes in water quality.

Anthropogenic activities that contribute to the degradation of water quality often occur near the shoreline. The near shore habitat is influenced greatly through landscape changes, effluent discharges, fishing, boating and wading activities (Bellinger & Sigee 2010). Drainages from faulty sewage/septic systems located near the edge of the water body also can reach lake water. As previously mentioned, since periphyton communities generally grow in the fringe areas of the lake, they are the communities exposed to these anthropogenic activities directly and therefore are the ideal community to observe water quality changes. The quick response to water quality changes (or any stressors) is reflected on periphyton community, such as taxa shifts, species richness and species diversity variation, therefore acting as an ideal indicator of water quality changes.

The health of Lake Simcoe has a long history of water quality degradation due to the influence of excessive nutrient input originating from anthropogenic activities such as intensive agriculture, septic tank leakages, and effluent output from waste water treatment plants, in addition to the occurrence of biological invasions e.g. *Dreissenia polymorpha* and *Bythotrephes longimanus* (Ozersky et al., 2013; Kelly et al., 2013). Lake Simcoe has experienced high phosphorus load throughout the last few decades due to urbanization, agricultural runoff and effluent release (Winter et al., 2007; Young et al., 2010; Palmer et al., 2011; North et al., 2013; Palmer et al., 2013)

Excessive nutrient input from intensive agriculture has led to eutrophication in some parts of Lake Simcoe (North et al., 2013). Literature review showed that there is a limited amount of data on water quality from the northern part of Lake Simcoe compared to southern or central parts. In addition, data on periphyton research showed very little information available from the littoral zone of Lake Simcoe. Therefore, this study aims to fill this gap by generating base line data on periphyton community dynamics from the littoral zone of northern Lake Simcoe. The data generated will help to design a periphyton based water quality index for this part of Lake Simcoe. The study will emphasize on microalgae especially on diatoms and non-diatoms of the periphyton community. This study thus will help to design a more cost effective management strategy for Lake Simcoe through the examination of periphyton community changes in relation to water quality.

Study Design:

In order to understand the periphyton community dynamics (biofilm thickness, biomass, species density, species richness and species diversity) with season, location of study (degree of exposure to anthropogenic activities), and duration of exposure field studies were carried out from three different locations in the northern part of Lake Simcoe. The study was repeated 4 times over a 2 year period to represent different seasons of a year (SP1=Fall 2011, SP2=Spring2012, SP3=Summer 2012, SP4=Fall 2012). Once autogenic species processes have been understood, influences of temperature and lake turnover processes on periphyton community have been determined from the data. In addition, the influence of environmental parameters such as nutrient concentrations and other water chemistry parameters on periphyton community was also studied from the data.

The hypotheses tested were:

1. The periphyton community dynamics (biofilm thickness, biomass, species density, species diversity, species richness) vary with season and location (degree of exposure to anthropogenic activities) of study.

2. The periphyton species diversity decreases as a result of increased nutrient availability.

3. The periphyton community dynamics are influenced by autogenic processes.

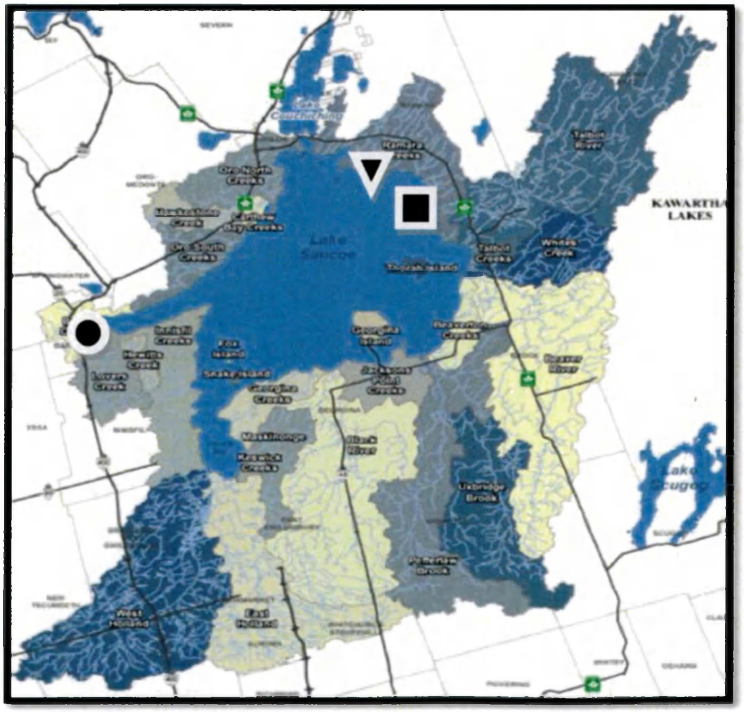
4. Diatom abundance and species composition will increase in spring and fall seasons as a result of lake turnover processes.

The following part of the thesis is divided into 4 chapters. Chapter 2 describes the general methodology employed in this study. Chapter 3 focuses on the colonization and successional patterns in periphyton including spatial and temporal dynamics. Chapter 4 describes periphyton community dynamics as an index of water quality changes. Finally, chapter 5 provides an overall summary, conclusion and future research suggestions.

Chapter 2: Materials and General Methods

Site Selection

The study was conducted at three sampling sites located in the northern part of Lake Simcoe, namely Kempenfelt Bay (KB), Barrie, ON (44.377858,-79.689331), Concession Point 10 (C10), Ramara Township, ON (44.590956,-79.317856), and Lagoon City (LC), Brechin, ON (44.546931,-79.209366) (Figure 3). Lake Simcoe is a hard water, dimictic lake with a surface area of 722km^2. The entire watershed area consists of 2899km^2 (Palmer et al., 2011; North et al., 2013). It is divided geographically by two large bays, Cook's Bay, located at the southern tip of the lake (mean depth 13m, maximum depth 15m, surface area 44km), Kempenfelt Bay near Barrie, Ontario (mean depth 14m, maximum depth 42m, surface area 34km^2), and the large shallow main basin which covers the northeastern portion of the lake (mean depth 14m, maximum depth 33m, surface area 643km^2) (Winter et al., 2007; Young et al., 2010; North et al., 2013). It is a main connecting passage of the Trent Severn Waterway (North et al., 2013). The lake's water retention time is approximately 11 years as it drains into Lake Couchiching by way of the Atherley Narrows (Young et al., 2010; North et al., 2013).



● Kempenfelt Bay, Barrie (KB)

▼ Concession Point 10,Ramara Township (C10)

■ Lagoon City, Brechin, ON (LC)

Figure 3. Map of Lake Simcoe and the three sampling locations.

Sampling site descriptions

The sampling sites were chosen according to varying degrees of anthropogenic disturbances that they are exposed to. The disturbances include effluent discharge from a sewage treatment plant (KB, Barrie), a fairly undisturbed area (C10, Ramara Township) and an intense lakeshore residential development area (LC, Brechin).

Site 1. Kempenfelt Bay, Barrie (KB)

This site is located in the treated effluent release discharge canal that empties into Kempenfelt Bay. This site is less than a kilometre away from the Barrie Water Pollution Control Centre (WPCC). The WPCC treats effluents for the city's population of approximately 196,000 (StatsCan 2014). The site also has a high traffic tourist public park/beach, Centennial Beach, which is next to a heavily used boat ramp.

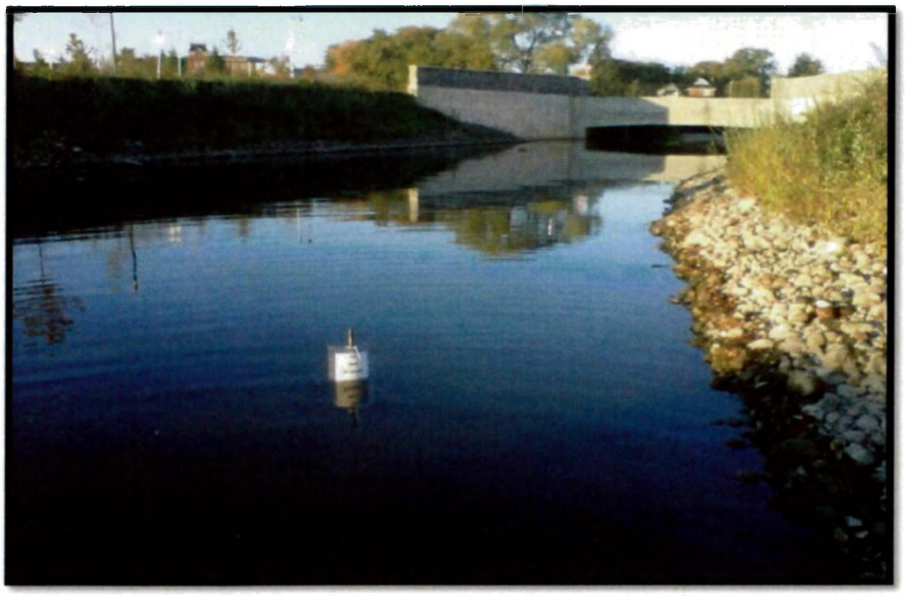


Figure 4. Site 1. Kempenfelt Bay, Barrie, ON.

The public beach is often under a swimming advisory during summer months as a result of unsafe levels of bacteria (Simcoe District Health Unit 2014) and provides habitat for many Canadian geese and various duck species. Due to the occurrence of the above mentioned environmental disturbances, including a point source nutrient input (effluent discharge from water treatment plant), this site is considered as a highly disturbed area.

Site 2. Concession Point 10, Ramara Township (C10)

The second site (C10) is located in the nearshore of northern Lake Simcoe in the Ramara Township. This is a quiet bay surrounded by few cottages (approximately 40 dwellings). The mode of sewage waste treatment in this vicinity is through holding tanks and septic beds. Ditches on both sides of the main gravel road provide easy run-off to the lake, as one of the ditches connects directly with the lake.

Figure 5. Site 2. Concession 10, Rama, ON.

Many boats and personal watercraft reside in this bay in addition to supporting a takeoff and landing area of local floatplanes. Anthropogenic influences are considered at a lesser degree at this site compared to other two therefore this site is considered as the least disturbed one in this study.

Site 3. Lagoon City, Brechin (LC)

Site three (LC) is located in the north western area of Lake Simcoe, in Lagoon City, Brechin, Ontario. Lagoon city is a popular tourist location for boaters and cottagers due to its easy access to Lake Simcoe and the Trent Severn waterway via long and winding interconnecting canals (LCCA, 2014). The area of Lagoon City was formerly a wetland (pre 1970s).

Figure 6. Site 3. Lagoon City, Brechin, ON.

The sampling site is located in the main canal leading to Lake Simcoe approximately 500m away from the water treatment centre. Lagoon city also provides its ~ 3,000 residents with sewage treatment services via a treatment plant built in the 1990s (Town of Ramara, 2014). As a

result of anthropogenic stressors such as excessive boating, nutrient enrichment and degradation of natural terrain, this site is considered a moderate to highly disturbed one.

Periphyton collection

Periphyton samples were collected over a 1 year period during 4 sampling periods representing ice free seasons (Table 3) using collection rigs (Figure 7), containing fifty (50) glass slides (10cm x 3cm x 0.3cm). The rigs with cleaned slides (slides were cleaned using detergent devoid of phosphate, rinsed with tap water followed by deionized water and air dried before suspension), were submerged in the littoral zone of the three sampling locations (approximately 10-20cm below the surface water). Six glass slides each were randomly collected at a five day interval to a maximum of 30 days. The slides were carefully removed from the rig and inserted into clean 150 ml polyethylene bottles containing surface water collected from the sampling location (separate polyethylene bottles were used for each slide). Slides were collected in unfiltered water and delivered into the sample containers underwater to avoid the collapse of the natural biofilm. All samples were stored in a cooler box containing ice until arrival at the lab.

Table 3. Schedule of sampling dates.

Sampling Period	**Dates (30 day duration)**
1	Fall 2011 (October 7 to November 11)
2	Spring 2012 (May 11 to June 15)
3	Summer 2012 (August 16 to September 11)
4	Fall 2012 (October 12 to November 7)

Glass slides were used as they are considered an inert substrata and an inexpensive easy way to collect and examine microalgae (Oemke & Burton 1986; Kanavillil et al., 2012; Kanavillil & Kurissery 2013).

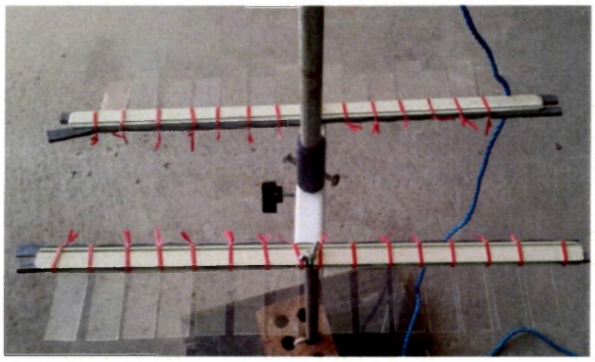


Figure 7. Periphyton collection rig. The rig is deployed on day zero of the study with 50 slides. The steel pole is driven into the sediment and the slides trays are submerged at depths of 10- 20cm below the water surface.

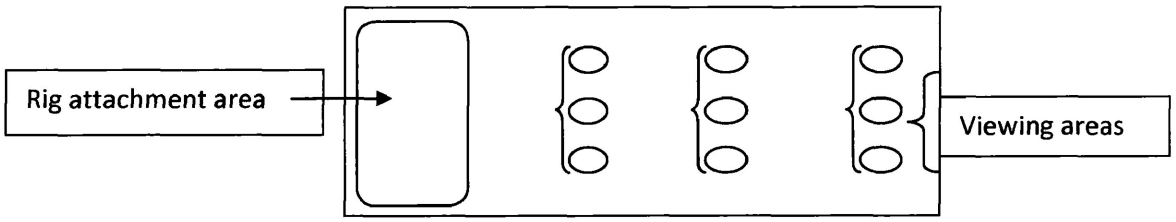
Periphyton analysis

Periphyton analysis during this study involved microalgae (diatom and non-diatom groups) and protozoa. Bacteria were not enumerated during this study. The microalgae were quantified and identified to the species level using identification keys and manuals.

Periphyton enumeration and identification were carried out by the following method; one side of the glass slide was cleaned by wiping with cotton (chosen randomly) and the intact periphyton was examined live under a compound light microscope (Ken-a-vision). Triplicate slides were analysed for periphyton community analysis. The surface of the glass slide was continuously watered (filtered surface lake water) by using a Pasteur pipette (Kanavillil & Kurissery, 2013). Nine predetermined viewing areas (Figure 8) were examined for taxonomic composition (diatom and non diatom species), species density, and biofilm thickness. Identifications were made using 400X or 1000X magnification, and microalgae were identified to genus and, if possible, species level, by using identification keys and manuals (Prescott 1978;

Round et al., 1990; Kelly et al., 2005; Wehr et al., 2002; Spaulding et al., 2010). Motile cells were counted first to avoid omission. Cell over lay and optical quality was handled by constant refocusing within the many layers of the biofilm. Each layer was independently counted to avoid duplication of cells. Where growth was too thick to observe clearly the viewing co-ordinates were adjusted accordingly. On two separate occasions thick growth and inorganic depositions made it impossible to count on the substrate and the sample slide had to be scraped with a razor and the scraped slide was then rinsed 5mls of filtered lake water and enumeration was performed on a haemocytometer in triplicate (PHE 2014). Biofilm thickness was measured in micrometres at each viewing area by recording the measurement at substratum surface (A) and at the top of the biofilm (B) (Sekar et al., 2004). The thickness was calculated by subtracting B from A. The nine (9) viewing area values were averaged to get a mean wet biofilm thickness (Sekar et al., 2004).

Figure 8. Viewing areas of a glass slide.



Periphyton biomass was determined by following the APHA method (APHA 2005). Each periphyton slide was scraped (one side) with a hard bristle brush and rinsed with 10ml of filtered lake water. An additional aliquot of 5ml of water was used to rinse the brush. The scraped extract was filtered through a pre-weighed Whatman GF/B 47mm glass fibre filter. The filter paper was dried at 50°C in an oven for 24-48 hours and re-weighed. The biomass was finally expressed as mg (dry weight)/cm^2.

Measurement of periphyton chlorophyll *a* concentration was done by following APHA protocol (APHA, 2005). This involved scraping of the periphyton from one side of the glass slide (in triplicate) by using 10 ml of filtered lake water and filtering through Whatman GF/B 47mm glass fibre filter. The filter paper with the periphyton was extracted using 90% acetone (12 ml) in a refrigerator for 16-24hr. Upon extraction, the samples were centrifuged for 15 minutes at 1055.10 (g), the supernatant was extracted using a Pasteur pipette and transferred into a 10ml acid washed, glass test tube which was inserted directly into a Beckton Dickinson Spectrophotometer (DU700) where the corrected absorbance measurements (750,664,647, and 630nm) were recorded and the chlorophyll *a* concentration was calculated and expressed as mg/cm^2.

Water parameters

During each sampling day, water samples were collected to measure biochemistry parameters such as dissolved oxygen, conductivity, pH and temperature. Water pH, dissolved oxygen and conductivity were measured in-situ by using a hydro lab (VWR Symphony SB9 0M5), ambient water temperatures were measured by using a Fisher Scientific thermometer. Water samples were also collected for nutrient analysis and chlorophyll *a* using clean 1L polyethylene bottles from each sampling area (10-20 cm below the water surface, the same depth at which slides were suspended; Kanavillil & Kurissery, 2013). Unfiltered water samples for nutrient analysis were stored in freezer until analyses were carried out. Total phosphorus was analyzed (in duplicates) following the standardized persulfate oxidation digestate method (APHA, 2005). Ascorbic acid reagent pillows (HACH) were used for final determination.

Chlorophyll *a* measurements give an estimation of primary productivity of the water column and that of periphyton. In this study, chlorophyll *a* from the water column was measured

according to the APHA standard protocol (APHA, 2005). This was done by filtering 1 litre of water sample through a 0.045mm Whatman GF/B47mm glass fibre filter and extracting chlorophyll *a* in 12 ml of 90% acetone at 4°C for 16-24hr. The remaining steps were the same as that described for periphyton samples. Chlorophyll *a*, concentrations were expressed as mg/cm^3 (APHA2005).

Data analysis

Species density was expressed as an average of cells/organisms observed per cm^2. Dominant species were determined from the average density of each species present. In this study dominance is described as the species having highest density and present consistently throughout the 30 days compared to rest of the species. Species diversity was calculated by using the Shannon-Weiner Index (Ricklefs, 2001).

The Shapiro-Wilk test for normality resulted in the failure to meet parametric assumptions for species density data. To meet parametric assumptions periphyton communities were subdivided into 2 groups, diatoms and non diatoms. Species density was $\log_{10}$ transformed to down weigh species dominance effects and resulted in a normal distribution (Kilroy et al., 2006). This procedure also assisted in the management of outliers. Additionally, rare species that appeared in low numbers >1 and present only once during the entire sampling period were removed from the analysis.

The values for biofilm thickness were $\log_{10}$ transformed while the values for chlorophyll *a* (periphyton and the water column) and biomass were transformed to square root to allow for linearity during data comparison. Data for total phosphorus, and hydrological parameters such as temperature, DO, pH, and conductivity remained unchanged and raw values were used and resulted in normal distribution.

The biofilm parameters such as thickness, species density, species richness, species diversity, chlorophyll *a,* and biomass within study period and between sampling locations were compared using repeated measure two way ANOVA with Greenhouse-Geisser Correction (IBM SPSS Statistics, Ver19, SPSS Inc, Armonk, New York, USA). *Post hoc* tests using a Bonferroni Correction were performed to test pair wise comparisons (Kanavillil & Kurissery, 2013). One way analysis of variance was performed on all biochemistry parameters between sampling periods and sites (Microsoft Excel, 2007).

Species densities of periphyton communities (grouped into diatoms and non diatoms) and were ordinated using Canonical Correspondence Analysis (R Project for Statistical Computing http://www.r.project.org, Vegan Package) to summarize the species composition of each sampling period in addition to testing the possible relationships between environmental factors and the species distribution (Palmer 1993; ter Braak 1995) .

In addition, relationships between all parameters studied such as species density (periphyton & phytoplankton), biofilm thickness, chlorophyll *a* (periphyton & water column), biomass, DO, TP, pH, conductivity and temperature were tested using linear regression analysis (r^2) (Microsoft Excel, 2007).

Chapter 3: Colonization and succession of periphyton community on glass slides in Northern Lake Simcoe.

Introduction

Periphyton communities are ubiquitous in nature and are part of a larger dynamic natural biofilm matrix of both photoautotrophic and heterotrophic organisms, including diatoms, green algae, bacteria, Cyanophyceae, protozoa and other invertebrates (Wetzel 1983; Azim et al., 2005; Wu et al., 2011). Many members are considered primary producers and contribute to various trophic levels within the food web.

Periphyton growth dynamics exhibit various colonization, growth and successional patterns (Sekar et al., 2004; Szabo et al., 2008). Periphyton community composition is heavily influenced by nutrient and light availability (Gustina 1996; Von Schiller et al., 2007), physical disturbances such as flow rate, wave action etc. (Peterson, 1986; Stevenson, 1990; Iwaniec et al., 2006; Gottlieb et al., 2006; Cecala et al., 2008; Izagirre et al., 2009; Wiklund et al., 2010) and biotic factors such as grazing pressure (Rosemond et al., 1993; Burgmer, 2010). Thus, the taxonomic composition and species succession are influenced by water quality, immigration/emigration and reproduction rates of the periphyton members (Pan et al., 1996; Sekar, 2004; King et al., 2006; Bellinger & Sigee, 2010). Different modes of attachment, especially those of Bacillariophyceae, will also influence the species dominance of periphyton community during its different phases of development (Kanavillil et al., 2014).

The literature survey (chapter 1) indicates that there is a limited amount of data on periphyton community dynamics from temperate area compared to tropical area, especially from lentic water systems. Data on this is thought to help us better understand the health of water

systems being studied and therefore to take adequate ecosystem restoration strategies if needed. In order to understand periphyton community succession with season, location of study (degree of exposure to anthropogenic activities), and duration of substratum exposure, 30 days field exposure studies were carried out from three different locations in the northern part of Lake Simcoe. The study was repeated four times to represent different seasons of a year. The hypotheses tested were:

1. The periphyton community dynamics vary with season and location (degree of exposure to anthropogenic activities) of study.

2. The periphyton species diversity decreases at areas with increased nutrient availability.

3. The periphyton community dynamics are influenced by autogenic processes.

4. Diatom abundance and species composition increase in spring and fall seasons as a result of lake turnover processes.

This chapter describes the successional patterns of periphyton communities in three locations in northern Lake Simcoe exposed to varying degrees of anthropogenic influences. The community dynamics have been studied from the data on taxonomic composition, biomass, chlorophyll *a* and biofilm thickness during the growth period of 30 days.

Methods

Detailed general methodology is described in chapter 2. The study was conducted at three sampling locations in the northern part of Lake Simcoe, namely Kempenfelt Bay, Barrie, ON (44.377858,-79.689331), Concession Point 10, Ramara Township, ON (44.590956,-79.317856), and Lagoon City, Brechin, ON (44.546931,-79.209366) (Figure 3).

Periphyton samples were collected with the help of collection rigs (Figure 4), containing fifty (50) glass slides (10cm x 3cm x0.3 cm). Extra slides were used for replicates and in case of

breakage or loss. The rigs with cleaned slides were submerged in the littoral zone of the three sampling locations (approximately 10-20 cm below the surface water).

Periphyton members mainly diatoms, green algae and cyanobacteria were quantified and identified to species level by using various identification keys and manuals (Prescott 1978; Round et al., 1990; Wehr et al., 2002; Kelly et al., 2005; Spaulding et al., 2010). The data obtained were taxonomic composition (diatom and non diatom species), species density, relative abundance, and biofilm thickness.

Hydrological parameters such as dissolved oxygen, conductivity, pH and temperature were measured during each sampling day. Ambient water temperatures were measured by using a Fisher Scientific thermometer. Unfiltered water samples were also collected for nutrient and chlorophyll *a* analyses in clean 1L polyethylene bottles from the sub-surface area (10-20 cm below the water surface, the same depth at which slides were suspended) (Kanavillil & Kurissery, 2013). Water samples for nutrient analysis were stored in a freezer until the analyses were performed.

Data analysis

Species density was expressed as an average of number of cells observed per cm^2. Species dominance was determined from the average density of each species present in the community. Species diversity was calculated using Shannon-Weiner Index (Ricklefs, 2001). The data were analyzed with the help of various statistical tests as described in the chapter 2.

Results

Hydrological parameters

Temperature varied significantly over the four sampling periods, ranging from 5-28°C ($F_{3,72}$=30.68 , p<0.05). The maximum temperature was recorded during sampling period 3 at LC (28°C). The lowest temperature value was recorded during sampling period 4 at C10 (5°C). The dissolved oxygen (DO) concentration varied significantly ranging from 3.44-10.7mg/L between sampling periods ($F_{3,72}$=10.61, p<0.05), but did not vary significantly between the days in each sampling period or sites. Conductivity values varied from 334-1183µS/cm. It varied significantly between sites ($F_{3,72}$=10.12, p<0.05), but did not vary significantly between sampling periods of a particular site or with the days of observation in each sampling period. The highest conductivity values recorded was at KB (Table 4), located near the treated effluent release area of the City of Barrie's Waste Water treatment plant. The conductivity values of other two locations were approximately half that of KB.

Over the entire study period water TP concentrations ranged from 0.006 to 0.200mg/L. One way ANOVA results showed a significant variation between sites ($F_{2,69}$= 2.99, p<0.05), and days of observation within each sampling period ($F_{2,69}$=3.88, p< 0.05), but did not vary significantly between sampling periods. More specifically KB and LC, situated in the vicinity of effluent release from waste water treatment plants, had consistently higher TP concentrations than C10. In terms of compliance with provincial environmental standards, all sites exceeded the Provincial guideline, i.e. concentration of 0.02mg/L, with the exception of sampling period 2 at KB.

Chlorophyll *a* concentrations of periphyton varied from 0.09 to 1.41mg/m^3. Periphyton chlorophyll *a* concentrations varied significantly between sites ($F_{2,47}$=322.45, p<0.05) and days

of growth ($F_{5,48}$=4.92, p<0.05, Table 5). The maximum concentration of periphyton chlorophyll *a* was recorded at KB during sampling period 3 (1.41mg/m^3). Chlorophyll *a* concentrations of periphyton showed a general increase up to 20-25 days. This was followed by a decrease to the end of the sampling period (30 days). The trend was similar for periphyton micro-algal density and the relationship between periphyton density and periphyton chlorophyll *a* in sampling period 1, 2, & 4 was significant (r^2=0.44, p<0.05, r^2=0.55, p<0.05, r^2=0.33, p<0.05, respectively for sampling period 1, 2,and 4). Interaction results of two- way rmANOVA between sites and days, sites and sampling period, days and sampling period and sites and days and sampling period also resulted in significant variation in periphyton chlorophyll *a* concentrations (Table 5).

Chlorophyll *a* concentrations in the water column during the study period varied from 0.05 to 40.80mg/m^3. It varied significantly between sampling periods, (one way ANOVA $F_{3,68}$=9.49, p<0.05) and reached moderate significance between sites (one way ANOVA $F_{2,69}$=2.48, p=0.09).

The maximum concentration of chlorophyll *a* from the water column was recorded during sampling period 2 at LC (40.80mg/m^3). Generally, chlorophyll *a* concentrations in the water column was more stable than those of the periphyton. For example, during sampling period 3 there was a general increase over the days of observation in all sites. Exceptions to this trend were observed during sampling period 4 at sites C10 and LC where a steep decrease in chlorophyll *a* concentrations was observed on day 30 of the sampling period.

Table 4. Mean values and standard deviations in parenthesis of hydrological parameters at all sites and sampling periods (SP). DO refers to dissolved oxygen and TP refers to total phosphorus.

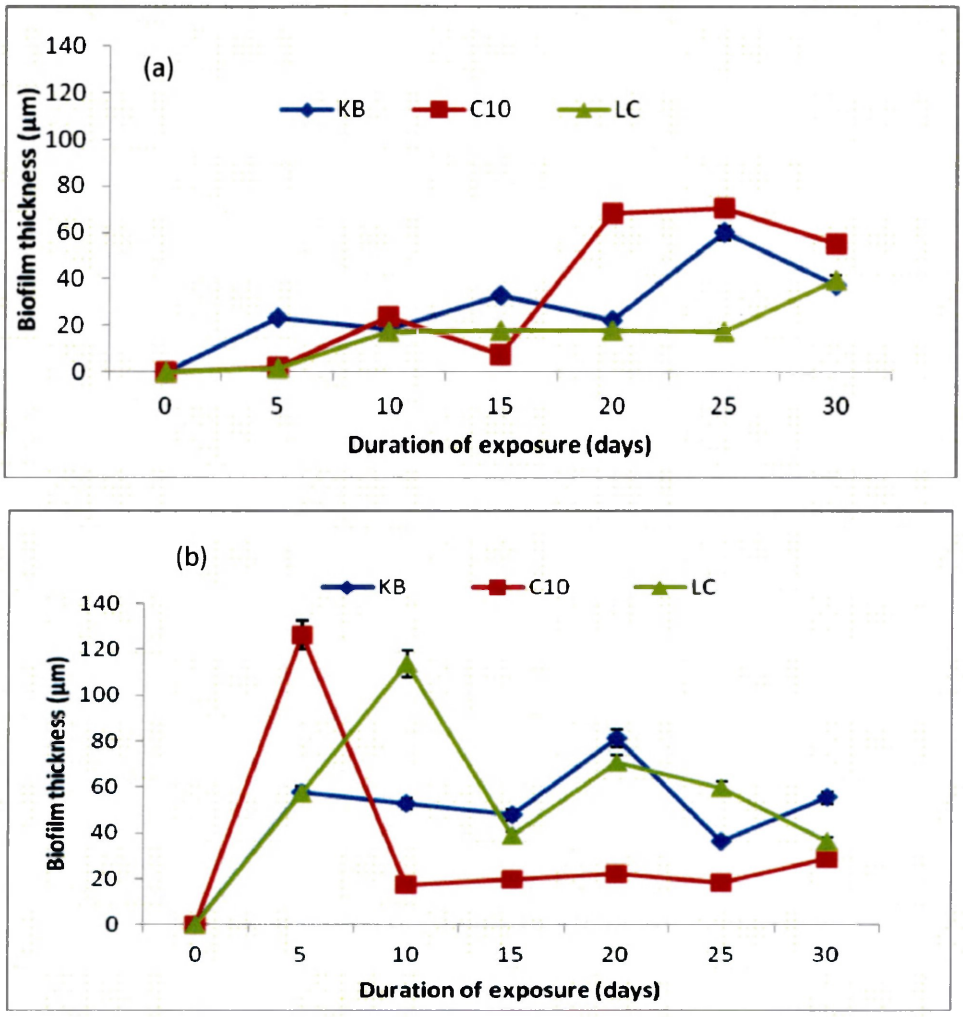
KB	Temperature °C	DO	pH	Conductivity µS/cm	TP (mg/L)	Chlorophyll *a* (Periphyton) mg/m^3
SP1	11.42 (3.01)	4.80 (0.82)	7.85 (0.15)	636.83 (228.83)	0.05 (0.03)	0.61 (0.28)
SP2	14.83 (3.97)	8.33 (1.42)	7.58 (0.14)	803.33 (271.83)	0.07 (0.06)	0.91 (0.41)
SP3	20.17 (1.47)	5.62 (2.12)	7.71 (0.29)	475 (74.23)	0.06 (0.04)	0.82(0.39)
SP4	9.25 (1.84)	8.4 (0.85)	7.25 (0.39)	NA	0.10 (0.60)	0.89 (0.21)
C10						
SP1	11.33 (3.08)	5.37 (1.08)	7.83 (0.35)	389.33 (60.85)	0.05 (0.02)	0.48 (0.26)
SP2	16.5 (2.51)	8.3 (0.56)	734 (0.27)	403 (5.36)	0.05 (0.05)	0.34 (0.15)
SP3	21.5(2.59)	7.73 (0.55)	7.83 (0.20)	386.67 (10.33)	0.03 (0.02)	0.21 (0.05)
SP4	8.833 (2.93)	7.95 (1.70)	7.25 (0.32)	NA	0.07 (0.03)	0.45 (0.06)
LC						
SP1	11.5 (3.39)	5.65 (0.97)	7.93 (0.20)	401.67 (34.91)	0.07 (0.04)	0.18 (0.06)
SP2	20.33 (2.88)	6.87 (1.09)	7.68 (0.24)	435.05 (17.95)	0.04 (0.01)	0.38 (0.28)
SP3	23 (2.68)	7.53 (0.94)	7.83 (0.20)	395 (16.43)	0.06 (0.04)	0.22 (0.15)
SP4	9.42 (2.25)	8.05 (0.67)	7.50 (0.22)	NA	0.04 (0.02)	0.42 (0.23)

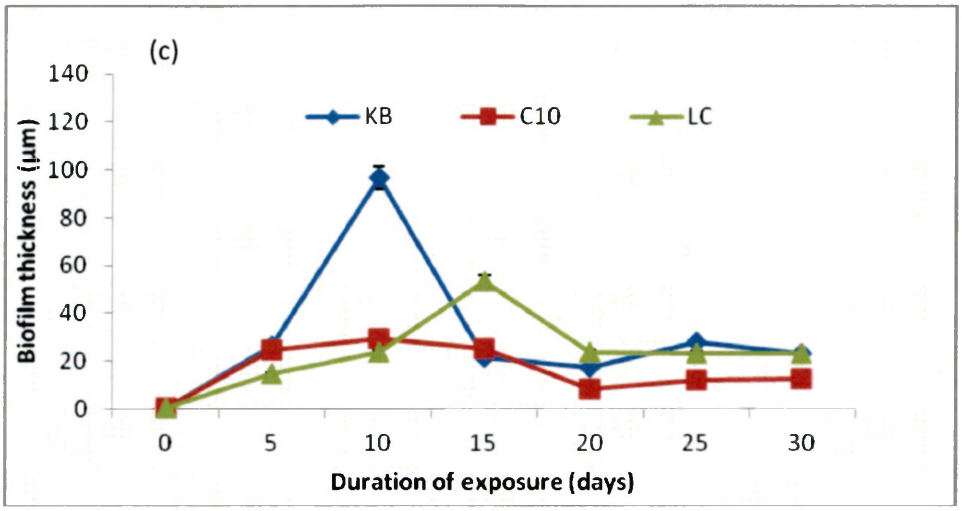
Periphyton characteristics (biofilm thickness, biofilm biomass, species density, species richness and species diversity)

Biofilm thickness values showed a normal growth pattern with one distinct peak demonstrating a gradual increase until 15-20 days followed by a decrease (Figure 9). The variation in biofilm thickness during the study was minimum at KB (18.11-96.67µm) while maximum at C10 (1.78-126.33µm). According to the two way rmANOVA results, biofilm thickness varied significantly over the duration of study period ($F_{3,48}$=187.92, p<0.05; Table 5).

Thickness also varied significantly between sampling periods ($F_{3,48}$=177.44, p<0.05; Table 5) and between sites ($F_{1,48}$=605.68, p<0.05, Table 5). The *post hoc* analysis showed that the thickness varied significantly only between days 5 and 15 during the sampling periods ($F_{3,48}$=786.15, p<0.05). Interaction results of two way rmANOVA between sites and days, sites and sampling period, days and sampling period and sites and days and sampling period resulted in significant variation of biofilm thickness (Table 5).

Figure 9(a-d). Variation in biofilm thickness observed on glass slides during the study period. Biofilm thickness values of 4 sampling periods (sampling periods 1-4) were averaged independently and plotted against duration of slide exposure. Error bars represent the 95% confidence limit.





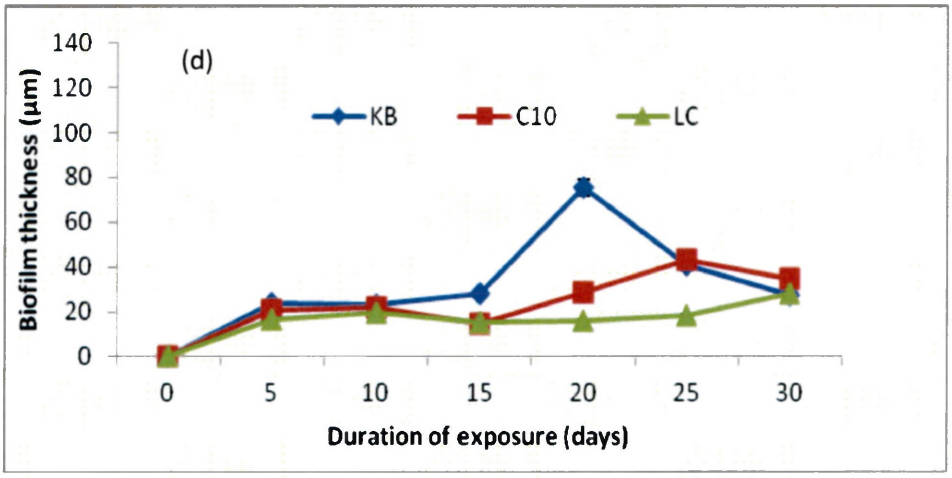


Table 5. Results of two way repeated measure ANOVA for biofilm thickness, species density, chlorophyll *a*, biomass, and species richness observed in three study sites during four sampling periods. The resultant *F* values and *p* values were obtained after Greenhouse-Geisser correction. *Post-hoc* tests on pair-wise comparison were carried out using Bonferroni correction (the significant results are mentioned in the text).

Source	**ss**	**df**	**ms**	***F***	***p***
Total species density (Slides)					
Sites	20.921	2	19.879	835.209	0
Days	21.595	5	4.319	8.099	0
Sampling Periods	2.201	3	0.734	1.376	0.261

Sites X Days	6.308	10	1.199	50.366	0
Sites X Sampling period	12.438	6	3.94	165.524	0
Days X Sampling period	16.624	15	1.108	2.078	0.028
Sites X Days X Sampling period	11.943	30	0.757	31.786	0
Chl a Slides					
Sites	4.068	2	2.657	503.851	0.000
Days	2.043	5	0.409	4.923	0.001
Sampling Periods	0.982	3	0.327	3.945	0.014
Sites X Days	0.792	10	0.103	10.503	0.000
Sites X Sampling period	0.835	6	0.182	34.491	0.000
Days X Sampling period	0.528	15	0.035	0.424	0.964
Sites X Days X Sampling period	1.019	30	0.044	8.417	0.000
Biomass					
Sites	0.101	2.0	0.068	1.027	0.344
Days	14.243	5	2.849	1.75	0.141
Sampling Periods	191.444	3	63.815	39.202	0
Sites X Days	5.94	10	0.803	12.132	0
Sites X Sampling period	3.242	6	0.731	11.034	0
Days X Sampling period	34.087	15	2.272	1.396	0.188
Sites X Days X Sampling period	26.548	30	1.197	18.072	0
Species Diversity (Slides)					
Sites	3.89	2	2.498	28.5	0
Days	3.899	5	0.78	2.68	0.032
Sampling Periods	13.319	3	4.44	15.28	0
Sites X Days	5.086	10	0.653	11.53	0
Sites X Sampling period	6.971	6	1.492	17.03	0
Days X Sampling period	5.563	15	0.371	1.23	0.253
Sites X Days X Sampling period	21.625	30	0.926	10.56	0

Species Richness (Slides)					
Sites	635.68	2	380.091	226.388	0.000
Days	467.343	5	93.469	3.392	0.011
Sampling Periods	1342.772	3	447.591	16.241	0
Sites X Days	288.03	10	34.444	20.516	0.000
Sites X Sampling period	1059.564	6	211.181	125.783	0.000
Days X Sampling period	908.687	15	60.579	2.198	0.02
Sites X Days X Sampling period	831.688	30	33.153	19.746	0.000
Biofilm Thickness					
Sites	1.699	2	1.042	647.759	0.000
Days	1.517	5	0.303	187.972	0.000
Sampling Periods	3.808	3	1.269	786.151	0.000
Sites X Days	1.048	10	0.129	79.933	0.000
Sites X Sampling period	1.872	6	0.383	237.912	0.000
Days X Sampling period	8.218	15	0.548	339.359	0.000
Sites X Days X Sampling period	5.937	30	0.243	150.938	0.000

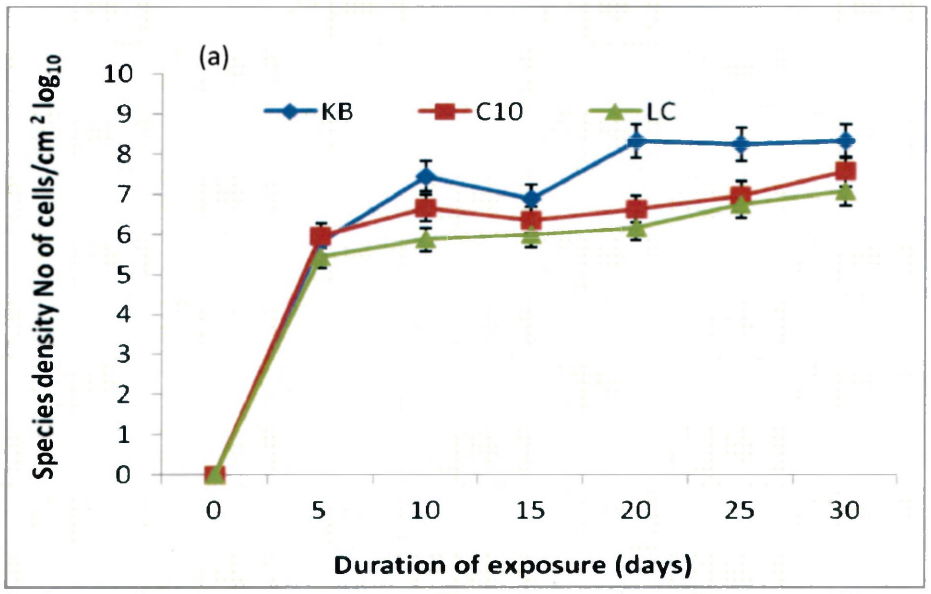
Total species density

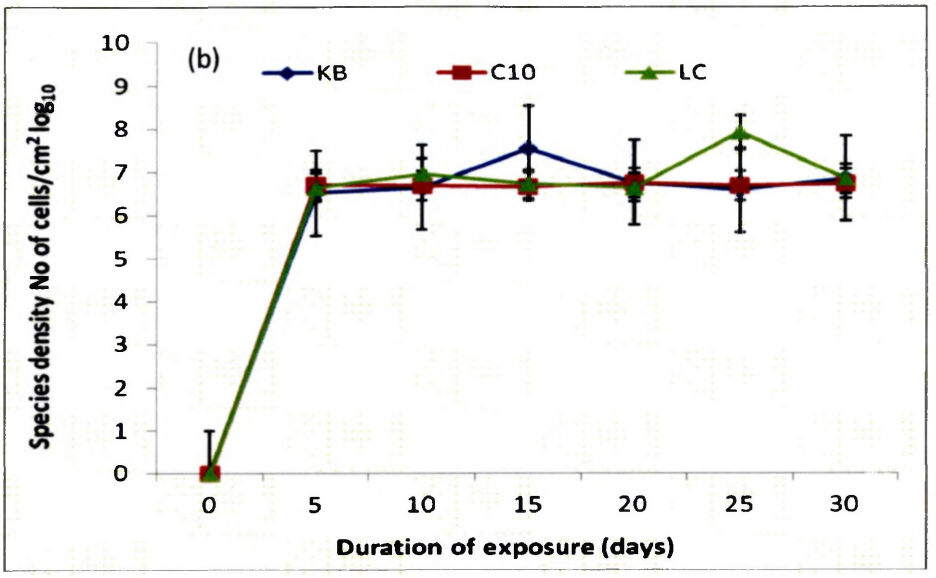
Species density followed a similar trend of variation as that of biofilm thickness and was significantly correlated with the latter during sampling periods 1, 3, and 4 (r^2=0.35, p<0.05, r^2= 0.16, p<0.05, r^2=0.34, p<0.05, respectively). Species density was also positively correlated with periphyton chlorophyll *a* concentrations for the same sampling periods (r^2=0.44, p<0.05, r^2= 0.55, p<0.05, respectively). Figures10(a-d) show the distribution of species density with the duration of study with values ranging from $\log_{10}$ 5.81/cm^2 to $\log_{10}$ 6.29/cm^2 during the early growing phase (5 days) and $\log_{10}$ 6.90/cm^2 to $\log_{10}$ 7.36/cm^2 during the mid growth phase (15 to 20 days). The late growth phase (25 to 30 days) showed a reduced density probably due to sloughing off of cells. An exception to this trend was observed during sampling period 1 at site

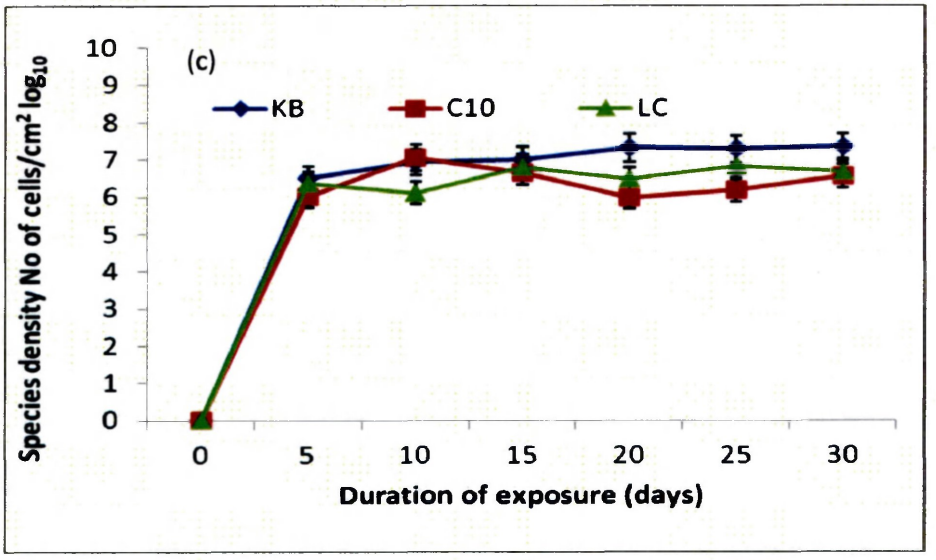
LC (Figure 10a). At this location a continuous increase of species density over the days of growth was observed during sampling period 1 (Figure 10a).

The maximum and minimum species density values recorded were at site KB during sampling period 4 ($\log_{10}$ 8.25 and $\log_{10}$ 4.84, respectively). According to the results of two way rmANOVA, species density showed significant variation within sampling periods ($F_{3,48}$=8.10, p<0.05) and between sites ($F_{1,48}$=835.21, p<0.05), but did not vary significantly between sampling periods ($F_{3,48}$=1.38, p=0.261). However, the interaction results of two way rmANOVA between sites and days, sites and sampling periods resulted in significant variations (Table 5). However, the *post hoc* analysis indicated significant variation in species density for only day 5 between all other days of growth.

Figure 10(a-d). Variation in average periphyton species density during the study period. Four sampling periods are independently averaged and plotted against the duration of the slide exposure. Error bars represent the 95% confidence limit.




(b)
KB
C10
LC
Species density No of cells/cm² log10
Duration of exposure (days)
0
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30


(c)
KB
C10
LC
Species density No of cells/cm² log10
Duration of exposure (days)
0
1
2
3
4
5
6
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8
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10
0
5
10
15
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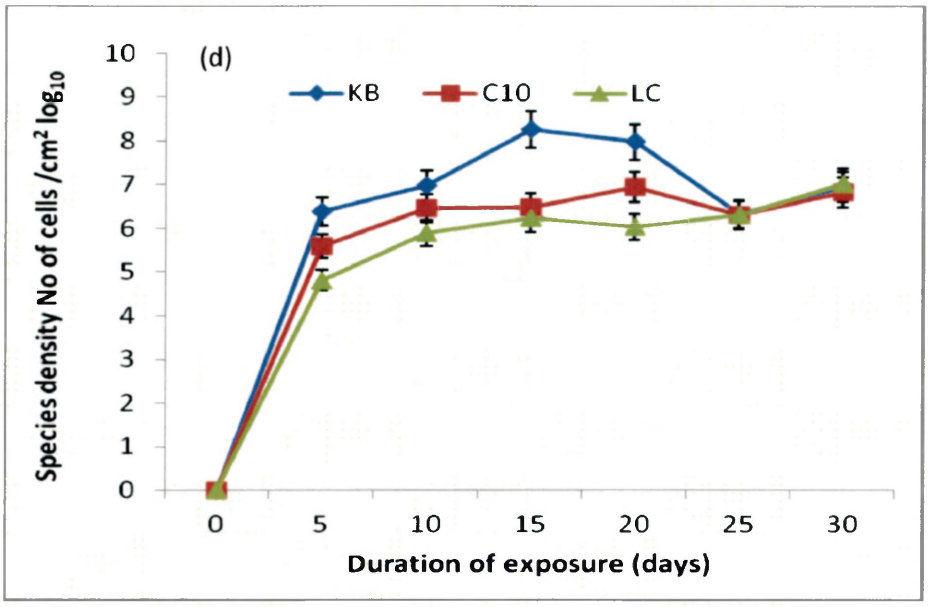
25
30



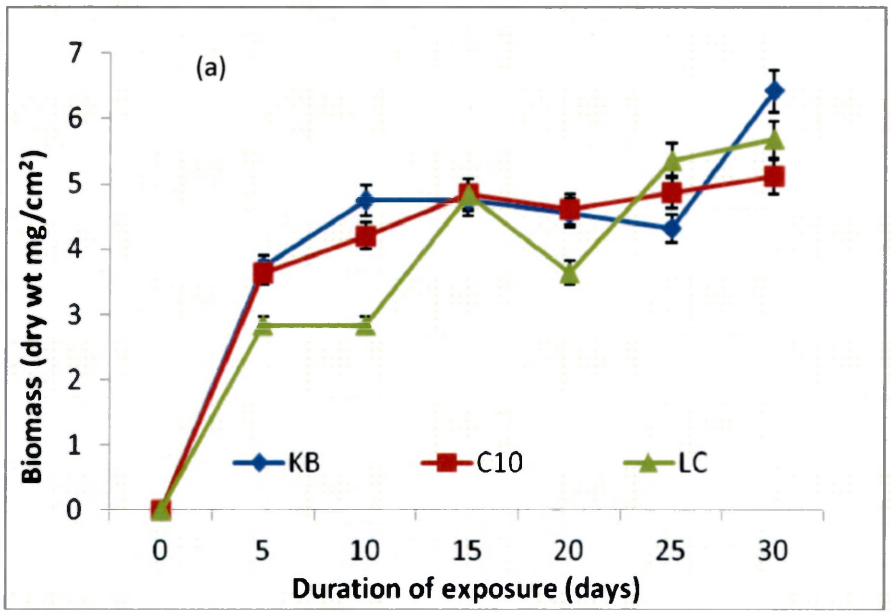
Biomass

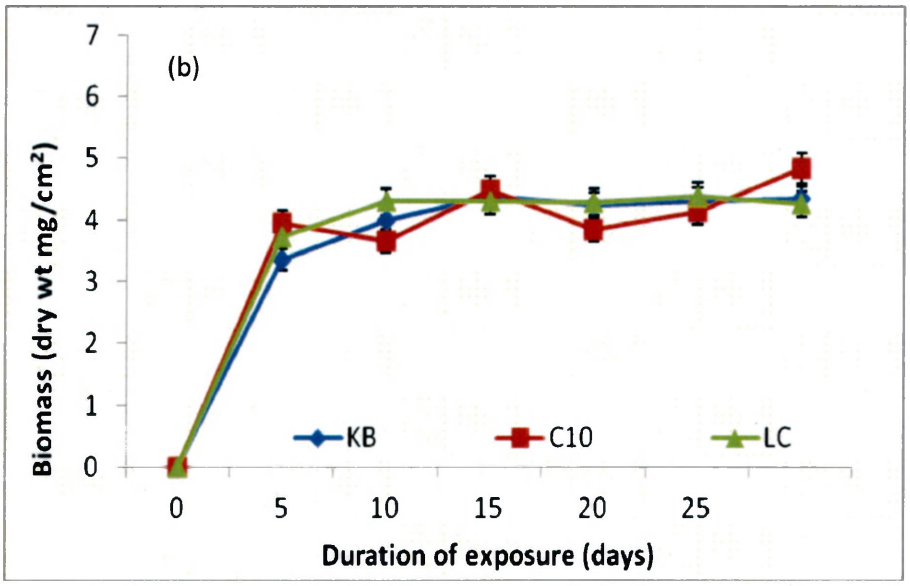
Biomass showed a similar pattern of variation as seen in biofilm thickness and species density. The biomass gradually increased until 15-20 days, but showed a reduction by day 25 (Figure 11a-d). The maximum value of biomass recorded was during sampling period 1 at KB (2643.00mg/L) while the minimum was recorded at the same site (KB) during sampling period 4 (0.05mg/L).

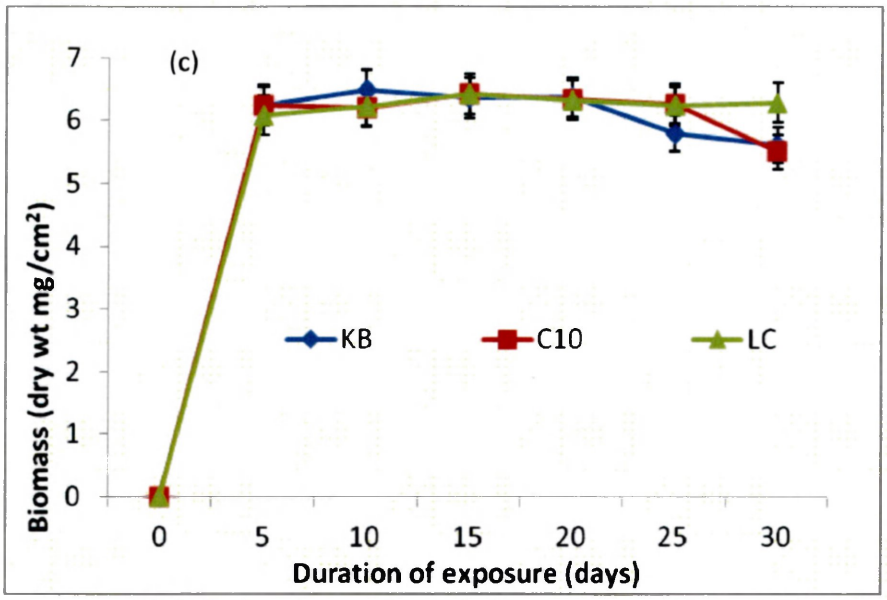
The two way rmANOVA of periphyton biomass values showed significant variation between sampling periods ($F_{3,48}$=11.03, p<0.05), but the variation between sites and days of growth was not significant (Table 5). However, interaction results of two way rmANOVA between sites and days, sites and sampling period resulted in significant variation (Table 5). *Post hoc* analysis showed that sampling period 3 was the only period that varied significantly from the other sites (p<0.05). Sampling period 3 was in summer and therefore experienced higher growth

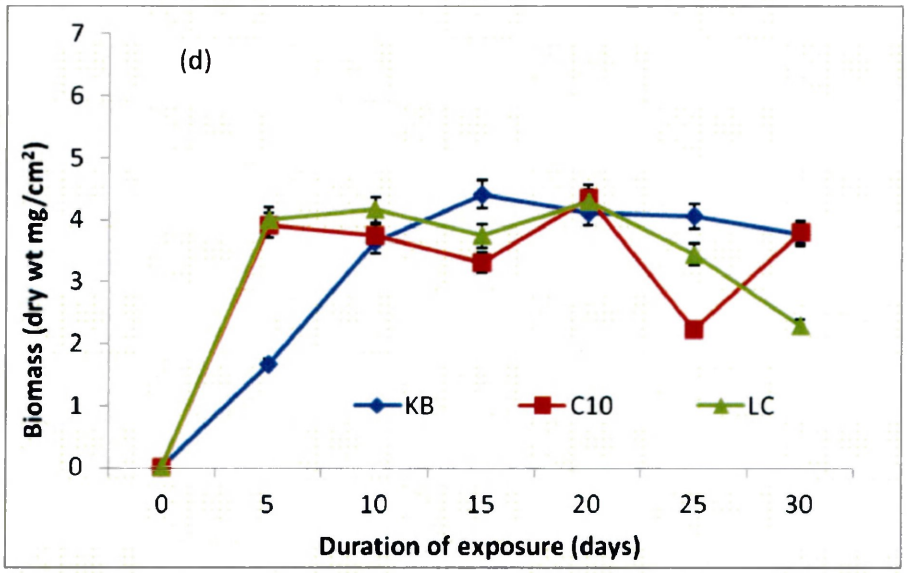
of Chlorophyceae, more specifically the filamentous green algae than the rest of the sampling periods (Figure 11c).

Figure 11(a-d). Variation in average periphyton biomass during the study period. Four sampling periods are independently averaged and plotted against the duration of the slide exposure. Error bars represent the 95% confidence limit.









Species richness

Species richness followed more or less the same trend as that of species diversity. Species richness and diversity were significantly correlated (r^2=0.42, p<0.05). Figure 12(a-d) shows a growth pattern containing two distinct peaks of species richness during the growth period demonstrating the two maxima observed during the early and late successional phases. The

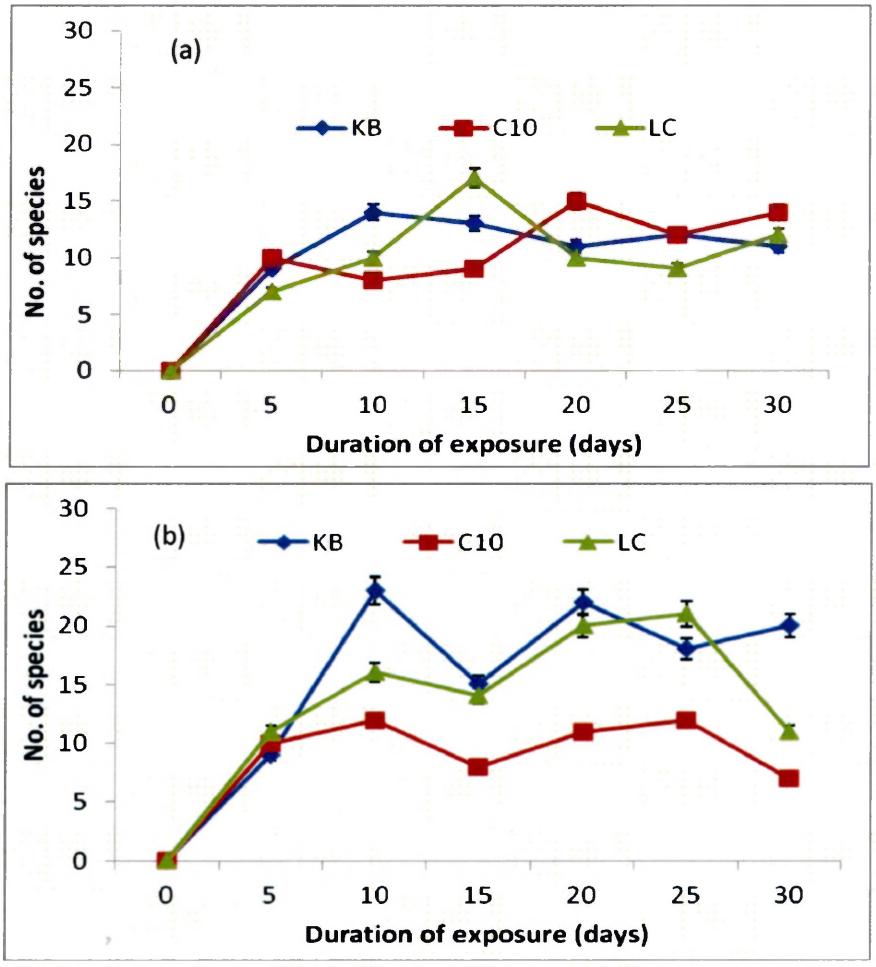
exception to the dual peaked pattern is observed during sampling period 3 at KB where the species richness values followed a general growth curve which corresponded to the highest species density values of the same period (Figure 10c).

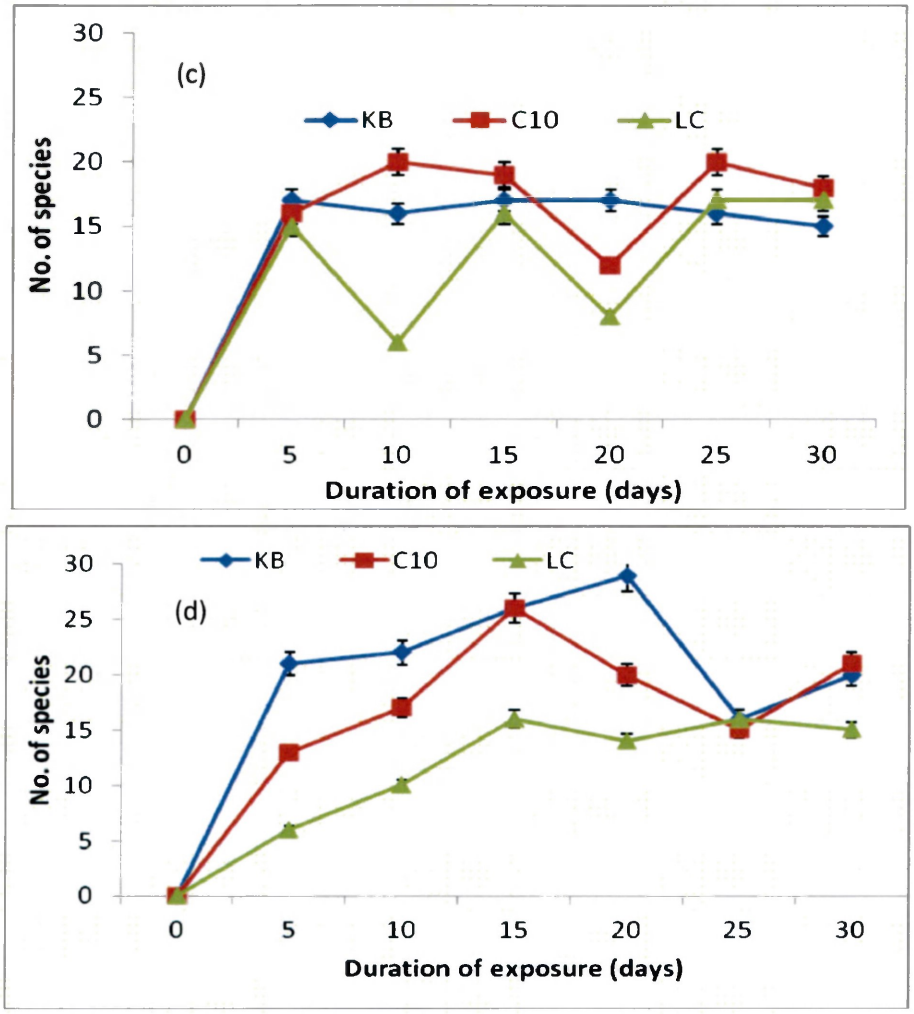
The two way rmANOVA results showed significant variation in species richness with the duration of slide exposure ($F_{5,48}$=3.39, p<0.05, Table 5) and between the sites ($F_{2,48}$=226.39, p<0.05, Table 5). The highest species richness value was recorded on day 20 at site KB (29 species) and the minimum recorded on day 5 at LC (6 species). There was a consistent increase in species richness up to days 20-25. *Post hoc* analysis showed significant variation between day 5 and all other days of growth. Species richness values for day 5 ranged from 6 to 21 which were recorded during sampling period 4.

The two way rmANOVA also showed a significant variation in species richness between sampling periods ($F_{3,48}$=16.24, p<0.05). Although, *post hoc* analysis showed significant variation between sampling periods 1, 2 and 4 (p<0.05), site specific trends played a role in this variation. For example, KB ranked number one for overall species richness for all sampling periods. In contrast C10 demonstrated relatively lower species richness during sampling periods 1 and 2 and seemed to follow the trend observed for biofilm thickness and species density at this site. Species richness values were high during sampling periods 3 and 4 and followed a general growth pattern with 2 distinct peaks. At site LC the richness values with duration of slide exposure followed a bimodal distribution. An exception to this trend was observed during sampling period 3 day 10 which showed a sharp decline in species richness (Figure 12c). This decline was not detected in species density, but was seen in the species diversity values. During this time, the species dominance showed an increasing shift due to the dominance of *Cocconeis placentula* (an

abundance value of 0.50 compared to an earlier abundance value of 0.02 on day 5 and a value of 0.26 on day 15).

Figure 12(a-d). Variation in the average periphyton species richness during the study period (sampling periods 1-4 respectively). Four sampling periods are independently averaged and plotted against the duration of the slide exposure. Error bars represent the 95% confidence limit.





Species diversity

Species diversity values exhibited a normal growth pattern with two distinct peaks, one early and the other in the late successional phases of the biofilm development (Figure 13a-d). For example, at C10 during sampling period 2 species diversity increased rapidly from day 1 to day 5 then progressively decreased to a minimum value during day 20 followed by an increase towards the end of the growth period. A second minima was recorded in some cases, especially by the end of the growth period, as observed at C10 during sampling period 4. However, it is unclear if this is a trend or rather a part of data "noise". Although, the two peaks of diversity values can be

noticed in most sites, but there were some noticeable exceptions, mainly during sampling period 4. For instance, at LC species diversity values followed a classic single peaked growth pattern (Figure 13d), while at site KB the species diversity variation during the growth period suggested a repeated increase and decrease resulting in a cyclic pattern (Figure 13d).

As expected the variation of species diversity and species dominance followed opposite patterns. For example, on days 20-25 there was a decrease of species diversity possibly due to sloughing off of organisms from the community. Exceptions to this trend were observed during sampling period 1 at KB where an observable shift in diversity from day 15 to day 20 was seen (Figure 13a). The shift included a decrease in diversity value from 1.79 on day 15 to 0.19 on day 20. This shift in diversity may be due to the dominance of the diatom species *Cocconeis placentula* which accounted for 97% of the entire community. This dominance lowered slightly by day 25, however a decline in non diatom members by day 30 resulted in a community mainly composed of diatoms. Site KB is the other example of deviation from the general dual peaked trend observed during sampling period 4 (Figure 13d). Diversity values showed a sudden decrease during day 15 corresponding to a shift in dominance of *Navicula lancelota* which accounted for 97% of the entire community (Figure 14a). This dominance suddenly reversed by day 20 pushing the diversity values higher. Linear regression results showed a significant positive relationship between the species diversity and density during sampling period 1 (r^2= 0.46, p<0.05).

Results of two way rmANOVA showed significant variation between sampling periods which directly reflects seasonality ($F_{3,48}$=15.28, p<0.05). Overall species diversity measurements showed higher values during sampling periods 1 and 4 which coincided with the fall turnover.

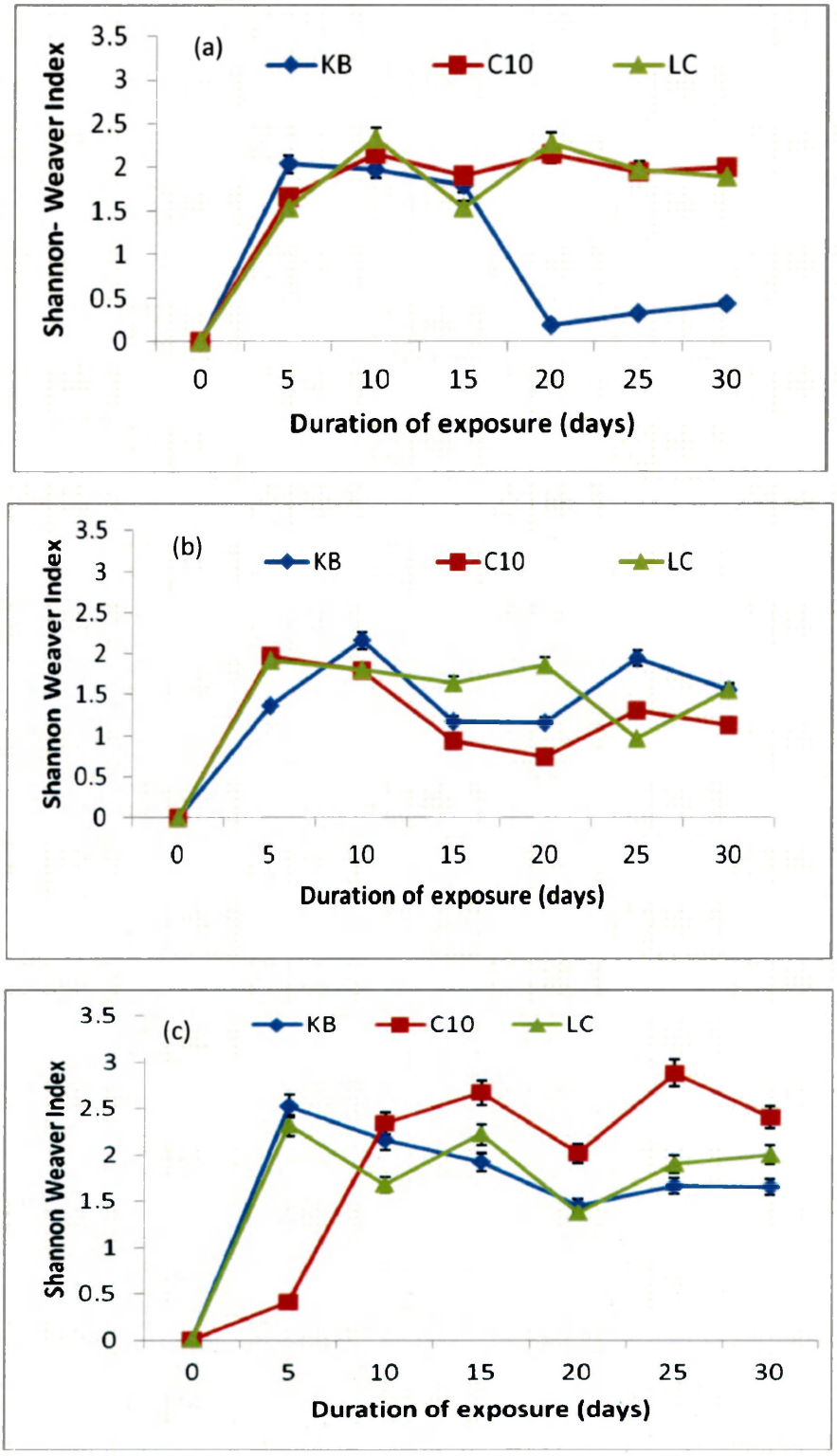
Sampling period 3 gave rise to the highest diversity during the sampling periods (2.97). Additionally, *post hoc* analysis showed that sampling period 3 was the only period to vary significantly from the other 3 sampling periods ($p<0.05$). Sampling period 3 was summer and it experienced a high growth of Chlorophyceae due to the warm water temperature and longer insolence period. Additionally, there was a significant correlation between species diversity of periphyton and species density of phytoplankton ($r^2=0.32$, $p<0.05$). This is an interesting relationship as it points to the role of phytoplankton as a supplier of propagules to periphyton.

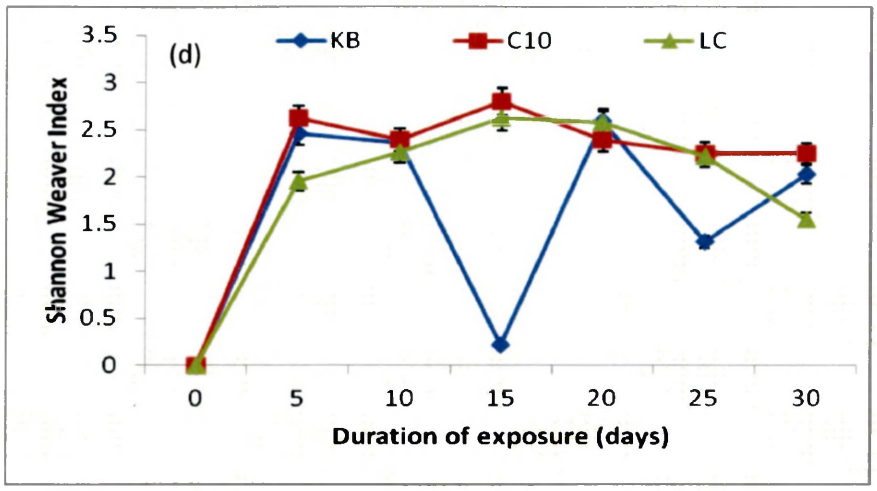
The two way rmANOVA results also showed significant variation in the species diversity values between sites ($F_{2,96}=28.50$, $p<0.05$). As mentioned earlier, the fluctuation in species diversity values corresponded to the shifts in diatom dominance (Figure 15a-d). The lowest diversity value was observed at KB (sampling period 4). The diversity value at KB was significantly correlated (positively) to TP in water ($r^2=0.35$, $p<0.05$). It is important to note that site KB had the highest overall TP concentrations among the sites during the entire study period (Table 4).

The diversity values at sites LC and C10 showed lesser fluctuation compared to KB. Among the three sites, C10 consistently showed highest values during sampling periods 1, 3 and 4, while site LC maintained a stable diversity value throughout the study period.

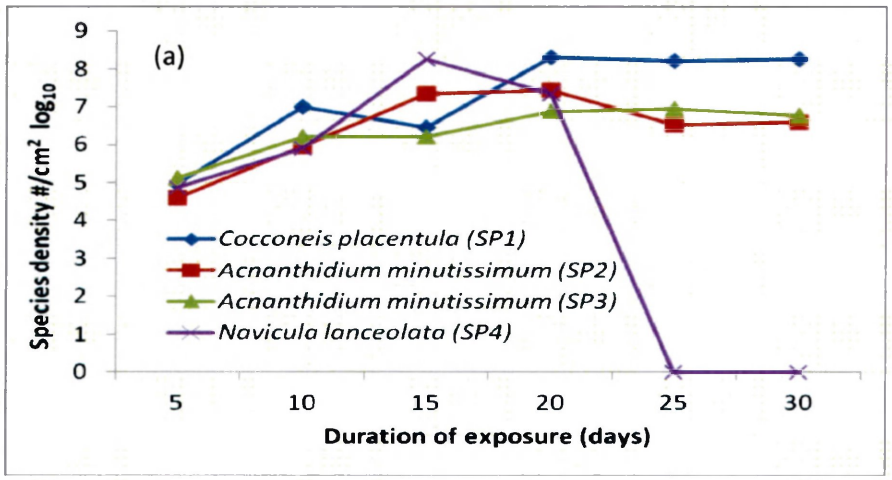
Interaction results of the two way rmANOVA between sites and days, sites and sampling period, days and sampling period and sites and days and sampling period resulted in significant variation of species diversity (Table 5).

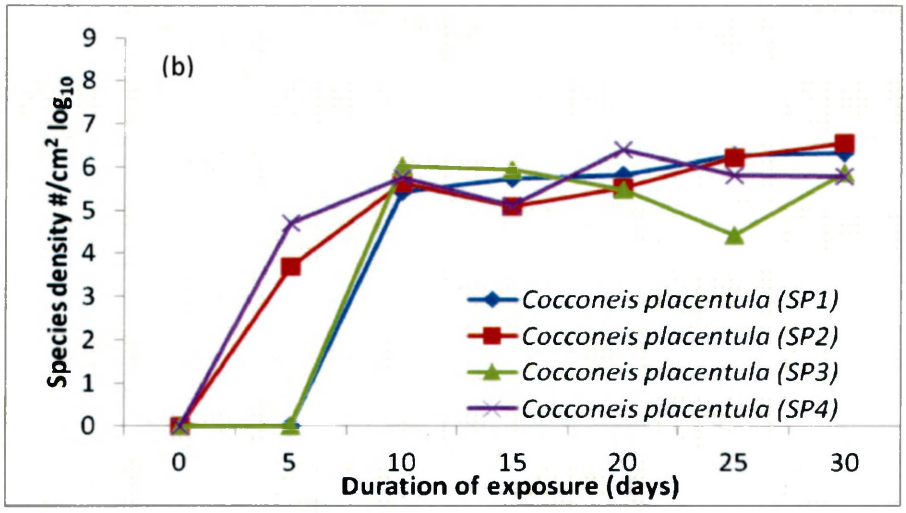
Figure 13 (a-d). Variation in average periphyton species diversity during the study period. Four sampling periods are independently averaged and plotted against the duration of the slide exposure. Error bars represent the 95% confidence limit.

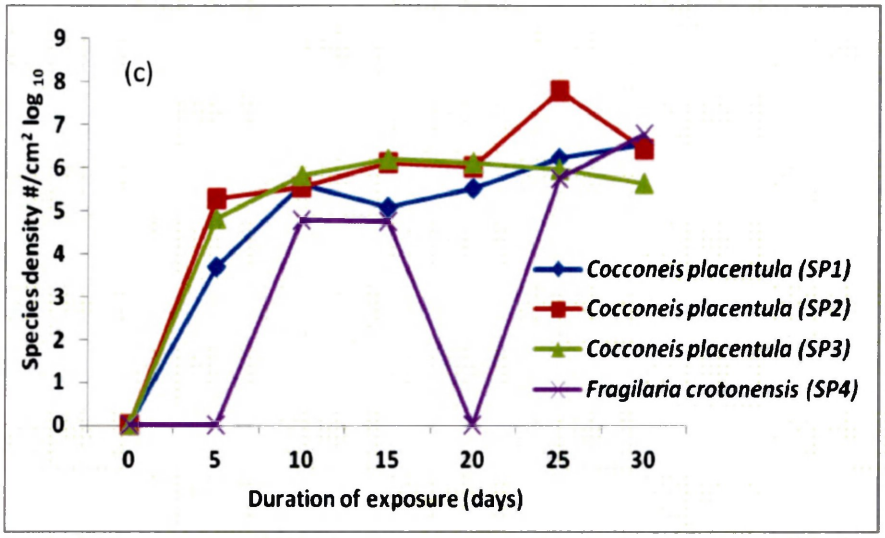




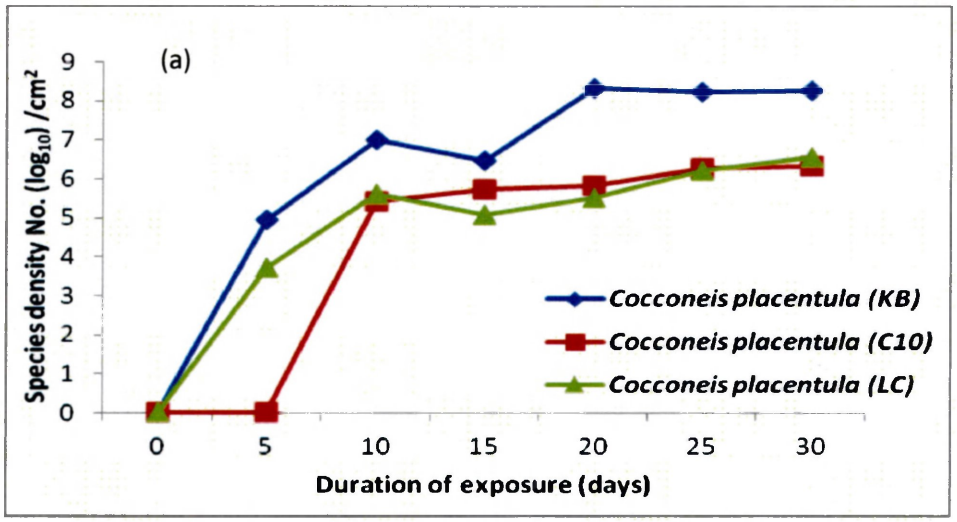
14(a-c). Density variation of dominant diatoms species at KB, C10 and LC respectively, observed during the study period. Each of the four sampling period values were averaged.

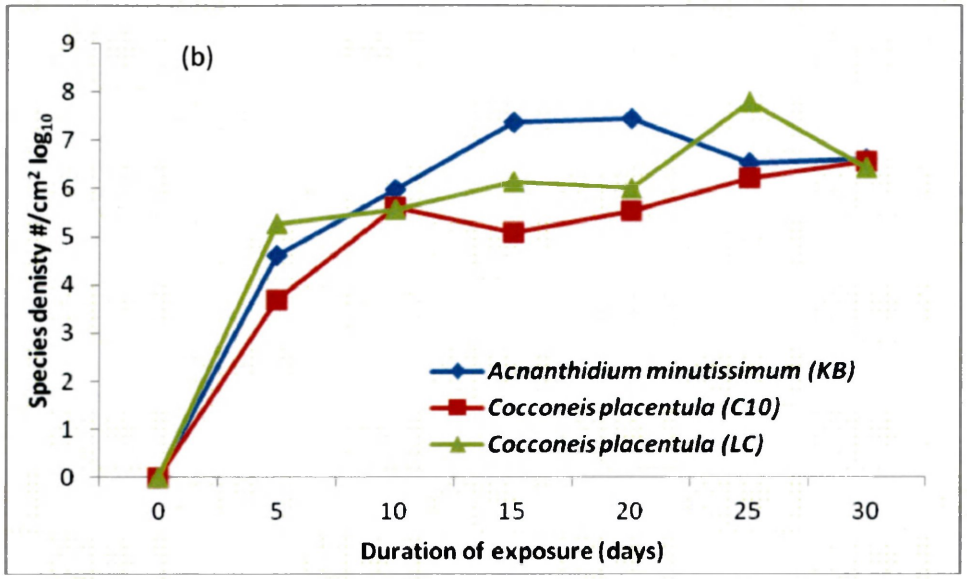


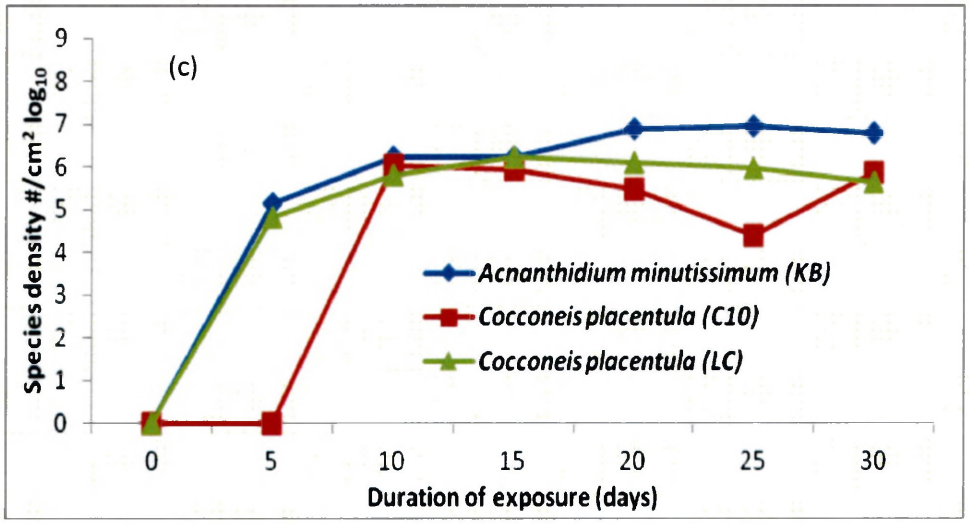


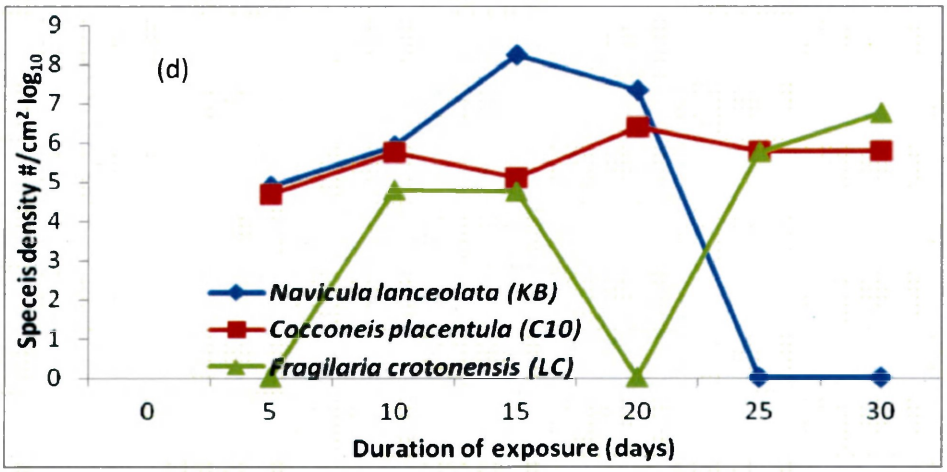


15(a-d). Density variation of dominant diatom species in each sampling period (1-4), according to site observed during the study period.




(b)
Species denisty #/cm² log10
Acnanthidium minutissimum (KB)
Cocconeis placentula (C10)
Cocconeis placentula (LC)
Duration of exposure (days)


(c)
Species density #/cm² log10
Acnanthidium minutissimum (KB)
Cocconeis placentula (C10)
Cocconeis placentula (LC)
Duration of exposure (days)


(d)
Speceis density #/cm² log10
Navicula lanceolata (KB)
Cocconeis placentula (C10)
Fragilaria crotonensis (LC)
Duration of exposure (days)

Taxonomic Composition

Table 6 lists all species observed in the periphyton communities during the study. In total, there were 65 Bacillariophyceae members, 29 members of Chlorophyceae, 19 members of protozoa, 4 genera of Cyanophyceae, 2 genera of Chrysophyceae and 1 rotifer in addition to various macroinvertebrates.

The relative abundance of these groups expressed as percentage densities were calculated from species density variables, varied between sampling periods, and sites (Figure 16a-d). Bacillariophyceae was the dominant group throughout the study followed by the Chlorophyceae and protozoa. A diversion from this trend was found in sampling period 3 at C10 where Cyanophyceae accounted for almost the same percentage as that of Chlorophyceae and the protozoa (Figure 14b).

Bacillariophyceae

The most dominant species in all sites during sampling period 1 was *Cocconeis placentula* (Figure 14a). However, the remaining sampling periods showed site specific species dominance (Figure 15b-d). For example during sampling period 2 although sites C10 and LC showed dominance of *Cocconeis placentula,* site KB showed dominance of *Acnanthidium minutissimum*. Sampling period 3 also showed similar result as that of sampling period 2 (Figure 15c). Sampling period 4 showed variation in species dominance at site KB and LC, but site C10 was similar to sampling periods 2 and 3 (Figure 15d). The shift in species dominance at KB showed a dominance of the cold water species *Navicula lancelota* during sampling period 4 (Figure 14a). At site LC the shift resulted in the dominance of *Fragilaria crotensis* (Figure 14c).

Figure 16(a-d). Variation in average percentage density of algal groups observed at three sampling locations during the four sampling periods within the study.

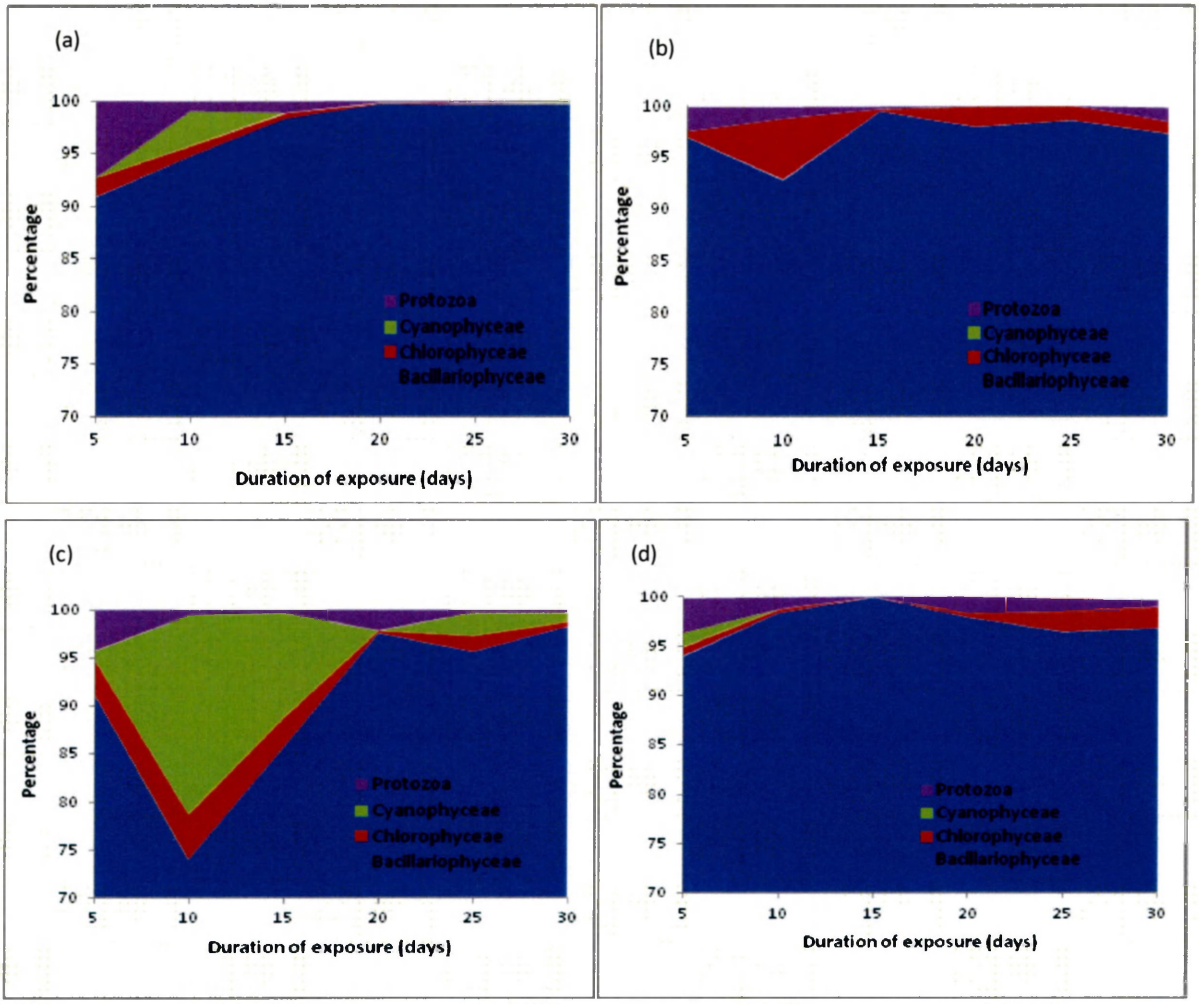
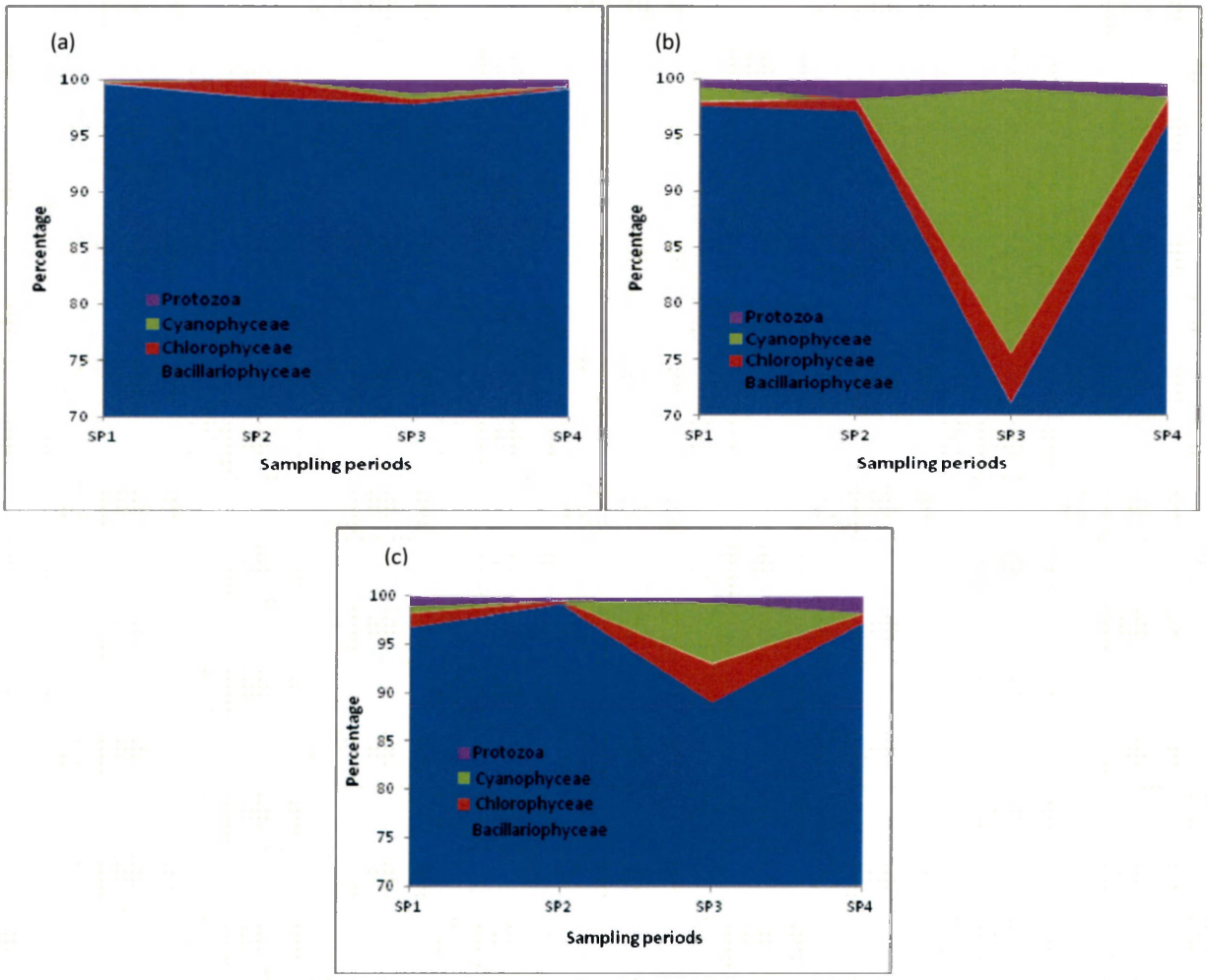


Figure 17 (a-c). Variation in average percentage composition of various taxonomic groups according to site (KB, C10, LC respectively).



The diatom species recorded has been divided into 4 categories; *present*, *frequent*, *abundant*, and *absent*. *Present* is defined as a species observed at least 50% of time, *frequent* is defined as a species observed 60-80% of the time and *abundant* is observed 90% of the duration of study. *Cocconeis placentula, Cymbella tumida, Gomphonema truncatum, Acnanthidium minutissimum and Amphora ovalis* were observed as abundant while, frequently observed within the community were *Cymbella lanceolota, Epithemia turgida, Navicula* spp., *Fragilaria crotensis, Synedra* spp. and *Synedra ulna* (Table 6).

During the entire growth period, *Navicula lanceolota, Aulacoseira varians* (*Melosira varians*), *Nitzschia* spp., *and Gyrosigma acuminatium* were frequently present during the early growth phase while *Eucocconeis* spp, *Navicula tripunctata*, *Pinnularia* sp., *Placoneis eigens*, *Rhopolodia gibba* were relatively more frequent during the late growth phase (Table 6).

In general the periphyton community development was highly dependent on the site and environmental factors such as TP, DO, and conductivity. Some sites shared common initial colonizers such as *Acnanthidium minutissimum, Cocconeis placentula, Cymbella tumida* and *Diatoma vulgaris,* however variability between sites and between sampling periods (seasonal influence) was observed.

Diatom taxonomic composition showed seasonal variation in species abundances (Table 7). For example, fall samples (sampling period 1) showed species such as *Cosmioneis* spp., and *Cymatopleura solea* while the spring samples (sampling period 2) showed the presence of different species such as *Cymatopluera elliptica*, *Entomoneis paludosa*, *Eucocconeis* spp., *Gomphoneis* spp., *Meridion circulare*, *Stauroneis* spp., and *Synedra cyclopium.*

The samples collected during summer (sampling period 3) gave rise to a highly diverse taxonomic composition which included many common species and species that were only observed during summer (Table 7). These species were *Amphipleura pellucida*, *Cocconeis pediculus*, *Cosmioneis reimeri*, *Eutonia* spp., *Fragilaria capucina*, *Fragilaria* spp., *Frustulia* spp., *Gomphonema parvulum*, *Gomphenesis* spp., *Navicula stesvicensis*, and *Nitzschia calida* (Table 7). Species that overlapped between sampling periods 3 and 4 (sampling period 4 – second fall), but were not observed in any other sampling periods were *Navicula tripunctata*, *Neidium dubium*, and *Tabellaria flocculosa.* Finally, species present only during second fall

included *Amphipleura* spp., *Cocconeis pediculus*, *Cymatopleura ovalis*, *Epithemia sorex*, *Eucocconeis* spp., *Navicula gastrum*, *Nitzschia amphibia*, *Sellaphora pupulla*, and *Synedra capitata*.

Site specificity also played a role in diatom taxonomic composition. Most of the common diatoms such as *Cocconeis placentula*, *Cymbella tumida*, *Gomphonema truncatum*, *Navicula* spp., and *Synedra* spp. were observed in all 3 sites, however some were site specific. For example, some site specific taxa included *Meridion circulare* (KB and C10), *Nitzschia amphibia* (C10 and LC) and *Synedra cyclopium* (KB and C10).

Non-diatom groups

Members other than diatoms observed in the periphyton community included Chlorophyceae, Cyanophyceae, Chrysophyceae and protozoa groups. The species varied between sampling periods and sites. For example, sampling period 3 (summer) gave rise to the highest abundance of Chlorophyceae ($\log_{10}$ 7.23/cm^2). Species such as *Coleastrum* spp., and *Cosmarium* spp. were observed abundantly throughout the entire study period followed by the frequent presence of *Coleachaeta* spp., Filamentous green algae (FGA) spp., and *Pediastrum simplex*. The Chlorophyceae appeared in certain growth phases of periphyton community. For example, species such as *Closterium* spp., *Crucigenia tetrapedia,* Green algae (GA) spp., *Monorhaphidium convolutum,* and *Staurastrum* spp. were observed during the early phase of growth while *Scenedesmus quadricauda* were observed mostly in the late growth phase of the periphyton (Table 6).

Various protozoa were observed in the periphyton community during the entire growth period. Species such as *Amoeba* spp., *Stylonchia* spp. *and* some unknown protozoan species were

abundant during the entire growth phase. Species such as *Actinosphaerium* spp., *Euglena* spp., *Lacrymaria* spp., and *Euplotes* spp. were more frequently observed during the early growth phase while species such as *Amoeba* spp., *Stylonchia* spp. and some unknown protozoan species were observed more frequently during the late phase of biofilm development (Table 6).

Cyanophyceae species were present in the periphyton community in all sites and sampling periods. *Merismopedia glauca* was the most abundant species and was the main contributor to the high density values ($\log_{10}$ 5.45) of Cyanophyceae on day 10 during summer. *Anabaena* spp. was present during the early growth phase while *Chroococcus* spp. *and Spirulina princeps* was recorded during the late growth phase (Table 6).

Several species of Nematodes were abundantly present during the entire growth period, however this was restricted to site C10 and LC.

The presence-absence of non-diatom species showed a seasonal trend. While certain non-diatom species were present during all sampling periods, some species were observed only in certain sampling period. For instance, the sampling periods 1 and 2 included common taxa (observed in all 4 sampling periods) such as *Coleastrum* spp., and *Cosmarium* spp., in addition to rare ones such as *Crucigenia tetrapedia*. The spring sampling period recorded various non-diatom taxa such as; *Diplochloris* spp., *Kirchneriella* spp., *Spirogyra* spp., *Spirulina* spp., *Chroococcus* spp., *Chlamydomonas* spp. and *Dinobryon divergens* which were not present during the other sampling periods.

The summer sampling period exhibited the most diverse non-diatom species. They included *Characium* spp., *Coelastrum microporum*, *Pediastrum tetras*, *Spirulina* spp., *Stigeclonium tenue*, *Amphileptus* spp., in addition to the presence of unknown copepods and

ostracods. The species that were present in spring and summer included *Characiopsis* spp., *Kirchneriella* spp., *Monorhaphidium convolutum*, and *Spirulina* spp.

The second fall sampling period included species such as *Staurastrum gracile*, *Stigeoclonium* spp., *Arcella* spp., *Platycola vaginella*, *Stentor* spp. It is important to note that Chironomidae larvae were present during spring and summer only. Species that were commonly present during the first and second fall sampling periods included *Dictyosphaerium pulchellum*, *Coleastrum* spp., *Coleochaete* spp. and the protozoa species *Stentor* spp.

Site also played a role in non-diatom taxonomic composition. Though many common non-diatom species were present in all sites, some were seen only in certain sites. For example, some species recorded only at KB included *Diplochloris lunata* and the protozoa species, *Platycola vaginella* in addition to the only recorded unidentified ostracods. The lowest species richness value (average over all sampling periods) of non-diatom species during the study period was at KB (5.4 species) while C10 had a much higher average species richness value (7.5 species). C10 also had the highest density of Cyanophyceae species, *Merismopedia glauca*, among all three sites. Other species present only at C10 included *Staurastrum gracile, Pediastrum tetras,* and the testate amoeba *Arcella* spp. The average species richness value for site LC was on par with C10 (7.5 species). Site LC also showed the only presence of Chrysophyceae genus, *Dinobryon divergens* and the protozoa species *Lecane lunaris.*

Table 6. Algal species present in the periphyton community during the duration of the study (October 2011- November 2012). *Present* represents species observed at least 50% of time during the entire study, *frequent* represents presence of 60-80% of the time during the entire study, and *abundant* represents presence of > 90% of the time during the study.

Algal Species	**Days of Exposure**		**x=present; xx= Frequent, xxx= abundant, -=absent**				
	5	**10**	**15**	**20**	**25**	**30**	
Acnanthes spp	-	-	x	x	-	-	
Acnanthidium minutissimum	xx	xxx	xxx	xx	xxx	xxx	
Amphipleura pellucida	xx	-	-	-	-	x	
Amphipleura solea	-	-	-	x	x	-	
Amphora ovalis	xx	xxx	xxx	xx	xxx	xxx	
Asterionelia formosa	x	xxx	x	x	x	x	
Aulacosiera granulata	xx	x	x	x	x	x	
Cocconeis pediculus	x	x	-	x	-	x	
Cocconeis placentula	xxx	xxx	xxx	xxx	xxx	xxx	
Cosmioneis reimeri	x	x	x	x	-	-	
Craticula cuspidata	-	x	x	x	x	x	
Cyclotella meneghiniana		-	x	-	-	-	
Cyclotella spp.	x	x	x		x	x	
Cymatopluera elliptica	-	-	x	x	x	x	
Cymatopleura ovalis	-	-	x	-	-	-	
Cymatopleura solea	x	-	xx	xx	x	x	
Cymbella lanceolota	xx	xx	xx	xx	xx	xx	
Cymbella tumida	xxx	xxx	xxx	xxx	xxx	xxx	
Diatoma vulgaris	xxx	xxx	xxx	xx	x	xx	
Entomoneis paludosa	-	x	-	-	-	x	
Epithemia sorex	x	-	x	-	-		
Epithemia turgida	xx	xx	xx	xxx	x	xx	
Eucconeis spp.	-	xx	-	xxx	xxx	x	
Eutonia spp.	x	-	-	-	x	-	
Fragilaria capucina	-	-	x	-	x	-	
Fragilaria crotensis	xx	xxx	xxx	xx	x	xx	
Fragilaria spp.	-	-	x	-	xx	-	
Frustulia spp.	-	-	-	-	-	x	
Gomphenema acuminatium	x	x	xx	x	x	-	
Gomphonema parvulum	-	-	x	-	x	-	
Gomphonema truncatum	xxx	xxx	xxx	xxx	x	xxx	
Gomphoneis spp.	-	-	-	x	xxx	-	
Gyrosigma acuminatium	x	xx	xx	xx	x	x	
Hippodonta capitata	x	x	x	-	x	x	

Meridion circulare	-	x	x	x	-	x	
Navicula gastrum	x	x	x	x	x	x	
Navicula lanceolota	xx	xx	x	x	x	x	
Navicula spp.	xxx	xxx	xxx	xx	x	xxx	
Navicula stesvicensis	-	x	-	x	x	-	
Navicula tripunctata	x	x	x	x	xxx	xx	
Neidium dubium	-	-	x	x	-	-	
Nitzschia acicularis	x	x	x	x	x	x	
Nitzschia amphibia	-	x	x	x	-	x	
Nitzschia calida	x	x	-	-	x	-	
Nitzschia lanceolata	-	-	-	-			
Nitzschia sigmoidea	x	x	x	x	x	x	
Nitzschia spp.	xxx	xx	xx	x	x	x	
Pinnularia sp.	x	x	x	xx	x	xx	
Placoneis eigens	xx	x	xx	xxx	xx	x	
Rhoicosphenia curvata	-	x	x	x	x	x	
Rhopolodia gibba	x	x	x	x	xx	xx	
Sellaphora pupulla	-	-	x	-	-		
Stauroneis spp.	-	x	x	x	x	-	
Suriella ovalis	x	x	x	x	x	-	
Synedra acus	x	x	x	x	x	x	
Synedra capitata	-	-	x	-	-	-	
Synedra cyclopium	-	x	-	x	x	-	
Synedra spp.	xxx	xxx	xxx	xx	x	xxx	
Synedra ulna	xxx	xxx	xxx	xx	x	xx	
Tabellaria fenestra	-	-	x	x	xxx	-	
Tabellaria flocculosa	x	x	-	-	xx	x	
Unknown diatom	-	-	-	x	x	-	
Ankistrodesmus spp.	-		x	x	x	x	
Characiopsis spp.	-	x	x	x		x	
Characium spp.	x		x	x			
Chroococcus spp.					x	x	
Closterium lunata		x	-				
Closterium spp.	x	x	x		x	x	
Coleachaeta spp.	-	xx	xx	xxx	xx	xx	
Coelastrum microporum	-					x	
Coleastrum spp.	x	xxx	xx	x	x	xxx	
Cosmarium spp.	x	xx	xx	x	xxx	xx	
Crucigenia tetrapedia	-	xx	x	x	-	-	
Dictyosphaerium pulchellum	-	x	x	x	x	x	
Diplochloris lunata		x		x	-	-	

Eurastrum spp.	-	x	-	x	-	x	
FGA spp.	xxx	x	x	x	xxx	xxx	
GA spp.	xxx	xx	-	x	x	x	
Kirchneriella spp.	-	x					
Monorhaphidium convolutum	x	x	x	x	x		
Pediastrum simplex	x	x	xx	xx	x	x	
Pediastrum tetras		xx	-	x		x	
Scenedesmus quadricauda	x	-	xxx	xxx	x	xxx	
Spirogyra spp.	-	xxx	-				
Spirulina princeps	-	x	-		x	x	
Spirulina spp.	-	x	-	-	-	x	
Staurastrum gracile		-	x			-	
Staurastrum spp.	x	x	x		x	x	
Stigeoclonium spp.		x	x	x		x	
Anabaena sp.	-	x	x	-	-	-	
Merismopedia glauca	xx	x	x	x		x	
Dinobryon spp.						x	
Actinosphaerium spp.	x	x	xx	x	-	-	
Amoeba spp.	xx	x	x	x	x	x	
Amphileptus spp.	x		x			x	
Arcella spp.	x	x			x	x	
Chlamydomonas sp.	-	-	-	-	-	-	
Colpidinium spp.	x	x	-	-	-	-	
Daphnia spp.	-	-		-	-	x	
Euglena spp.	xx	-	x	-	-	-	
Euplotes spp.	xxx	x	xx	x	-	xx	
Lacrymaria spp.	x	xxx	x		-	x	
Lecane lunaris		x		-		x	
Litonotus spp.	x	x	x	x	-	x	
Phacus spp.	x		x	x			
Platycola vaginella		x	x			x	
Rotifer spp.	xx	x	x		x	x	
Stentor spp.	x	xx	-	x	x	x	
Stylonchia spp.	xxx	x	xxx	x	x	x	
Unknown protozoa spp.	xxx	xxx	xxx	xxx	xxx	xxx	
Vorticella spp.	-	x	x	x	x	x	
Nematode spp.	xxx	xx	xxx	x	x	xx	
Unknown copepod spp.					x	x	
Unknown ostracod spp.			x				
Chironomid spp.						x	

Table 7. Presence of diatom species in different sampling periods (seasons).

SPRING (SP2)	SUMMER (SP3)	FALL (SP1 & SP4
Cymatopluera elliptica	*Amphipleura pellucida*	*Amphipleura* spp.
Entomoneis paludosa	*Cocconeis pediculus*	*Cocconeis pediculus*
Eucconeis spp.	*Cosmioneis reimeri*	*Cymatopleura ovalis*
Gomphoneis spp.	*Eutonia* spp.	*Epithemia sorex*
Meridion circulare	*Fragilaria capucina*	*Eucconeis* spp.
Stauroneis spp.	*Fragilaria* spp.	*Navicula gastrum*
Synedra cyclopium	*Frustulia* spp.	*Navicula tripunctata*
	Gomphonema parvulum	*Neidium dubium*
	Gomphoneis spp.	*Nitzschia amphibia*
	Navicula tripunctata	*Synedra capitata*
	Neidium dubium	*Tabellaria flocculosa*
	Nitzschia calida	*Cosmioneis reimeri*
	Tabellaria flocculosa	*Cymatopleura solea*

Discussion

Periphyton succession

The successional patterns of periphyton communities are often similar to those observed in terrestrial ecosystems (Macarthur & Wilson 1967). Periphyton succession follows predictable

sequences or phases during its development including early, middle and late phases of growth (Stevenson 1990; Biggs et al., 1998; Sekar et al., 2004; Kanavillil et al., 2014).

Species succession with duration of slide exposure:

The growth of periphyton community in this study showed pioneer species such as *Cocconeis* spp., and *Acnanthidium minutissimum* which was also observed by other researchers from Oak Creek, Arizona, USA (Korte & Blinn 1983). However, the present study differed from the previous study in that *Cocconeis* spp. and *Acnanthidium minutissimum* remained dominant during majority of time over the study duration. These results were similar to the study performed by Kanavillil et al (2012) which looked at the succession of diatoms in natural biofilms on glass slides. These two species were reported to possess an adaptation to succeed in disturbed habitat with a high to moderate supply of resources (Biggs et al., 1998). Small cell size, rapid replication rates and fast colonization could be certain characteristics that helped these species to succeed as primary colonizers during the early phase (day 5-10) of periphyton growth and in the subsequent period (from day 25-30). However, species such as *Cymbella tumida, Gomphonema truncatum* and *Amphora ovalis* generally appeared during the mid phase of periphyton growth were characterized by large cell size, slow colonization, slow growth and attaining high biomass. Obviously, they required higher resource supply and less disturbance than the early colonizers. A similar growth pattern of for *Cymbella* spp. *and Gomphonema* spp. was observed by previous researchers (Korte & Blinn 1983). According to them, these taxa are able to uptake nutrients at a more efficient rate, resistant to herbivory via endotoxins, low palatability, stronger adhesion to substrate and growing tall or being filamentous. These features helped them to form high biomass in periphyton community (Biggs et al., 1998). The *Cymbella* spp. and *Amphora* spp. also employ additional reproductive strategies through producing

mucilaginous protective tubing for protection from degradation/ desiccation and perhaps reduced palatability to detract predators (Round et al., 1990).

The mid- late phase (days 10-20) of the periphyton community gave rise to taxa such as *Nitzschia palea* and *Gomphonema* spp., *Cymbella lanceolota*, and the rosette forming *Synedra* spp. These species employ adaptations to succeed in highly stressful/less productive undisturbed habitats by using attributes such as small-medium cells sizes, slow colonisation, slow growth, coupled with strong attachment capabilities (Biggs et al., 1998). Once the community has been established, by approximately 15-20 days, solitary diatoms with high mobility started to appear in the community. They include *Gyrosigma acuminatium*, *Pinnularia* sp. and the chain forming *Fragilaria crotonensis*. These diatoms are successful at maintaining a high growth rate at a lower irradiance level (Bellinger & Sigee 2010).

The present study observed Bacillariophyceae as the dominant group throughout the period of study which is similar to the findings of Kanavillil et al (2012), but varied from the findings of Sekar et al (2002) which included a co-dominance of Chlorophyceae and Bacillariophyceae during the early to mid phase subsequently leading to a Cyanophyceae dominated community. The variations in taxonomic composition could be due to the differences of substrata used in addition to the varying environments where these studies were carried out. Sekar et al (2002) studied in a typical tropical freshwater reservoir while the present study is in a temperate open freshwater system.

Seasonal variation in periphyton community development:

The periphyton community development is dependent on seasonal factors such as variation in nutrient concentration, temperature, duration of irradiance etc. In the present study,

the increased nutrient concentration that was detected during fall may be due to the fall of leaf litters and subsequent degradation of them releasing nutrients into the water. In addition, the fall turnover of water column also brings in nutrients to the shallow areas. Site C10 experienced the largest quantity of leaf litter among the 3 sampling locations. Similarly, the increase in atmospheric temperature increased water runoff due to melting of snow and water turn over resulting in higher nutrient concentration during spring. The summer season was when the highest growth occurred and could be due to the influence of more than one parameter.

The abundance of a species in a sampling period would suggest a strong preference for growth parameters that vary with seasons. There are several studies that reported seasonal abundance of certain periphyton species (Oemke & Burton 1986, Passey et al., 1999, Artigas et al., 2012 and Kanavillil et al., 2012). In the present study, the results of one way ANOVA showed that water temperatures varied significantly between spring, summer and fall sampling periods which were reflected on the presence/absence of diatom and non diatom species. For example, there was a shift in diatom dominance at KB during fall sampling period (4) *Navicula lancelota* (from $\log_{10}$ 5.90 to $\log_{10}$ 8.24) and the presence of *Meridion circulare* which is a cold water diatom species (Round et al., 1990; Kelly et al., 2005). Some other groups that exhibited a temperature preference, especially during the summer (water temperature ~28°C), were Chlorophyceae and Cyanophyceae (Figure 16c).

Among the other environmental factors that varied seasonally TP played a influential role in the taxonomic composition. Previous studies demonstrated that TP variation acts as an important driver of growth and taxonomic shifts in microalgae in an aquatic ecosystem (Liboriussen & Jeppesen, 2006; Vis et al., 2008; Ferragut & de Campos Bicudo, 2010). The

increased TP concentration as observed during certain sampling period might have played a role in species dominance. In the present study the maximum number of species (29 species on day 20) at KB was observed during sampling period 4 (second fall) coinciding with the highest concentration of TP in the water column (Table 4). There was a significant correlation between TP and species richness for all sampling periods. All sites and sampling periods exceeded the provincial guideline concentration of 0.02 mg/L, with the exception of sampling period 2 at KB. This relatively low value may have been due to an error occurred during sample analysis.

Thus, the present study results disagreed with Passy and Blanchet (2007) who found that an increase in nutrient concentration resulted in a decrease in species richness. This study observed that other factors such as light availability and autogenic processes such as sloughing off and immigration and emigration rates might have played important roles in determining the species richness in the periphyton communities studied.

Though nitrogen analysis results were not ready in time for the writing of this thesis it may play a role in various species presence according to site. For example, the presence of *Anabaena* spp., and high abundances of *Merismopedia glauca* and *Rhopolodia gibba* are exclusive to site C10 and all these species are nitrogen specialists (Stevenson et al., 1996).

Periphyton Characteristics (biofilm thickness, chlorophyll *a*, biomass, species density, species diversity and species richness)

Biofilm thickness values showed a normal growth pattern with a gradual increase until 15-20 days followed by a decrease until the end of the sampling period (Figure 9a-d). The biofilm thickness varied between sampling periods as a result of seasonality. Biofilm thickness

demonstrated a relationship with hydrological parameters such as temperature, pH, DO and conductivity as seen in the results of regression analysis.

There was significant correlation between the biofilm parameters biofilm thickness and biomass at site C10 only and may have been due to the higher disturbance levels as a result of intense wave action at this site. Species diversity showed a near significant correlation with biofilm thickness at site LC, while species density correlated significantly with biofilm thickness at sites C10 and LC. The biofilm thickness at site KB may have been negatively influenced by the constant sedimentation load from the effluent release from the Waste Water Treatment Centre in Barrie, ON.

Periphyton chlorophyll *a* concentrations followed a normal growth pattern and showed a general increase from the early stage of periphyton growth to days 20-25. This was followed by a decrease to the end of the sampling period (30 days). Repeated measure ANOVA results verified that there was significant variation between the days of growth. The trend was similar for periphyton micro-algal density and the correlation between periphyton micro-algal density and periphyton chlorophyll *a* in sampling period 1, 2, & 4.

Chlorophyll *a* concentrations in the water column varied significantly between sampling periods, sites and the duration of study in each sampling period. The general trend of chlorophyll *a* distribution exhibited a normal single peak growth pattern except for sampling period 1 when all sites exhibited 2 distinct peaks. The peaks at KB were more distinct than the other 2 sites and this may be due to the higher TP concentrations at KB. During sampling period 2 the maximum concentration of chlorophyll *a* was recorded at LC. This high concentration might be representing the spring turnover and this resulted in an increase in diatom abundance.

The periphyton chlorophyll *a* concentrations during sampling period 3 at site KB were related to the biomass values. This was in contrast to the species density values which did not follow the chlorophyll *a* variation during sampling period 3. The weak relationship between periphyton chlorophyll *a* and species density during sampling period 3 at KB may be related to the lower biomass values of diatom and non diatom species found at site KB (Stevenson et al., 1996; Biggs et al., 1998). Additionally, KB periphyton during sampling period 3 demonstrated a continuous growth until the end of the study (30 days) as opposed to the other two sites and sampling periods. This indicates the periphyton community at site KB during sampling period 3 might still be in a transition period (Lamberti & Resh, 1985; Porter-Goff 2010). Additionally, the chlorophyll *a* concentrations in the water column during sampling period 3 followed a similar variation over time and this resulted in a significant positive correlation between chlorophyll *a* in water column and periphyton biomass. The results thus show influence of water column micro-algal biomass on the periphyton biomass.

As mentioned above biomass values generally followed the same trend as that of biofilm thickness, species density and periphyton chlorophyll *a* throughout the study period. The maximum biomass recorded was during sampling period 3 at KB (3035.56mg/L) which is summer and included higher populations of Chlorophyceae, Cyanophyceae and protozoa. While the minimum value was recorded at the same site (KB) during fall (sampling period 4, 0.05mg/L) which was the beginning of decreasing water temperatures and insolence. The results of regression analysis showed periphyton biomass was significantly related to TP concentration.

As described by Stevenson et al (1996), biomass is a fundamental measure of the interaction between species composition, abiotic environmental factors and grazing. However, they also proposed that biomass measurement is a poor indicator of benthic algal mass when

there are large inputs of sediment and inorganic matter in addition to the dominant presence of heterotrophic and detrivore organisms within the periphyton community. Sites KB and C10 reflected Stevenson's proposal as the slides at KB were consistently covered with an inorganic layer of sediment. This was the result of effluent release from the City of Barrie's effluent treatment plant into the lake. Site C10 also received suspended load from land water run-off and other anthropogenic sources. Site C10 had the lowest water levels among the three locations. The slides often showed sand deposition. The accumulation of inorganic deposits may have skewed the biomass results and therefore resulted in a weak relationship with algal density and biofilm thickness.

The species density increased with the duration of study up to 15-20 days (Figure 10). The late successional growth phase (25 to 30 days) showed a decreasing trend probably under the influence of sloughing off processes. Many other researchers have observed the same trend in species density over time (Stevenson et al., 1996; Biggs et al., 1998; Catteano et al., 1990; Kanavillil et al., 2012). An exception to this general trend occurred only during sampling period 1 at LC where a continuous increase of density was observed throughout the period. Any particular reason could not be described for this exception.

The temporal variation in periphyton density was significant (rmANOVA, Table 5). The highest value of species density was observed during sampling 1 (first fall) at KB. Since KB was prone to most anthropogenic disturbance compared to other two locations, the variation in periphyton density could indicate the influence of these factors on the community build-up. In addition to the anthropogenic factors, physical factors such as water movement and wave actions could influence the periphyton community formation. Previous studies by Catteano et al (1990) demonstrated that physical factors such as wave action can influence populations in periphyton.

Species richness and diversity.

Species richness and diversity are important indicators of the biofilm development processes which respond to external stimuli/ stressors such as pollution (Kitner and Poulickova 2003; Poulickova et al., 2004). The overall trend of species diversity showed a growth curve with 2 distinct peaks during the 30 day period and varied significantly between days of growth (5 to 30).

The first peak of diversity was recorded (consistently in all sampling periods) during the early phase of periphyton development i.e. by day 10 and the second peak during the late growth phase immediately after the sloughing off period (25-30 days). The similarities in diversity measurements during the two peaks may be attributed to the availability of space and less competition. An exception to this trend was observed during sampling period 4 at KB where a cyclic pattern of high and low diversities was observed. The fluctuation in species diversity at KB (sampling period 4) corresponded to intense diatom dominance shifts ending in the lowest values of species diversity during the study. It is important to note that site KB had the highest overall TP concentrations among the sites during the entire study period. As mentioned earlier TP has a strong influence on periphyton growth dynamics. However, the cyclic pattern at site KB may also be related to the effluent release and the turbidity. At site KB the rig and slides were often observed to have a layer of sediment deposit. Probably, this deposit might have resulted in the collapse of the biofilm thereby opening new space for the new recruits. The collapse or disturbance of periphyton has been studied well in flood plains and lotic environments, but very rarely in lentic ecosystems. The observed single peak growth pattern of species diversity found at site LC during sampling period 4 suggests a stable environmental condition during the period of study (sampling period 4).

Species richness followed the trend of a double peaked growth pattern similar to that of species diversity over the growth period. It was generally higher during the early and late phases of periphyton growth. Artigas et al (2012) found similar results and observed a great increase of species richness on days 1 to 7 as a result of early colonization processes.

Species richness varied significantly between days, sites and sampling periods. The variation may be attributed to species tolerances to the existing environmental variables including hydrological parameters such as temperature, dissolved oxygen and availability of nutrients from the water column. The greatest overall species richness was observed during sampling period 4 which is fall. The fall turnover may result in the recycling of various nutrients such as silica and phosphorus which might influence the growth of periphyton communities, in particular the Bacillariophyceae. The days with higher biomass (usually 20-25 days) were observed to have higher species richness, and they were significantly correlated in sampling period 1 and 4. This may be an expected outcome due to higher immigration rates (more motile diatoms) and lower emigration rates in addition to the arrival of environmentally tolerant taxa (Peterson et al., 1996; Sekar et al., 1997; Bellinger and Sigee 2010).

Sites KB and C10 during sampling period 3 exhibited a normal growth curve for species richness with a significant decrease in species richness during the late phase of development. Species richness and TP were significantly correlated in all 4 sampling periods. Sampling period 3 showed the highest TP concentration during the study. The species that were present at a high abundance during the late phase of periphyton growth at KB included the diatom species *Acnanthidium minutissimum* and *Fragellaria crotensis* which are indicators of high nutrient concentrations (Vis et al., 1998; Geoffroy et al., 2000; Duong et al., 2007). At C10 species composition included the pollution tolerant diatoms *Acnanthidium minutissimum* and *Navicula*

lanceolata (Kelly et al., 2005). Phosphorus is the limiting factor in lentic environments and may influence the growth of periphyton communities (Winter and Duthie 2000). As mentioned before, there were also some rare species that appeared only during certain sampling periods. For example, *Meridion circulare,* a rosette forming diatom with strong attachment was only observed during sampling period two. According to Smucker & Vis (2013), the community composition at the end of succession represents the typical environmental conditions existing at a site location.

Periphyton communities take varying time periods to reach maturity. This depends on the location of study, study period, hydrological conditions, the substratum used etc. According to Bernhardt & Likens (2004) periphyton communities reach maturity in 28 days on a natural substratum such as rocks, however, the duration of maturity on artificial substratum can take much longer (Lamberti & Resh, 1985; Porter-Goff 2010). However, the present study showed community reached stability by 15-20 days, as the parameters such as the density, diversity, species richness and biofilm thickness started stabilizing by this time. Therefore, the duration of slide exposure in this study is thought to produce a good account of periphyton community development in this area.

Non Diatom succession

The succession of taxonomic groups other than diatoms in periphyton community observed in this study was different from those observed by Sekar et al (2002) and Liboriussen and Jeppesen (2006), however these previous studies used plexi-glass and plastic strips as substrata and may also play a contributing role to the variation of non diatom contributions to the over community. In the present study, Bacillariophyceae dominated the community throughout

the study period in contrast to the co-dominance of Chlorophyceae and Bacillariophyceae during the initial phase and dominance of Cyanophyceae towards the end of the study as observed by the above workers. However, this study also showed abundances of Chlorophyceae and Cyanophyceae during different sampling periods. For example sampling period 3 showed high densities of Chlorophyceae (25%) and Cyanophyceae (20%) as part of the periphyton community instead of 7-10% of the entire community observed during the other sampling periods. Furthermore, the variation observed among non diatom groups was site specific. For instance, the highest abundance of Cyanophyceae (~30% of the entire community) was observed at site C10. As C10 was considered the least disturbed one in this study these results may suggest the need for further investigation and ongoing water quality monitoring.

The successional pattern of non-diatoms showed presence of less motile Chlorophyceae such as *Coleachaeta* spp., *Cosmarium* spp., and *Coleastrum* spp. during the early stages of periphyton community development followed by motile or strongly attached forms such as *Closterium* spp., Filamentous green algae (FGA) spp. and the Cyanophyceae group. This successional pattern may be influenced by various factors such as competition for light and space (Sekar et al., 2004; Bellinger & Sigee 2010).

Cyanophyceae were present during all sampling periods except sampling period 2 Cyanophyceae have the ability to produce toxic lipopolysaccharides which can cause gastric distress and various dermatitis responses (NOAA 2014). As reported earlier, increased nutrient input can lead to a dense growth of certain Cyanophyceae (Schindler, 1977; Wetzel, 1983; Winter et al., 2011). As reported by Nurnburg et al (2013) Lake Simcoe's consistently high TP concentration in the recent past might have resulted in a dense growth of Cyanophyceae at certain locations.

Protozoa are the major consumers of microalgae and bacteria in the periphyton communities (Rosemond et al., 1993; Burgmer et al., 2010). Thus, the dominance of protozoa may have a significant impact on the periphyton community composition (Rosemond et al., 1993; Burgmer et al., 2010). As observed in other non-diatom groups, the population densities of protozoa also exhibited variation with time. The growth rate of periphyton became slow with lowering of water temperature during sampling periods 1 and 2 (Table 4) and this agrees with earlier studies (Rosemond et el., 1993; Mieczan et al., 2013).

Conclusion

The general trend of periphyton growth was highly influenced by natural autogenic succession processes in addition to the time of year (seasonality), site and duration of slide exposure in each sampling period. The overall periphyton growth exhibited an increase in density during the early phase; a climax during the mid phase; a reduction during sloughing off period (mid-late phase) and an increase towards the end due to re-colonization. The present study showed consistent dominance of Bacillariophyceae during the entire study period as opposed to the initial co-dominance of Bacillariophyceae and Chlorophyceae, leading to Chlorophyceae during the middle phase and to Cyanophyceae towards the end as observed by Sekar et al (2002). Therefore, the hypothesis that the periphyton community dynamics are influenced by autogenic processes was well supported, however a longer duration of study might have provided evidence of a longer term successional pattern such as taxonomic group shifts in periphyton communities.

This study observed great variability in periphyton species composition throughout the four sampling periods. Certain species were present only during certain season. Seasonality also had an influence on the overall density and periphyton taxonomic group dominance. The summer season (sampling period 3) resulted in high abundance of non diatom groups such as

Chlorophyceae and Cyanophyceae. The spring and fall sampling periods (SP2-Spring, SP1 & SP4- Fall) recorded high density of Bacillariophyceae may be due to the lake turnover processes and availability of nutrients. Therefore, the hypothesis that periphyton community dynamics vary seasonally can be justified.

The variation in species diversity distribution followed a normal growth pattern with two peaks demonstrating high diversities during the initial phase and towards the end of the study. This would suggest that species diversity depends on environmental disturbances and space availability. It was hypothesized that periphyton species diversity would decrease as a result of increased nutrient availability. This hypothesis seems to be true only with respect to Bacillariophyceae whose diversity is decreased with an increase in TP concentration, however, over all the diversity of the periphyton community did not change. This is because a reduction in Bacillariophyceae resulted in an increase in diversity in other groups such as Chlorophyceae, Cyanophyceae, protozoa and other organisms. Therefore, this hypothesis is not fully accepted. However, it is important to note that overall species diversity is also influenced by seasonal changes in hydrological factors especially the water temperature and insolence period.

This study showed that periphyton development is influenced by a variety of hydrological factors that may vary with site and season. This influence can relay important information about the water quality and the possibility of using periphyton community dynamics as water quality indicator of a certain location. The presence of indicator species such as the nutrient pollution tolerant species *Fragilaria crotensis* or *Anabaena* spp. (Cyanophyceae) indicates the condition of water in that location. Thus, the results from this study provide important and useful information to the ecosystem managers to consider periphyton community dynamics as an environmentally benign method of assessment of water quality in this area.

Chapter 4: An exploration of periphyton as a possible bio-indicator of water quality in the littoral zones of Northern Lake Simcoe

Introduction

Degradation of freshwater bodies is a global problem. Anthropogenic pressures have contributed significantly to the degradation of fresh water systems. Intensive mining and agricultural practices, damming, river diversion and increased human population are all contributors to the modification of aquatic ecosystems. The preservation of water quality is a global concern due to the limited availability of consumable fresh water. Fresh consumable surface water is one of the most important requirements to the quality of human life and it is decreasing on a daily basis (Chislock et al., 2013). As the human population keeps growing, the consequent anthropogenic impacts will exert more pressure on our fresh water bodies and therefore it is imperative that reliable monitoring tools be adopted to protect our water systems.

In Canada, Federal and Provincial governments spend millions of dollars annually to mitigate water quality issues (e.g., Lake Simcoe Clean Up Fund). Many factors (e.g. pollutants) that affect the quality of our water can be reduced through monitoring strategies. Bio-monitoring is a complimentary monitoring tool that can be used in combination with chemical monitoring methods. Periphyton is a useful tool that can be used to bio-monitor water systems (Azim et al., 2005; Schneider et al., 2009; Rotter et al., 2013). Various periphyton community characteristics that can be used to bio-monitor water systems include biomass, taxonomic composition, species diversity, chlorophyll *a* and species abundance/succession. The addition of periphyton as a monitoring tool in combination with chemical and other biological methods will result in a comprehensive approach of water quality monitoring.

Factors influencing water quality

Abiotic factors influencing water quality include chemical contaminants such as pharmaceuticals, industrial waste, fertilizers, physical factors such as temperature and light intensity while biotic influences include biological factors such as pathogens, invasive species, etc.

The methods currently being used to monitor our water resources include chemical analysis (i.e., assessing the amount of contaminants, such as heavy metals, pharmaceuticals, etc), the measurement of abiotic parameters (e.g. dissolved oxygen, pH, etc.), and measurement of nutrients (e.g., Total Phosphorus (TP), Total Nitrogen (TN), Calcium (Ca), etc). In addition to these assessments, microbiological analysis (such as periphyton, phytoplankton, pathogens and coliforms bacteria) and macro-biological analysis (i.e. macro-invertebrates, fish communities and macrophytes) may also be used.

From the studies carried out in Europe, it has become evident that although the introduction of General Diatom Index (GDI) (Coste & Ayphassorho 1991: Kwandrans et al., 1998) adds validity to bio-monitoring, there is a rising concern of unreliability of this index to other geographic locations due to variation of environmental conditions and different taxa. This necessitates the development of monitoring metrics specific to geographical location of interest (Gaiser et al., 2006; Potapova & Charles, 2002; Reavie, 2010). Additionally, Kwandrans et al., (1998) suggests that the characteristics of river type (i.e: alpine vs. lowland, low vs. high velocity) must be considered while developing water quality indices. This opinion is shared by Winter and Duthie (2000); Lewis et al. (2002); Komulaynen (2002); and Hill et al (2000). Pan et

al (1999) adds that benthic diatoms are mainly regulated by local influences. Therefore, indices chosen to be used in a study must represent the geographical sampling location suitably.

Currently in Canada the Eastern Canadian Diatom Index (IDEC) has been developed for biomonitoring streams in Eastern Canada. The IDEC shows good correspondence with nutrient values and various diatom species for each geographic location, but does not take the non diatom members of periphyton into consideration (Lavoie et al., 2014).

Periphyton as a lentic system bio-indicator

The literature review showed far less research on the use of periphyton as a tool for lentic system bio-monitoring (see chapter 1 for details). However, there is a copious amount of literature available on the use of periphyton as a biomonitoring tool for the lotic environments (Table 1). Most bio-monitoring protocols for the pelagic zone of lakes consist of phytoplankton or epilithic forms on the sediments (Poulickova et al., 2004; Liboriussen & Jeppeson, 2006).

Excessive nutrient loading of water bodies is a leading cause of the impairment of freshwater and coastal marine ecosystems worldwide (Schindler, 1977; Cardinale, 2011; Chislock et al., 2013). In a local context Lake Simcoe, Ontario is still suffering from a 3 fold increase in phosphorus levels since pre settlement (North et al., 2013). This has led to the decline of the cold water fishery due to lower dissolved oxygen levels. However, as a result of the Lake Simcoe phosphorus reduction plan that targets a 40% reduction of P by 2045 (North et al., 2013), the conditions showed significant improvement.

High levels of nutrients and other pollutants can be retained in the littoral zone through macrophytic, epilithic and epiphytic buffers (Hadwen & Bunn 2005). This often means that nutrient increase may be detected in the pelagic zone especially during the early stages of

pollution. Periphyton generally grows in the littoral areas of lakes and is the set of organisms getting directly exposed to the land originated pollutants. Therefore using them as an early detection tool of pollutants is highly logical. In turn, this would help to design intervention/prevention strategies for the spreading of pollutants offshore (Hadwen & Bunn, 2005).

The littoral zone algae can represent the trophic status of lakes. Poulickova et al (2004) observed that the littoral periphyton samples could accurately reflect the trophic status of the lakes being studied (55.5% to 66.6% of the total lakes studied). Kitner and Poulickova (2003) evaluated the use of littoral diatoms as good indicators of water quality with positive results. They used two different trophic diatom indices as suggested by van Dam et al (1994) and Rott (1999) as well as the saprobity index of Slodecek and Sladekova (1996) (Poulickova & Kitner 2003). It was concluded that the littoral diatoms represented the trophic status of the littoral environment. Additionally, they noted that the index created by van Dam et al. (1996) was the best index to use, as it fits well with the data collected.

Many studies have been performed on diatom taxonomy and its response to biological, chemical and physical stressors. Diatoms have been suggested as strong indicators of water quality due to their single cell structure, narrow optima and tolerance levels for environmental variables as a result of quick generation time (Dixit et al., 1992). In addition to these qualities, diatoms are inexpensive to analyse (compared to chemical analysis) and can be easily collected. Additionally, well documented literature for species identification, tolerance, and sensitivity levels are readily available. However, there is a lack of data on non diatom groups of periphyton and their usefulness as indicators of water quality. Non diatoms groups can provide important information about the microcosm that they grow in and can be used to detect ecological

degradation (Hill et al., 2000; Blinn & Herbst 2003; Schneider et al., 2011). However, the identification of algae other than diatoms is very difficult due to various growth and generative stages that they possess (Schneider et al., 2011).

Current use of periphyton as bio-monitoring tool

Periphyton is currently being used in several water quality bio-assessment protocols on a global level by the European Union, the United States of America Environmental Protection Agency, and the Ministry of the Environment in New Zealand (European Water Framework Directive 2014; EPA 2014; NIWA 2014). In a local context, periphyton (diatom focussed) based bio-assessments are gaining popularity in Ontario and Eastern Canada (Lavoie et al., 2014) However, these protocols focus on stream bio-assessments and therefore leave a gap for nearshore areas. Additionally, there is a lack of periphyton studies that include members other than diatoms such as bacteria, Chlorophyceae, Cyanophyceae, protozoa etc.

The current biological water quality monitoring protocol in the Lake Simcoe watershed consists of collecting planktonic diatoms from the nearshore areas of the lake and tributaries, however this protocol is relatively new and analysis has yet to be completed (personal communication LSRCA 2014). The Ministry of the Environment also monitors water quality of Lake Simcoe through chemical analysis as well as phytoplankton analysis (LSRCA 2014).

The literature survey (see chapter 1) indicates that there is a lack of data on periphyton as bioindicator of water quality from temperate areas, especially from lentic water systems. Therefore, data on periphyton is thought to help us better understand the health of the aquatic ecosystems being studied.

This chapter describes the taxonomic composition in relation to the natural and anthropogenic stressors of periphyton communities in three locations of northern Lake Simcoe with varying degrees of anthropogenic influences. The community dynamics have been studied from the data on taxonomic composition, biomass, chlorophyll *a* and biofilm thickness over a period of 30 days.

Methods (refer to Chapter 2- General Materials and Methods for details)

The detailed general methodology is described in chapter 2. Briefly, the study was conducted in three sampling locations in the northern part of Lake Simcoe, namely Kempenfelt Bay, Barrie, ON (44.377858,-79.689331), Concession Point 10, Ramara Township, ON (44.590956,-79.317856), and Lagoon City, Brechin, ON (44.546931,-79.209366) (Figure 2).

Periphyton samples were collected using collection rigs (Figure 7), containing fifty (50) glass slides (10cm X 3cm X 0.3 cm). Extra slides were used for replicates and in case of breakage or loss. The rigs with cleaned slides were submerged in the littoral zone of the three sampling locations (approximately 10-20 cm below the surface water). Six glass slides each were collected at a five day interval to a maximum of 30 days.

This chapter concentrates on periphyton species composition and dynamics from the three sampling locations and relates that to water quality parameters to specifically address the dynamics in periphyton as indicators of water quality changes. Therefore, if the evidence supports, periphyton community composition and dynamics may be suggested as a biological indicator of water quality for the northern part of Lake Simcoe. The methods used for measurements and analyses of data on periphyton density, species composition, biofilm thickness, diversity with sampling location, sampling periods and duration of slide exposure remain the same as that of chapter 3 and therefore are not described here to avoid repetition.

Results

The description of the variation of hydrological and biofilm parameters has been provided in chapter 3. To avoid repetition while understanding the relationship between the water quality parameters and biofilm parameters, this chapter mainly focuses on the inter-relationship between these parameters. Therefore this chapter describes data from a biological indicator of environmental conditions point of view.

Variation and inter-relationship between water quality and periphyton parameters

Temperature (°C) varied greatly over the seasons (fall, spring and summer, 5-28°C; one way ANOVA $F_{3,72}$=30.68, p<0.05), but did not vary significantly between sites in a given sampling period. Temperature correlated significantly with species density during sampling period one (r^2=0.33, p<0.05) and met moderate significance at site KB (r^2=0.14, p=0.08). Species diversity and temperature showed moderate significant correlation at sites C10 and LC (r^2=0.16, p=0.07; r^2=0.17, p=0.06). Biofilm thickness was significantly correlated with temperature at site LC (r^2=0.57, p<0.05) and showed moderate significance at site C10 (r^2=0.17, p=0.07)

Conductivity varied significantly between sites ($F_{3,72}$= 10.12, p<0.05), but did not vary significantly between sampling periods. The highest conductivity value was recorded at site KB (1183µS), located near the treated effluent release area for the City of Barrie's Waste Water treatment plant. There was significant correlation between conductivity and species density in sampling period 1 & 3 (r^2=0.30, p<0.05; r^2=0.41, p<0.05). Significant correlation between species density and conductivity was found at site C10 (r^2=0.59, p<0.05). Biofilm thickness significantly correlated with conductivity at LC (r^2=0.28, p<0.05). A significant negative correlation was found between conductivity and biomass (r^2=0.05, p<0.05) at site KB.

Dissolved oxygen values ranged from 4.0mg/L to 10.2 mg/L. The highest values were recorded at site KB during spring (sampling period 2) while the lowest value was recorded at the end of sampling period 4 (second fall) at site LC. Dissolved oxygen values varied significantly between sampling periods, but not between sites (One way ANOVA $F_{3,72}$= 10.62, p<0.05). There was a significant correlation between dissolved oxygen and species density at KB (r^2=0.24, p<0.05). The species diversity values at LC correlated with dissolved oxygen (r^2=0.45, p<0.05). A significant correlation with biofilm thickness was observed at KB and LC (r^2=0.24, p<0.05; r^2=0.24, p<0.05, respectively). Dissolved oxygen showed a significant negative correlation with biomass at all sites (r^2=0.02, p<0.05, r^2=0.02, p<0.05; r^2=0.09, p<0.05. Periphyton chlorophyll *a* showed a significant positive correlation with dissolved oxygen (r^2=0.14, p<0.05).

Total phosphorus (TP) concentrations ranged from 0.06mg/L to 0.200mg/L over the entire study period. The highest recorded TP concentration was at KB (0.200mg/L) during sampling period 4. One way ANOVA analysis showed a significant variation of total phosphorus (TP) concentration between sites ($F_{2.69}$=3.13, p<0.05), but not between sampling periods. Sites KB and LC, situated in the vicinity of treated effluent releases, had consistently higher TP concentrations than site C10.

Regression analysis showed a significant relationship between TP and species density for sampling periods 1 & 4 (r^2=0.34, p<0.05; r^2=0.35, p<0.05); biomass for sampling period 1 (r^2= 0.22, p<0.05); and species diversity for sampling period 4 (r^2=0.35, p<0.05). Biofilm thickness showed a moderately significant correlation with TP at C10 (r^2=0.14, p=0.07). Species diversity showed a significant correlation with TP at KB only (r^2=0.30, p<0.05).

Chlorophyll *a* concentrations of periphyton varied from 0.09 to 1.41mg/m^{3}over the study period. Periphyton chlorophyll *a* concentrations varied significantly between sites ($F_{2,47}$=322.45, p<0.05) and days of growth ($F_{5,48}$=4.92, p<0.05, Table 5). There was a significant negative correlation with periphyton chlorophyll *a* and temperature at site KB (r^2=0.06, p<0.05) while at site C10 the relationship was positive (r^2=0.14, p<0.05). Periphyton chlorophyll *a* correlated with dissolved oxygen and conductivity at C10 only (r^2=0.13, p<0.05; r^2= 0.11, p<0.05).

Chlorophyll *a* concentrations in the water column varied significantly between sampling periods and sites ($F_{3,48}$=410.00, p<0.05; $F_{2,48}$=7611.67, p<0.05, respectively; Table 5). The maximum concentration of chlorophyll *a* was recorded during sampling period 2 at site LC (40.80 mg/m^3). Chlorophyll *a* showed a significant correlation with periphyton biomass at all three sites (r^2=0.28, p<0.05; r^2=0.63, p<0.05; r^2=0.24, p<0.05. A significant correlation was also found between chlorophyll *a* (water) and periphyton species density at sites KB and C10 (r^2= 0.19, p<0.05; r^2=0.36, p<0.05). Finally, chlorophyll *a* (water) and periphyton species diversity had a significant negative correlation at site KB (r^2=0.07, p<0.05)

The biofilm thickness varied significantly with the duration of slide exposure ($F_{3,48}$= 187.92, p<0.05; Table 5), between sampling periods ($F_{3,48}$=177.44, p<0.05; Table 5) and between sites ($F_{1,48}$=605.68, p<0.05, Table 5). There was a significant correlation between the biofilm parameters biofilm thickness and biomass at site C10 (r^2= 0.21, p<0.05). Species diversity showed a moderately significant correlation with biofilm thickness at LC (r^2=0.15, p=0.07), while species density correlated significantly with biofilm thickness at sites C10 and LC (r^2= 0.16, p<0.05; r^2=0.43, p<0.05).

Species density

Species density increased during the early and mid phases followed by a decrease towards the end due to sloughing off of cells (Figure 10a-d). The maximum and minimum species density values were recorded at site KB during sampling period 4 ($\log_{10}$ 8.25, $\log_{10}$ 4.84). According to the results of rmANOVA, species density showed significant variation between days ($F_{3,48}$=8.10, p<0.05, (Table 5) and between sites ($F_{1,48}$=835.21, p<0.05, Table 5), but did not significantly vary between sampling periods ($F_{3,48}$=1.38, p=0.261). As mentioned before species density showed significant correlation with the various water quality and biofilm parameters such as conductivity, pH, temperature, dissolved oxygen and chlorophyll *a*. Species density also showed a significant correlation with biomass at LC (r^2=0.20, p<0.05).

Biomass

Biomass followed a similar pattern of variation as biofilm thickness and species density; it increased gradually until 15-20 days then reduced by day 25 (Figure 11a-d). The maximum biomass value was recorded during sampling period 1 at KB (2643.00mg/L) while the minimum was observed at the same site (KB) during sampling period 4 (0.05mg/L). The periphyton biomass values during the study varied significantly between sampling periods ($F_{3,48}$=11.03, p<0.05), but the variation between sites was not significant.

A significant correlation between temperature and biomass was observed in all sites, however, at site LC a negative correlation was detected (r^2=0.06, p<0.05) while sites KB and C10 were positively correlated (r^2=0.15, p<0.05; r^2=0.39, p<0.05, respectively). Biomass and dissolved oxygen were negatively correlated at all sites (r^2=0.02, p<0.05; r^2=0.02, p<0.05; r^2= 0.09, p<0.05, respectively). The relationship between biomass and conductivity showed a negative correlation at KB and LC (r^2=0.02, p<0.05; r^2=0.11, p<0.05) and showed moderate

significance at site LC (r^2=0.03, p=0.09). The pH correlated significantly with biomass at site C10 (r^2=0.47, p<0.05).

Species richness

Species richness followed more or less the same trend as that of species diversity. Species richness also showed two peaks within the growth pattern, one during the early and the other during the late growth phases (Figure 12a-d). The rmANOVA results showed significant variation in species richness with the duration of slide exposure ($F_{5,48}$=3.39, p<0.05; Table 5), between the sites ($F_{2,48}$=226.39, p<0.05) and between sampling periods ($F_{3,48}$=16.24, p<0.05). The highest species richness value was recorded at KB (29 species) and the lowest at LC (6 species). There was a consistent increase in species richness up to days 20-25. The species richness variation was site specific. For example site KB ranked number one for overall species richness among all sampling periods. In contrast site C10 showed relatively lower species richness during sampling periods 1 and 2 while high richness during sampling periods 3 and 4. At site LC the species richness values followed a typical growth pattern which exhibited 2 peaks except during sampling period 3.

Regression analysis results showed a significant negative correlation between species richness and TP for sampling periods 1, 2 and 3 (r^2=0.01, p<0.05; r^2=0.05, p<0.05; r^2=0.02, p<0.05). Site-wise regression analysis showed significant correlations between species richness and various hydrological parameters. Although, species richness and temperature showed a significant correlation at KB (r^2=0.15, p<0.05), there was a negative correlation at C10 and LC (r^2=0.01, p<0.05; r^2=0.05, p<0.05). Species richness values decreased as the temperature increased. The total phosphorus correlated significantly with species richness at KB and had an

inverse relationship at C10 and LC. As the TP concentrations increased the species richness decreased. For example, Figure 12c shows a decrease in species richness during sampling period 3 at LC on day 10 while there was an increase of TP. A significant correlation between richness and DO was observed at KB (r^2=0.14, p<0.05), while a negative relationship was observed with conductivity at the same site (r^2=0.04, p<0.05). Species richness values decreased as conductivity values increased (Figure 12b).

Species diversity

Species diversity values with days of growth showed two peaks, one during the early and the other during the late growth phases (Figure 13a-d). Species diversity values varied considerably with the sampling period. For example, during sampling period 1 at KB diversity fell suddenly from day 15 (1.79) to day 20 (0.19) (Figure 13a). This is due to the rapid increase in density (97%) of the diatom *Cocconeis placentula*. Site KB experienced another variation during sampling period 4 when the diversity decreased on day 15 due to the dominance of the diatom *Navicula lancelota* (97%) (Figure 16d).

Repeated measure ANOVA showed significant variation in diversity between sampling periods ($F_{3,48}$=15.28, p<0.05). Overall species diversity measurements were highest during sampling periods 1 and 4 which coincided with fall turnover. During this time the diatom diversity was very high, but the non-diatoms showed lowest diversity. Sampling period 3 (summer) gave rise to the overall highest diversity (2.97) due to the dominance of non-diatom groups. Additionally, there was a significant correlation between periphyton species diversity and the species density of phytoplankton (r^2=0.32, p<0.05). This is an interesting relationship supporting the view that phytoplankton act as propagule supplier of periphyton.

The variation in diatom diversity was related to TP as evidenced by a significant correlation between these two (r^2=0.35, p<0.05) during sampling period 4. Site-wise regression analysis showed a significant positive correlation with TP at KB (r^2=0.30, p<0.05) while site C10 and LC showed a moderately significant negative correlation (r^2=0.10, p= 0.08). The negative correlation indicates an inverse relationship between TP and diversity.

As mentioned previously species diversity significantly related to various hydrological parameters such as temperature and dissolved oxygen. Additionally, moderately significant relationships were found between diversity and biofilm thickness at LC (r^2=0.15, p=0.07), and biomass at C10 (r^2=0.15, p=0.07).

Taxonomic Composition

Table 6 lists all species observed in the periphyton communities over the entire study period. In total, there were 65 Bacillariophyceae members, 29 members of Chlorophyceae, 19 members of protozoa, 4 genera of Cyanophyceae, 2 genera of Chrysophyceae and 1 Rotifera member in addition to various macroinvertebrates. Bacillariophyceae was the dominant group throughout the study. Chlorophyceae and protozoa were the next abundant groups with the exception of sampling period 3, day 10, where Cyanobacteria accounted for a large percentage of the overall abundance. The relative abundance of these groups expressed as percentage densities, varied between sampling periods (Figure 16a-d) and sites (Figure 17a-d).

In general the periphyton community development was highly dependent on the specific site and environmental factors such as TP, DO, and conductivity. Some sites shared common initial colonizers such as *Acnanthidium minutissimum, Cocconeis placentula, Cymbella tumida*

and *Diatoma vulgaris,* however variability between sites and between sampling periods (seasonal influence) was observed.

Diatom taxonomic composition showed season specific abundances (Table 7). For example, fall samples (sampling period 1) showed species such as *Cosmioneis* spp., and *Cymatopleura solea* while the spring samples (sampling period 2) showed the presence of *Cymatopluera elliptica*, *Entomoneis paludosa*, *Eucocconeis* spp., *Gomphoneis* spp., *Meridion circulare*, *Stauroneis* spp., and *Synedra cyclopium*.

The samples collected during summer gave rise to a highly diverse taxonomic composition which included many common species and species that were observed only during sampling period 3 (Table 7). These species included *Amphipleura pellucida*, *Cocconeis pediculus*, *Cosmioneis reimeri*, *Eutonia* spp., *Fragilaria capucina*, *Fragilaria* spp., *Frustulia* spp., *Gomphonema parvulum*, *Gomphoneis* spp., *Navicula stesvicensis*, and *Nitzschia calida* (Table 7). Species that overlapped between sampling periods 3 & 4, but were not observed in any other sampling periods included species such as *Navicula tripunctata*, *Neidium dubium*, and *Tabellaria flocculosa.* Finally, species unique to the second fall included *Amphipleura* spp. *Cocconeis pediculus*, *Cymatopleura ovalis*, *Epithemia sorex*, *Eucocconeis* spp., *Navicula gastrum*, *Nitzschia amphibia*, *Sellaphora pupulla*, and *Synedra capitata*.

Site specificity also played a role in diatom taxonomic composition. Most of the common diatoms such as *Cocconeis placentula*, *Cymbella tumida*, *Gomphonema truncatum*, *Navicula* spp., and *Synedra* spp. were observed in all 3 sites, however some were site specific, for example, *Meridion circulare* (KB and C10), *Nitzschia amphibia* (C10 and LC) and *Synedra cyclopium* (KB and C10).

Various indicator species (diatoms) of high, moderate and low organic pollution were observed during the study, such as *Cymatopluera elliptica*, *Epithemia turgida*, *Eutonia* spp., respectively (Table 8a). Additionally, indicators of high conductivity such as *Navicula slevencisus* and *Entomoneis paludosa* were observed during this study. Non diatom species such as *Pediastrum* spp. and *Cosmarium* spp. may be described as bio-indicators of high nutrient concentrations (Table 9).

Table 8a. Diatom relative abundance values calculated from total abundance of the diatoms according to site within each sampling period (total abundance is 1.0 or 100%).

SP1	KB		C10		LC	
Days	**Species**	**Abundance**	**Species**	**Abundance**	**Species**	**Abundance**
5	*Acnanthidium minutissimum*	0.18	*Cymbella tumida*	0.21	*Diatoma vulgaris*	0.09
5	*Cocconeis placentula*	0.15	*Navicula* spp.	0.43	*Navicula* spp.	0.13
5	*Diatoma vulgaris*	0.22	*Synedra* spp.	0.16	*Synedra* spp.	0.16
10	*Cocconeis placentula*	0.36	*Acnanthidium minutissimum*	0.14	*Cocconeis placentula*	0.56
10	*Fragellaria crotensis*	0.17	*Epithemia turgida*	0.18	*Navicula* spp.	0.13
10	*Aulacoseira (Melosira) varians*	0.12	*Navicula* spp.	0.19	*Synedra* spp.	0.08
15	*Cocconeis placentula*	0.36	*Cocconeis placentula*	0.23	*NA*	
15	*Fragellaria crotensis*	0.23	*Epithemia turgida*	0.26	*NA*	
15	*Navicula* spp.	0.13	*Navicula* spp.	0.15	*NA*	
20	*Cocconeis placentula*	0.96	*Cocconeis placentula*	0.15	*Acnanthidium minutissimum*	0.14
20	*Gomphonema truncatum*	0.01	*Epithemia turgida*	0.33	*Cocconeis placentula*	0.22
20	*Synedra* spp.	0.01	*Navicula* spp.	0.11	*Navicula* spp.	0.20
25	*Cocconeis placentula*	0.94	*Acnanthidium minutissimum*	0.16	*Cocconeis placentula*	0.29
25	*Gomphonema truncatum*	0.01	*Cocconeis placentula*	0.20	*Epithemia turgida*	0.09
25	*Synedra* spp.	0.01	*Epithemia turgida*	0.32	*Synedra* spp.	0.24
30	*Cocconeis placentula*	0.88	*Acnanthidium minutissimum*	0.10	*Cocconeis placentula*	0.30

30	*Gomphonema truncatum*	0.08	*Cocconeis placentula*	0.18	*Fragellaria crotensis*	0.31
30	*Navicula* spp.	0.01	*Epithemia turgida*	0.36	*Synedra* spp.	0.09
SP2	**KB**		**C10**		**LC**	
5	*Diatoma vulgaris*	0.64	*Cymbella tumida*	0.38	*Diatoma vulgaris*	0.06
5	*Navicula* spp.	0.10	*Diatoma vulgaris*	0.16	*Fragellaria crotonensis*	0.47
5	*Nitzschia sigmoidea*	0.08	*Fragellaria crotonensis*	0.12	*Synedra* spp.	0.15
10	*Acnanthidium minutissimum*	0.21	*Acnanthidium minutissimum*	0.15	*Acnanthidium minutissimum*	0.08
10	*Diatoma vulgaris*	0.25	*Cymbella tumida*	0.49	*Diatoma vulgaris*	0.01
10	*Navicula* spp.	0.22	*Diatoma vulgaris*	0.08	*Fragellaria crotonensis*	0.57
15	*Acnanthidium minutissimum*	0.63	*Acnanthidium minutissimum*	0.28	*Acnanthidium minutissimum*	0.23
15	*Diatoma vulgaris*	0.22	*Cocconeis placentula*	0.65	*Cocconeis placentula*	0.16
15	*Navicula* spp.	0.05	*Epithemia turgida*	0.04	*Fragellaria crotonensis*	0.46
20	*Acnanthidium minutissimum*	0.61	*Cocconeis placentula*	0.82	*Amphora ovalis*	0.48
20	*Diatoma vulgaris*	0.25	*Cymbella lanceolota*	0.11	*Cocconeis placentula*	0.22
20	*Navicula* spp.	0.05	*Epithemia turgida*	0.02	*Fragellaria crotonensis*	0.05
25	*Acnanthidium minutissimum*	0.29	*Acnanthidium minutissimum*	0.33	*Acnanthidium minutissimum*	0.14
25	*Diatoma vulgaris*	0.23	*Cocconeis placentula*	0.48	*Cocconeis placentula*	0.73
25	*Navicula* spp.	0.20	*Cymbella lancelota*	0.12	*Cymbella tumida*	0.02
30	*Acnanthidium minutissimum*	0.58	*Acnanthidium minutissimum*	0.37	*Amphora ovalis*	0.09
30	*Fragellaria crotonensis*	0.17	*Cocconeis placentula*	0.54	*Acnanthidium minutissimum*	0.39
30	*Navicula* spp.	0.06	*Cymbella tumida*	0.02	*Cocconeis placentula*	0.38
SP3	**KB**		**C10**		**LC**	
5	*Cocconeis placentula*	0.19	*Cocconeis pediculus*	0.14	*Cymbella tumida*	0.08
5	*Cymbella tumida*	0.09	*Cymbella tumida*	0.12	*Navicula* spp.	0.22
5	*Navicula* spp.	0.32	*Navicula* spp.	0.18	*Synedra* spp.	0.11
10	*Acnanthidium minutissimum*	0.18	*Cocconeis placentula*	0.09	*Cocconeis placentula*	0.50
10	*Cymbella tumida*	0.20	*Cymbella tumida*	0.06	*Gomphonema truncatum*	0.11
10	*Fragilaria crotonensis*	0.18	*Synedra* spp.	0.14	*Navicula* spp.	0.03
15	*Acnanthidium minutissimum*	0.16	*Acnanthidium minutissimum*	0.11	*Cocconeis placentula*	0.26
15	*Cocconeis placentula*	0.33	*Cocconeis placentula*	0.18	*Epithemia turgida*	0.09
15	*Cymbella tumida*	0.20	*Epithemia turgida*	0.08	*Fragellaria crotonensis*	0.06
20	*Acnanthidium minutissimum*	0.36	*Cocconeis placentula*	0.31	*Cocconeis placentula*	0.43

20	*Cocconeis placentula*	0.41	*Epithemia turgida*	0.14	*Epithemia turgida*	0.30
20	*Gomphonema truncatum*	0.08	*Navicula* spp.	0.19	*Navicula tripunctata*	0.15
25	*Acnanthidium minutissimum*	0.45	*Cymbella tumida*	0.07	*Cocconeis placentula*	0.13
25	*Cocconeis placentula*	0.14	Navicula spp.	0.38	*Cymbella tumida*	0.41
25	*Fragilaria crotonensis*	0.20	*Synedra* spp.	0.08	*Epithemia turgida*	0.15
30	*Acnanthidium minutissimum*	0.27	*Acnanthidium minutissimum*	0.22	*Cocconeis placentula*	0.22
30	*Cocconeis placentula*	0.14	*Cocconeis placentula*	0.20	*Cymbella tumida*	0.30
30	*Fragilaria crotonensis*	0.42	*Navicula lanceolata*	0.11	*Epithemia turgida*	0.20
SP4	**KB**		**C10**		**LC**	
5	*Cymbella tumida*	0.14	*Cymbella tumida*	0.13	*Amphora ovalis*	0.23
5	*Fragilaria crotonensis*	0.20	*Fragilaria crotonensis*	0.12	*Cocconeis placentula*	0.23
5	*Synedra* spp.	0.19	*Synedra* spp.	0.15	*Cyclotella* spp.	0.08
10	*Fragilaria crotonensis*	0.29	*Fragilaria crotonensis*	0.20	*Navicula* sp	0.17
10	*Melosira varians*	0.14	*Melosira varians*	0.19	*Synedra acus*	0.19
10	*Synedra* spp.	0.11	*Synedra* spp.	0.13	*Synedra ulna*	0.16
15	*Fragilaria crotonensis*	0.00	*Fragilaria crotonensis*	0.17	*Cocconeis placentula*	0.16
15	*Navicula lanceolata*	0.97	*Navicula lanceolata*	0.12	*Navicula lanceolata*	0.10
15	*Synedra acus*	0.00	*Synedra acus*	0.12	*Synedra ulna*	0.10
20	*Asterionelia formosa*	0.03	*Asterionelia formosa*	0.10	*Cocconeis placentula*	0.12
20	*Fragilaria crotonensis*	0.19	*Fragilaria crotonensis*	0.29	*Navicula* sp	0.14
20	*Navicula lanceolata*	0.23	*Navicula lanceolata*	0.16	*Synedra ulna*	0.14
25	*Amphora ovalis*	0.71	*Amphora ovalis*	0.20	*Cocconeis placentula*	0.16
25	*Diatoma vulgaris*	0.04	*Diatoma vulgaris*	0.31	*Cymbella lanceolata*	0.15
25	*Navicula* spp.	0.06	*Navicula* spp.	0.13	*Fragilaria crotonensis*	0.28
30	*Amphora ovalis*	0.24	*Amphora ovalis*	0.36	*Cocconeis placentula*	0.20
30	*Cocconeis pediculus*	0.41	*Cocconeis pediculus*	0.10	*Fragilaria crotonensis*	0.57
30	*Nitzschia lanceolata*	0.05	*Nitzschia lanceolata*	0.16	*Synedra* spp.	0.05

Table 8b. Non diatom relative abundance values calculated from total abundance of the non diatoms according to site within each sampling period (total abundance is 1.0 or 100%).

Days	Species	Abundance	Species	Abundance	Species	Abundance
SP1	**KB**		**C10**		**LC**	
5	*Colpidium* spp.	0.05	*Closterium* spp.	0.5	*Staurastrum* spp.	0.35
5	*Daphnia* spp.	0.05	*Scenedesmus quadricauda*	0.25	*Merismopedia glauca*	0.30
5	*Unknown protozoan* spp.	0.91	*Unknown protozoan* spp.	0.25	*Unknown protozoan* spp.	0.15

10	*Coleochaete* spp.	0.14	*Scenedesmus quadricauda*	0.059	*Ankistrodesmus spp.*	0.13
10	*Coleastrum* spp.	0.03	*Unknown protozoa* spp.	0.82	*Coleochaete* spp.	0.13
10	*Anabaena* spp.	0.75	*Nematode* spp.	0.04	*Unknown protozoa* spp.	0.75
15	*Colpidium* spp.	0.5	*Coleochaete* spp.	0.78	*Coleochaete* spp.	0.78
15	*Vorticella* spp.	0.39	*Unknown protozoa* spp.	0.22	*Unknown protozoa* spp.	0.22
20	*Coleochaete* spp.	0.05	*Ankistrodesmus* spp.	0.07	*Ankistrodesmus* spp.	0.07
20	*Stentor* spp.	0.06	*Pediastrum simplex*	0.03	*Pediastrum simplex*	0.03
20	*Unknown protozoa* spp.	0.81	*Merismopedia glauca*	0.53	*Merismopedia glauca*	0.53
25	*Coleochaete* spp.	0.10	*Chroococcus* spp.	0.26	*Chroococcus* spp.	0.26
25	*Dictyosphaerium pulchellum*	0.12	*Unknown protozoan* spp.	0.57	*Coleochaete* spp.	0.04
25	*Unknown protozoa* spp.	0.65	*Nematode* spp.	0.043	*Coleastrum* spp.	0.04
30	N/A	0	*Chroococcus* spp.	0.11	*Chroococcus* spp.	0.11
30	N/A	0	*Scenedesmus quadricauda*	0.09	*Merismopedia glauca*	0.76
30	N/A	0	*Merismopedia glauca*	0.76	*Scenedesmus quadricauda*	0.09
SP2	**KB**		**C10**		**LC**	
5	*Scenedesmus quadricauda*	0.12	*Lacrymaria* spp.	0.15	*Scenedesmus quadricauda*	0.12
5	*Euplotes* spp.	0.10	*Stylonchia* spp.	0.23	*Euplotes* spp.	0.10
5	*Nematode* spp.	0.57	*Nematode* spp.	0.15	*Nematode* sp	0.57
10	*Spirogyra* spp.	0.28	*Unknown FGA*	0.12	*Spirogyra* spp.	0.28
10	*Unknown protozoa* spp.	0.20	*Euplotes* spp.	0.24	*Unknown protozoa* spp.	0.20
10	*Nematode* spp.	0.12	*Nematode* spp.	0.18	*Nematode* spp.	0.12
15	*Euplotes* spp.	0.31	*Coelastrum* spp.	0.29	*Euplotes* spp.	0.31
15	*Litonotus* spp.	0.15	*Stylonchia* spp.	0.29	*Litonotus* spp.	0.15
20	*Coleochaete* spp.	0.15	*Unknown protozoa* spp.	0.29	*Coleochaete* spp.	0.15
20	*Scenedesmus quadricauda*	0.185	*Unknown FGA* sp	0.74	*Scenedesmus quadricauda*	0.18
20	*Chironomid* spp.	0.184	*Amoeba* spp.	0.05	*Chironomid* spp.	0.18
25	*Cosmarium* spp.	0.44	*Rotifer* spp.	0.13	*Cosmarium* spp.	0.44
25	*Scenedesmus quadricauda*	0.22	*Unknown protozoa* spp.	0.5	*Scenedesmus quadricauda*	0.22
25	*Unknown protozoa* spp.	0.22	N/A		*Unknown protozoa* spp.	0.22
30	*Rotifer* spp.	0.19	*Unknown protozoa* spp.	0.27	*Rotifer* spp.	0.19
30	*Unknown protozoa* spp.	0.44	*Vorticella* spp.	0.13	*Unknown protozoa* spp.	0.44
SP3	**KB**		**C10**		**LC**	
5	*Unknown GA* spp.	0.17	*Merismopedia glauca*	0.34	*Merismopedia glauca*	0.83
5	*Stylonchia* spp.	0.13	*Euplotes* spp.	0.10	*Unknown GA* spp.	0.09
5	*Unknown protozoan* spp.	0.15	*Stylonchia* spp.	0.14	*Cosmarium* spp.	0.03

10	*Kirchneriella* spp.	0.45	*Dictyosphaerium pulchellum*	0.07	*Coleochaete* spp.	0.58
10	*Cosmarium* spp.	0.09	*Merismopedia glauca*	0.84	*Stigeoclonium* spp.	0.13
10	*Scenedesmus quadricauda*	0.09	*Spirulina* spp.	0.01	*Euplotes* spp.	0.08
15	*Cosmarium* spp.	0.67	*Dictyosphaerium pulchellum (cells)*	0.24	*Merismopedia glauca*	0.84
15	*Unknown ostracod*	0.33	*Merismopedia glauca*	0.65	*Coleochaete* spp.	0.07
15	N/A		*Coleochaete* spp.	0.02	*Unknown protozoa* spp.	0.02
20	*Cosmarium* spp.	0.03	*Coleochaete* spp.	0.5	*Pediastrum simplex*	0.2
20	*unknown GA* spp.	0.03	*Stigeclonium* spp.	0.5	*Unknown protozoa* spp.	0.8
20	*Unknown protozoa* spp.	0.91	N/A		N/A	
25	*Unknown FGA* spp.	0.67	*Cosmarium* spp.	0.67	*Coelastrum* spp.	0.40
25	*Unknown copepod*	0.11	N/A		*Unknown GA*	0.18
30	*Coelastrum microporum*	0.19	*Merismopedia glauca*	0.82	*Coelastrum* spp.	0.36
30	*Cosmarium* spp.	0.10	*Euplotes* spp.	0.04	*Scenedesmus quadricauda*	0.11
30	*Unknown protozoa* spp.	0.57	*Coleochaete* spp.	0.03	*Unknown GA* spp.	0.16
SP4	**KB**		**C10**		**LC**	
5	*Arcella* spp.	0.27	*Euplotes* spp.	0.25	*Pediastrum simplex*	0.33
5	*Unknown Protozoa* spp.	0.45	*Merismopedia glauca*	0.4	*Euplotes* spp.	0.67
5	N/A		*Unknown GA* spp.	0.15	N/A	
10	*Arcella* spp.	0.05	*Unknown protozoa* spp.	0.31	*Coelastrum* spp.	0.38
10	*Stylonychia* spp.	0.19	*Cosmarium* spp.	0.15	*Unknown protozoa* spp.	0.25
10	*Unknown protozoa* spp.	0.48	*Nematode* spp.	0.15	N/A	
15	*Scenedesmus quadricauda*	0.25	*Unknown protozoa* spp.	0.68	*Unknown protozoa* spp.	0.7
15	*Platycola vaginella*	0.5	*Actinosphaerium spp.*	0.08	N/A	
15	*Euplotes* spp.	0.25	*Nematode* spp.	0.08	N/A	
20	*Unknown protozoa* spp.	0.90	*Cosmarium* spp.	0.22	*Dictyosphaerium pulchellum*	0.41
20	*Pediastrum simplex*	0.02	*Scenedesmus quadricauda*	0.44	*Unknown protozoa* spp.	0.33
20	*Scenedesmus quadricauda*	0.08	N/A		*Coelastrum* spp.	0.11
25	*Coelastrum* spp.	0.33	*Coelastrum* spp.	0.30	*Unknown protozoa* spp.	0.67
25	*Arcella* spp.	0.33	*Unknown GA* spp.	0.255814	*Rotifer* spp.	0.25
25	*Nematode* spp.	0.22	*Unknown protozoa* spp.	0.139535	*Stentor* spp.	0.08
30	*Unknown FGA* spp.	0.83	*Unknown FGA* spp.	0.220779	*Vorticella* spp.	0.13
30	*Nematode* spp.	0.069	*Unknown GA* spp.	0.12987	*Coleochaete* spp.	0.17
30	N/A		*Nematode* spp.	0.168831	*Unknown protozoa.*	0.2

Table 9. Possible diatom and non diatom species as environmental indicators in Northern Lake Simcoe.

Environmental indication	Indicator Species	Reference
Diatom Group		
Heavy organic pollution	*Cymatopluera elliptica* (KB)	Kelly et al., 2005
	Synedra cyclopium (KB)	Kelly et al., 2005
	Nitzschia sigmoidea (LC)	Kelly et al., 2005
		Wu et al., 2011
Moderate organic pollution	*Epithemia turgida* (C10 dominant)	Kelly et al., 2005
Low nutrient concentration	*Eutonia* spp. (C10)	Kelly et al., 2005
	Gomphoneis spp. (C10)	Stevenson et al., 1996
	Tabellaria flocculosa (C10)	Kelly et al., 2005
High Electrolytes	*Navicula slevencisus* (KB & LC)	Potapova et al., 2013
	Entomoneis paludosa (KB & LC)	Kelly et al., 2005
Non Diatom Group		
High nutrient concentration	*Pediastrum* spp. (KB & LC)	Schneider et al., 2009
	Cosmarium spp. (KB & LC)	Schneider et al., 2009
	*Scenedesmus quadricauda (*KB & LC)	Schneider et al., 2009
	Dictyosphaerium pulchellum (KB & LC)	Schneider et al., 2009

Canonical Correspondence Analysis (CCA)

Diatom groups

Ordination was used to assess the correspondence between hydrological parameters (TP, DO, pH, temperature, conductivity), and periphyton community composition between sites (Table 10). The analysis was carried out for all 4 sampling periods separately. The data for diatom and non diatom groups was analysed separately by CCA.

Sampling period 1

Eigenvalues along the first and second axes were 0.3069 and 0.1464 respectively. This explained 46% of the total variation for sampling period 1. The relative positions of the species and site scores showed a strong influence of hydrological parameters on the species composition at each site. The most important predicators of species distribution, as indicated in their significant correlation with the first CCA axis were DO (r^2=0.69, p<0.001), pH (r^2=-0.20, p<0.001), temperature (r^2=-0.64, p<0.001), conductivity (r^2=-0.87, p<0.001), site (r^2=0.56, p<0.001) and TP (r^2=0.04, p<0.001). Although the largest variation among the three sites was attributed to the first CCA axis, the second CCA axis also exhibited strong correlations with the environmental variables such as, dissolved oxygen (r^2=0.72, p<0.001), pH (r^2=0.98, p<0.001), temperature (r^2=0.77, p<0.001), and total phosphorus (r^2= 0.99, p<0.001) (Table 10).

Table 10. Regression values for corresponding hydrological parameters in CCA analysis in all four sampling periods (Significance codes: '***' 0.001 '**' 0.01 '*' 0.05).

Diatoms				**Non diatoms**			
Parameter	**CA1 r^2**	**CA2 r^2**	***p***	**Parameter**	**CA1 r^2**	**CA2 r^2**	***p***
Sampling period 1							
DO	0.69	0.72	0.001 ***	DO	0.80	-0.60	0.001 ***
pH	-0.20	0.98	0.001 ***	pH	0.99	0.09	0.001 ***
Temp	-0.60	0.77	0.001 ***	Temp	0.96	0.28	0.001 ***
TP	0.05	0.99	0.001 ***	TP	0.99	-0.03	0.001 ***
Cond	-0.87	-0.49	0.001 ***	Cond	-0.33	0.95	0.001 ***
Sampling period 2							
DO	-0.88	-0.48	0.001 ***	DO	-0.30	0.95	0.666
Temp	0.95	0.31	0.001 ***	Temp	0.05	-0.99	0.001 ***
TP	-0.99	-0.04	0.001 ***	TP	0.36	0.93	0.825
Cond	-0.98	0.20	0.001 ***	Cond	0.65	0.76	0.001 ***
Sampling period 3							
DO	-0.13	0.99	0.484	DO	0.85	0.52	0.001 ***
pH	0.99	-0.07	0.001 ***	pH	0.80	0.61	0.001 ***
Temp	0.83	0.56	0.001 ***	Temp	0.25	0.97	0.001 ***

TP	-0.57	0.82	0.001 ***	TP	-0.96	0.28	0.001 ***
Cond	-0.99	-0.14	0.001 ***	Cond	-0.85	-0.52	0.001 ***
Sampling period 4							
pH	0.42	0.91	0.001 ***	pH	0.99	-0.07	0.001 ***
Temp	-0.56	0.83	0.672	Temp	0.83	0.56	0.001 ***
TP	-0.96	-0.26	0.692	TP	-0.57	0.82	0.001 ***

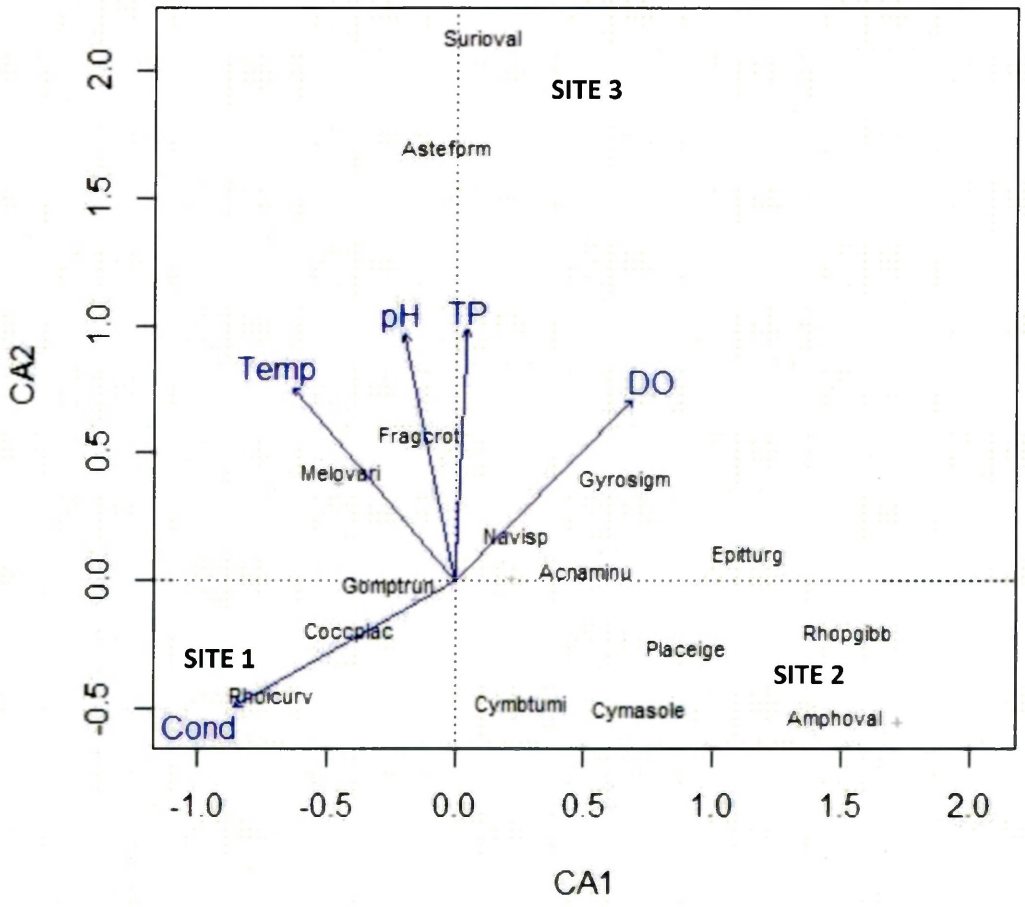
Figure 18 shows the position of site KB in the lower left corner of the diagram corresponding to the conductivity variable. Site KB exhibited the highest values of conductivity during the study (Table 4). The location of the diatom species *Rhoicosphenia curvata* in the lower left quadrant corresponded strongly to site KB as it was observed exclusively at site KB (Figure 18).

Site LC was plotted at the centre- top position of the diagram (Figure 18). The species associated with site LC were *Suriella ovalis* (exclusive to site LC) and *Asterionelia formosa* which was found in a higher abundance at this site than the other 2 sites (Figure 18). In terms of the influence of environmental factors TP corresponded most with site LC (Figure 18). This is due to higher concentrations of TP at site LC (0.04mg/L to 0.13mg/L) compared to the other two sites during sampling period 1 (Table 3). Figure 18 showed the location of site 2 (C10) in the lower left quadrant of the CCA diagram. Although C10 did not show correspondence with any of the environmental factors species such as *Pinnularia* sp. was exclusive to this site (Figure 18). The species *Amphora ovalis*, *Placoneis eigens*, and *Rhopolodia gibba* were observed in higher abundance at C10 than at the other two sites (Figure 18).

Figure 18 shows the diatom species *Aulacoseira varians* (*Melosira varians*) was corresponded to the temperature and was observed at sites KB and LC only. Higher temperatures were recorded at both these sites (15°C & 17°C respectively). A correspondence with *Fragilaria*

crotensis with pH can be seen in Figure 18. *Fragilaria crotensis* was present at all sites and ANOVA results showed that pH did not vary significantly between sites. The dissolved oxygen concentration was related to the diatom species *Gyrosigma acuminatium* (Figure 18). This particular species was present in all 3 sites, and ANOVA results showed no significant variation between sites.

Figure 18. CCA bi-plots of dominant diatom species composition with corresponding environmental factors for sampling period 1 by sites and species. Eigenvalues along the first and second axes were 0.3069 and 0.1464 respectively. This explained 46% of the total variation. The species names have been abbreviated to fit the bi plot.



Sampling period 2

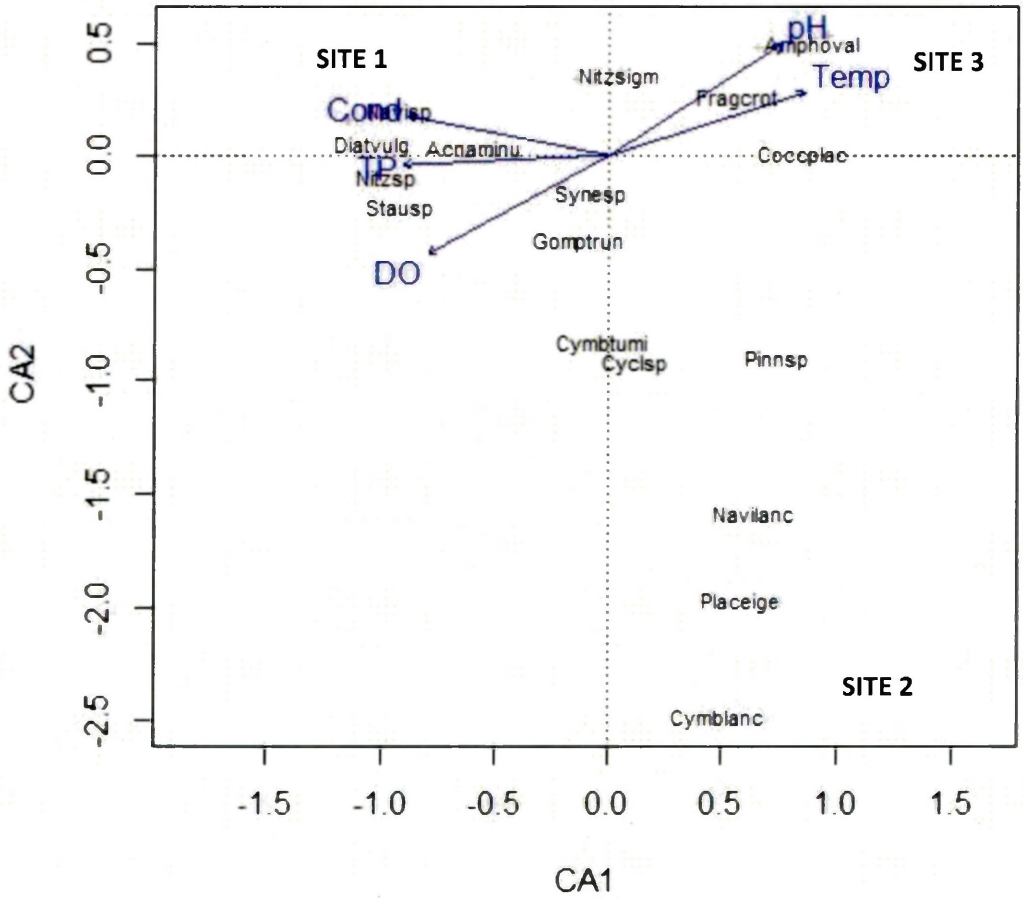
Eigenvalues along the first and second axes were 0.5663 and 0.1003 respectively. This explained 67% of the total variation for sampling period 2. The relative positions of the species and site scores showed a strong influence of the hydrological variables on the species composition of each site. The most important predicators of species distribution, as indicated in the significant correlation with the first CCA axis were DO ($r^{2=}$ -0.88, p<0.001), temperature (r^2= 0.95), conductivity (r^2= -0.98, p<0.001), and TP (r^2= -0.99, p<0.001). Although the largest variation among the three sites was attributed to the first CCA axis, the second CCA axis also exhibited strong correlations with the environmental variables such as conductivity (r^2= 0.20, p<0.001), and total phosphorus (r^2= -0.04110, p<0.001) (Table 10).

Figure 19 shows the position of site 1 (KB) in the upper left quadrant of the diagram corresponding to the conductivity variable. Site KB exhibited the highest values of conductivity during the study (Table 4). Figure 19 shows the location of the diatom species *Navicula* spp. and *Diatoma vulgaris* in the upper left quadrant related strongly to site KB as it was observed in high densities at site KB and as the dominant species at various points throughout sampling period 2 (Table 8a).

The TP variable corresponded most with site KB and in particular *Stauroneis* sp. and *Nitzschia* spp. Though the species *Acnanthidium minutissimum* was plotted in the same area it was present at all sites in high abundance, similar to the species *Cocconeis placentula* located on the first CCA axis on the right side of the bi-plot. These two species were co-dominant throughout the duration of study (30 days) (Table 8a). Figure 19 showed the position of site 2

(C10) in the lower left quadrant of the CCA diagram. The species *Cymbella lancelota* was associated with site C10 as it was observed in higher abundance compared to sites KB and LC. *Cymbella lancelota* was a dominant member of the taxonomic composition towards the late phase of the 30 day growth period (Table 8a).

Figure 19. CCA bi-plots of dominant diatom species composition with corresponding environmental factors for sampling period 2 by sites and species. Eigenvalues along the first and second axes were 0.5663 and 0.1003 respectively. This explained 67% of the total variation. The species names have been abbreviated to fit the bi plot.



Site 3 (LC) was plotted in the upper left quadrant of the bi-plot (Figure 19). The strongest corresponding species was *Amphora ovalis* which was observed at site LC in high densities and dominating the taxonomic composition by 50% at one point (during sampling period 2; Table 8a). *Placoneis eigens* was present exclusively at C10 during sampling period 2, while the diatom *Navicula lancelota* was observed at C10 and LC and was found in higher densities at C10. The species *Fragilaria crotensis* corresponded with LC also and dominated the taxonomic composition (50%) for the first half of sampling period 2 and also seemed to relate to the temperature variable (Table 8a).

The diatom species positioned in the centre of the bi-plot corresponded with all sites during the 30 day period (i.e., *Synedra* spp, *Gomphonema truncatum*, *Cymbella tumida*, *Cyclotella* spp.), except the species *Nitzschia sigmoidea* which had higher densities at KB and LC than at C10 (Figure 19).

Sampling period 3

Eigenvalues along the first and second axes were 0.2059 and 0.1005 respectively. This explained 31% of the total variation for sampling period 3. The relative positions of the species and site scores showed a strong influence of the hydrological variables on the species composition at each site. The most important predicators of species distribution, as indicated in their significant correlation with the first CCA axis were pH (r^2= 0.99, p<0.001), temperature (r^2= 0.83), and TP (r^2= -0.57, p<0.001). Although the largest variation among the three sites was attributed to the first CCA axis, the second CCA axis also exhibited strong correlations with the environmental variables such as DO (r^2= 0.99, p<0.001), and total phosphorus (r^2= 0.82, p<0.001) (Table 10).

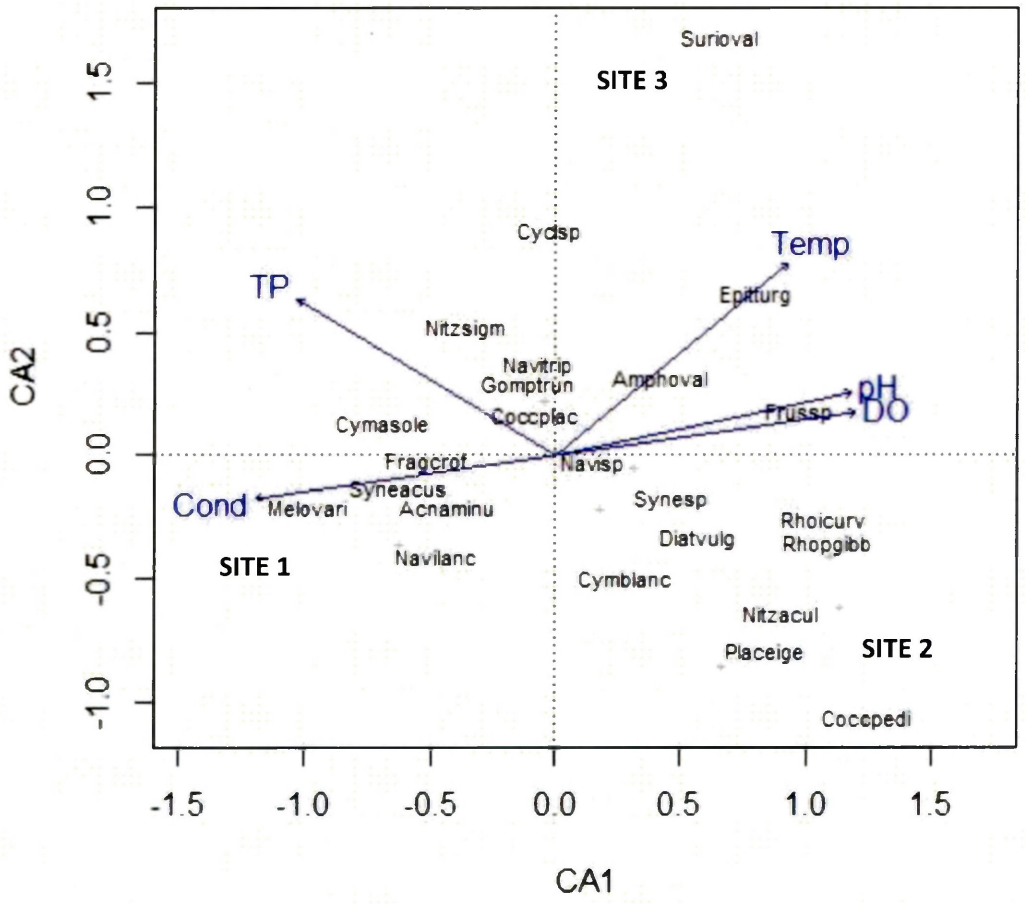
Figure 20 shows the position of site 1 (KB) in the lower left quadrant of the bi-plot corresponding to the conductivity variable. Site KB exhibited the highest values of conductivity during the entire study (Table 4). Figure 20 showed the location of diatom species *Aulacoseira varians (Melosira varians)* in the lower left quadrant corresponding strongly to site KB as it was exclusive to this site. Other species located in the lower left quadrant corresponded with KB with high abundance values at this site compared to the other two sites (i.e., *Synedra acus*, *Navicula lancelota*). The diatom species *Acnanthidium minutissimum* co-dominated with *Cocconeis placentula* throughout sampling period 3 at KB (Table 8a).

Figure 20 shows the location of site 2 (C10) in the lower right quadrant of the CCA diagram. The species *Cocconeis pediculus* was observed exclusively at site C10 and was located in the lower right quadrant. Species such as *Nitzschia acicularis*, *Placoneis eigens*, *Rhopolodia gibba*, *Rhoicosphenia curvata* and *Cymbella lancelota* were observed in all sites, but were in higher densities at site C10.

Site 3 (LC) was plotted in the upper left region of the diagram (Figure 20). The species observed exclusively at this site was *Suriella ovalis*, which was also situated in the upper right quadrant (Figure 20).

The species located in the centre of the CCA diagram were present at all sites in similar abundances (i.e, *Cocconeis placentula*, *Gomphenema truncatum*, *Navicula tripunctata*, *Cyclotella* spp.) (Figure 20).

Figure 20. CCA bi-plots of dominant diatom species composition with corresponding environmental factors for sampling period 3 by sites and species. Eigenvalues along the first and second axes were 0.2059 and 0.1005 respectively. This explained 31% of the total variation. The species names have been abbreviated to fit the bi- plot.



The species *Nitzschia sigmoidea* and *Cymatopleura solea* corresponded with TP. *Nitzschia sigmoidea* was found exclusively at site KB while Cymatopleura solea was observed at site KB and LC. Both sites KB and LC had the highest recorded TP values over the entire sampling period 3 (0.12mg/L & 0.13mg/L respectively).

The species *Epithemia turgida* and *Amphora ovalis* were associated with temperature (Figure 20). Both species were observed in high densities at sites C10 and LC. Although temperature did not vary significantly between sites (ANOVA) the average temperature at sites C10 and LC were almost 3°C higher than site KB (Table 4).

Sampling period 4

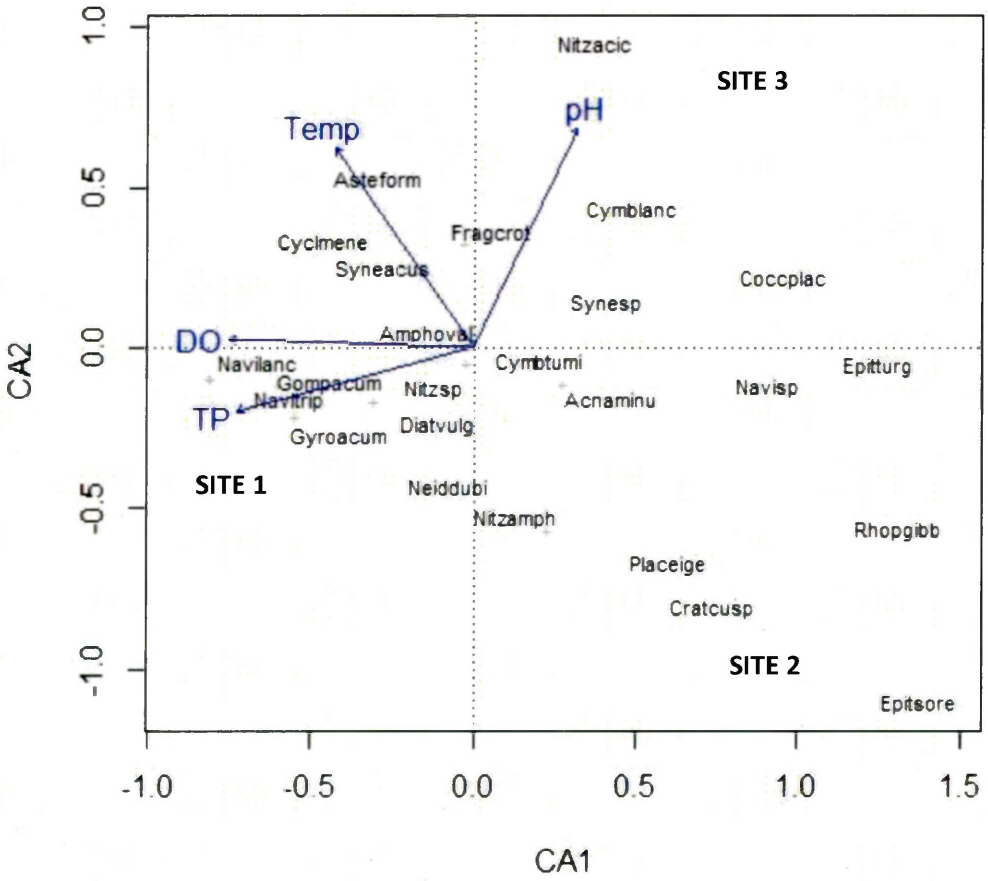
Eigenvalues along the first and second axes were 0.30883 and 0.07393 respectively. This explained 31% of the total variation for sampling period 3. The relative positions of the species and site scores showed a strong influence of the hydrological variables on the species composition of each site. The most important predicators of species distribution, as indicated in their significant correlation with the first CCA axis were site (r^2=0.86, p<0.001), pH (r^2=0.42, p<0.001), and TP (r^2=-0.57, p<0.001). Although the largest variation among the three sites was attributed to the first CCA axis, the second CCA axis also exhibited strong correlations with the environmental variables such as pH (r^2=0.91, p<0.001) (Table 10).

Figure 21 shows the position of site 1 (KB) in the lower left quadrant of the bi-plot corresponding to the TP and DO factors. Species that corresponded strongly with KB and the TP variable included *Navicula lancelota*, *Gomphonema acuminatium*, and *Navicula tripunctata*, which were present at all sites, but in higher abundances at KB. Additionally, the species *Gyrosigma acuminatium* was present exclusively at sites KB and C10, but was observed in higher densities at KB. The highest TP concentration over the four sampling periods was recorded at site KB (0.20mg/L) which is 10 times higher than the provincial guidelines concentration of 0.02mg/L. The species *Amphora ovalis* corresponded with the DO variable and

was present in all sites. One way ANOVA results showed that DO did not significantly vary between sites.

Figure 21 shows the location of site 2 (C10) in the lower right quadrant of the bi-plot. The species *Epithemia sorex* was observed exclusively at C10 and was positioned in the lower right quadrant. Species such as *Placoneis eigens*, *Rhopolodia gibba*, and *Craticula cuspidata* were observed across all sites, but they were observed in higher densities at site C10.

Figure 21. CCA bi-plots of dominant diatom species composition with corresponding environmental factors for sampling period 4 by sites and species. Eigenvalues along the first and second axes were 0.30883 and 0.07393 respectively. This explained 31% of the total variation. The species names have been abbreviated to fit the bi-plot.



Site 3 (LC) was plotted in the upper right region of the diagram (Figure 21). The corresponding species, *Nitzschia acicularis* was also positioned in the upper right quadrant (Figure 21). The species *Cymbella lancelota* was present at all sites, however it was observed in higher densities at site LC (Table 8a). The location of *Cocconeis placentula* in the upper right quadrant is indicative of its dominance at site LC throughout sampling period 4, although it was present at the other two sites in lower densities (Table 8a). Similarly, the other species plotted in this quadrant were present in all sites, but in higher densities at LC.

The species, *Fragilaria crotensis* was plotted on the first CCA axis in the upper region of the diagram and was observed at sites KB and LC in high densities and at times dominated the community (Table 8a). The species that were located near the second CCA axis on the right hand side were observed in all 3 sites in similar densities, except for the species *Epithemia turgida* located to the extreme right, which was only present at sites C10 and LC.

Non Diatoms

Sampling period 1

Eigenvalues along the first and second axes were 0.26448 and 0.03951 respectively. This explained 30% of the total variation for sampling period 1. The relative positions of the species and site scores showed a strong influence of the water quality variables on the species composition at each site. The most important predicators of species distribution, as indicated in their significant correlation with the first CCA axis were DO (r^2= 0.80, p<0.001), pH (r^2= 0.99, p<0.001), TP (r^2= 0.99, p<0.001), temperature (r^2= -0.57, p<0.001), and conductivity (r^2= -0.32, p<0.001. Although the largest variation among the three sites was attributed to the first CCA

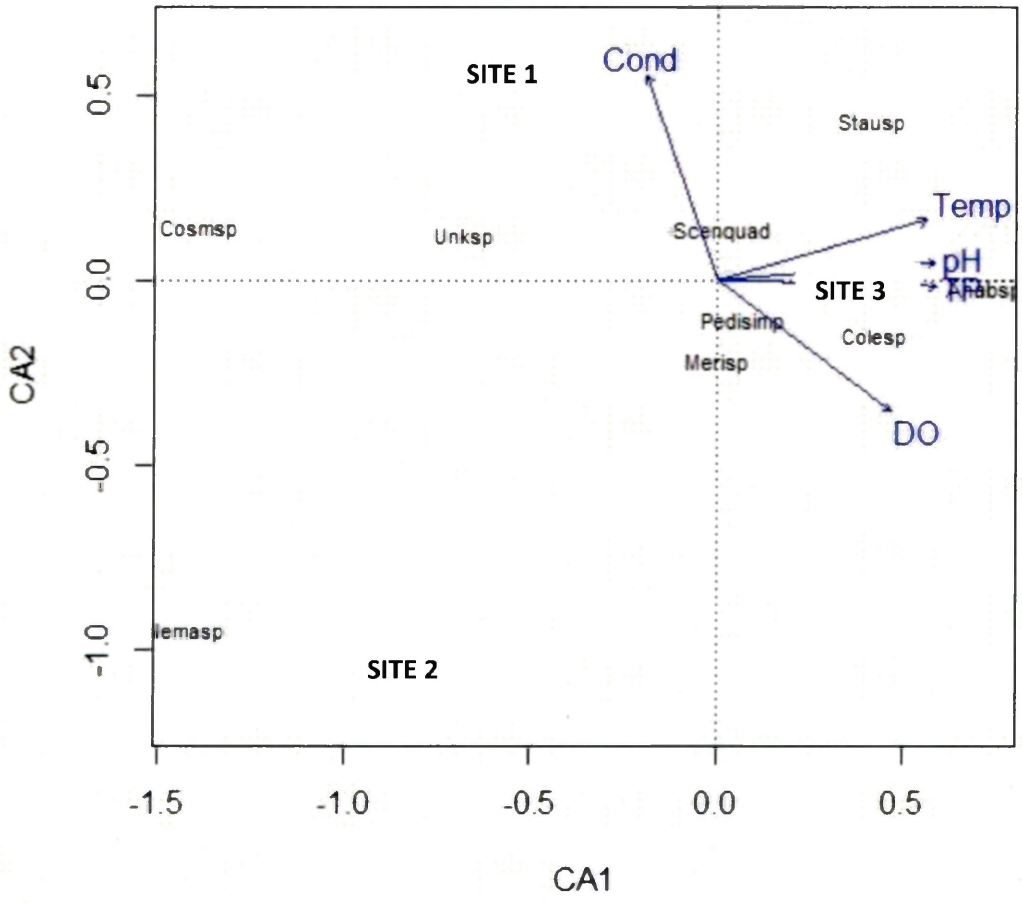
axis, the second CCA axis also exhibited correlations with the environmental variables such as conductivity (r^2= 0.95, p<0.001) (Table 10).

Figure 22 shows the position of site 1 (KB) in the upper left quadrant of the bi-plot corresponding with conductivity. Site KB exhibited the highest values of conductivity during the study (Table 4). The location of site C10 was in the lower right quadrant of the CCA diagram where *Nematode* spp. was plotted and subsequently corresponded with this site.

Site 3 (LC) was plotted in the middle right area of the bi-plot (Figure 21). *Anabaena* spp. was exclusive to LC and was positioned in the lower left quadrant of the bi-plot corresponding with site 3. The species dispersion at this site was under the influence of TP. The species *Coleastrum* spp. was present at sites KB and LC, but was in higher density at site LC than KB (Table 8b).

The species located on the first CCA axis near the lower quadrants was *Merismopedia glauca* which was present at C10 and LC only, while the species positioned on the same axis in the upper region was observed in all sites in similar proportions. The location of *Staurastrum* sp. which is located in the upper right quadrant of the bi-plot was associated with temperature. This species was observed in high densities during the first sampling day of the 30 day growth period at site LC (Table 8b).

Figure 22. CCA bi-plots of dominant non diatom species composition with corresponding environmental factors for sampling period 1 by sites and species. Eigenvalues along the first and second axes were 0.26448 and 0.03951 respectively. This explained 30% of the total variation. The species names have been abbreviated to fit the bi plot.



Sampling period 2

Eigenvalues along the first and second axes were 0.6369 and 0.4558 respectively and explained 100% of the variation. The relative positions of the species and site scores showed a strong influence of hydrological factors on species composition of each site. The most important predicator of species distribution as indicated in their significant correlation with the first CCA

axis were site (r^2= -0.15, p<0.001), and conductivity (r^2= 0.65, p<0.001. Although the largest amount of variation among the three sites was attributed to the first CCA axis, the second CCA axis also exhibited strong correlation with temperature (r^2= -0.99, p<0.001) (Table 10).

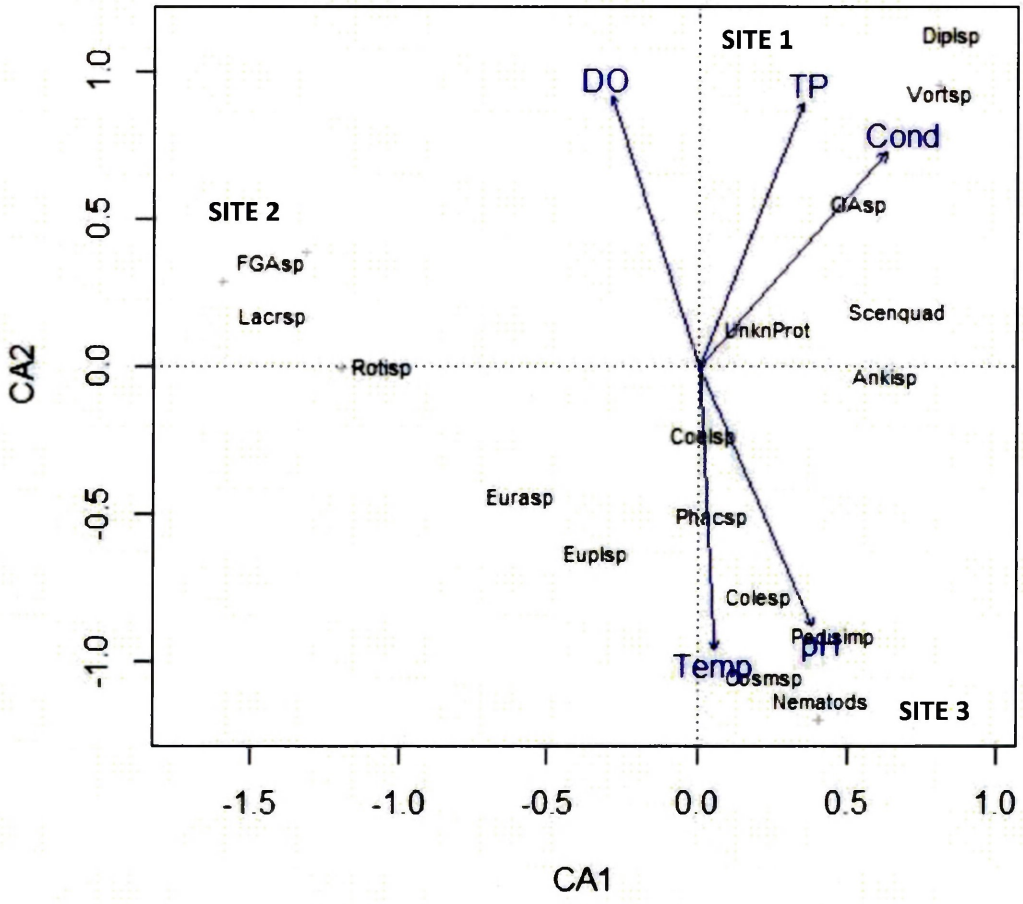
Figure 23 shows the position of KB in the upper right quadrant of the bi-plot corresponding to conductivity. Site 1 (KB) exhibited the highest values of conductivity during the study (Table 4). Species present in KB and associated environmental factors such as DO & conductivity were *Diplochloris lunata*, and unknown green algae spp. (GA) and the protozoan *Vorticella* spp. (Figure 23).

In the bi-plot site 2 (C10) was situated in the mid left quadrant (Figure 23). *Lacrymaria* spp. was observed at sites C10 and LC, but in higher densities at C10. Similarly, the filamentous green algae (FGA) spp. were observed at all sites, but higher densities were observed at C10 (Figure 23).

Site 3 (LC) was located in the lower left quadrant of the bi-plot (Figure 23). *Nematode* sp. corresponded with site LC and was exclusive to this site (Figure 23). The species dispersion at this site was under the influence of pH, which according to ANOVA results, did not significantly vary between sites. However, *Cosmarium* spp. corresponded to pH and was observed at C10 and LC in higher densities (Figure 23). The average pH value (7.68) for site LC was higher than at KB and C10 (7.58, 7.34, respectively).

Temperature corresponded with *Coleastrum* spp. which was present on day 15 of the sampling period, while the protozoa *Phacus* spp. was observed early in the sampling period only on day 5 (Figure 23). Species positioned on the lower left quadrant *Euplotes* spp. and *Eurastrum* spp. were observed at C10 and LC, but not at KB (Figure 23).

Figure 23. CCA bi-plots of dominant non diatom species composition with corresponding environmental factors for sampling period 2 by sites and species. Eigenvalues along the first and second axes were 0.6369 and 0.4558 respectively. The species names have been abbreviated to fit the bi plot.



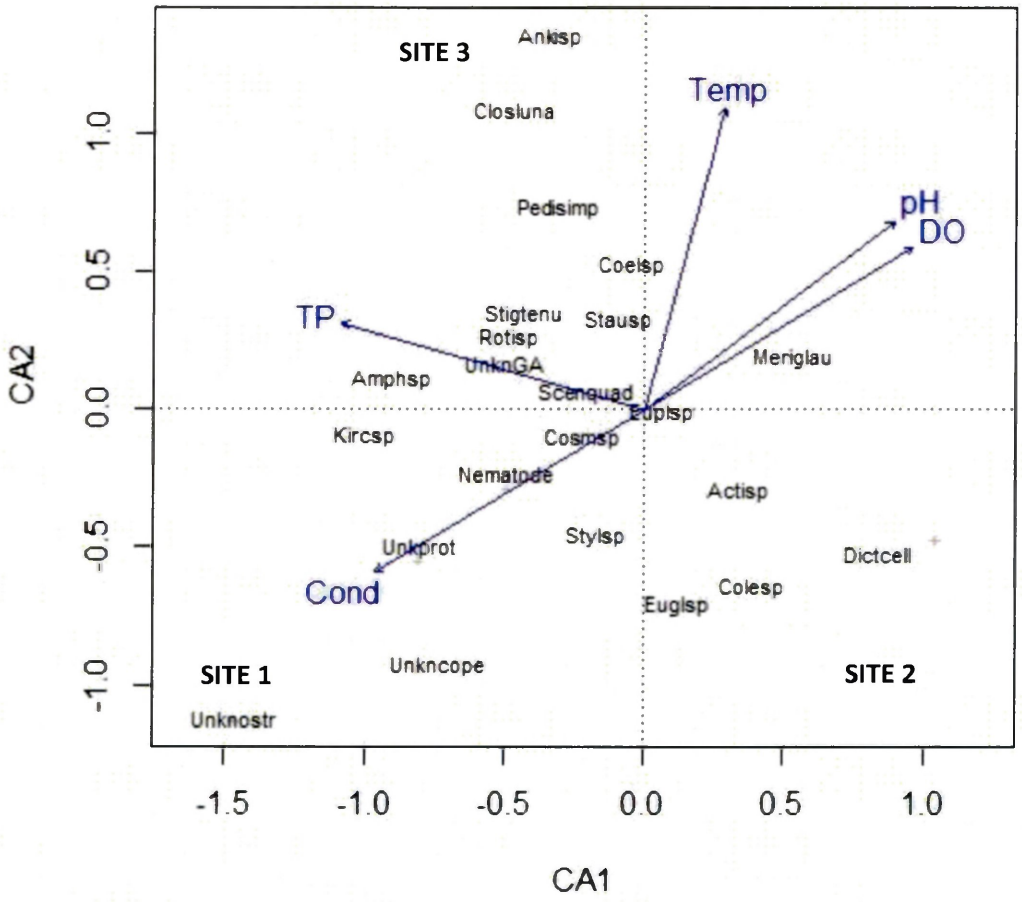
Sampling period 3

Eigenvalues along the first and second axes were 0.3263 and 0.2149 respectively. This explained 54% of the total variation for sampling period 3. The relative positions of the species and site scores showed a strong influence of the hydrological factors on the species composition of each site. The most important predicators of species distribution, as indicated in the significant correlation with the first CCA axis were DO (r^2= 0.85, p<0.001), pH (r^2= 0.80, p<0.001), TP (r^2= -0.96, p<0.001) and conductivity (r^2= 0.65, p<0.001. Although the largest variation among the three sites was attributed to the first CCA axis, the second CCA axis also exhibited strong correlations with temperature (r^2= 0.97, p<0.001) (Table 10).

Figure 24 shows site 1 (KB) located in the lower left quadrant of the bi-plot corresponding to conductivity. Site KB exhibited the highest values of conductivity during the study (Table 4). The unknown Ostracod sp. corresponded to site KB and the associated high conductivity levels which were exclusive to KB.

Site 2 (C10) was situated in the lower left quadrant of the bi-plot (Figure 24). *Dictyosphaerium pulchellum* corresponded to this site and was exclusive to C10. The other species positioned in this quadrant were *Euglena* spp., and *Actinosphaerium* spp. observed at KB and C10 only, and *Coleochaete* spp., that was observed at LC and C10 (Figure 24).

Figure 24. CCA bi-plots of dominant non diatom species composition with corresponding environmental factors for sampling period 3 by sites and species. Eigenvalues along the first and second axes were 0.3263 and 0.2149 respectively. This explained 54% of the total variation. The species names have been abbreviated to fit the bi-plot.



Site 3 (LC) was located in the upper left quadrant of the CCA bi-plot (Figure 24). Species exclusive to site LC included *Ankistrodesmus* sp., and *Closterium lunata*. The average TP concentrations at sites KB and LC were twice as high as the average value at site C10 (0.03mg/L). Species associated with TP included Chlorophyceae members, *Stigeclonium tenue*

and unknown green algae (GA) as well as the protozoan species *Amphileptus* spp., and a Rotifer species (Figure 24).

Species such as *Stylonchia* spp., and Nematode sp. situated in the lower left quadrant were observed at sites C10 and LC, while *Kirchneriella* spp., were observed at KB and LC, but not at C10 (Figure 24).

The Cyanobacteria species was located in the upper left quadrant of the bi-plot and was associated with the DO (Figure 24). It was observed at sites C10 and LC, but its density was 3 times higher than at LC (Table 8b).

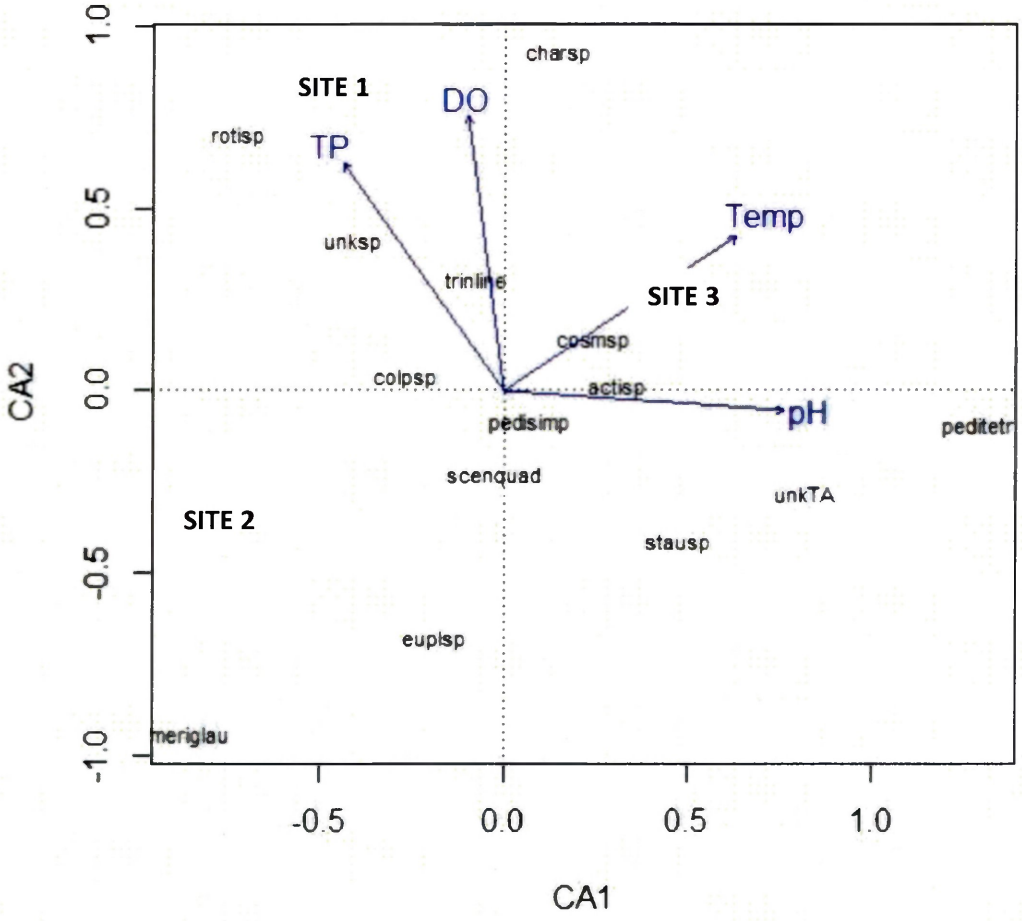
Sampling period 4

Eigenvalues along the first and second axes were 0.3110 and 0.1991 respectively. This corresponds to 51% of the total variation for sampling period 3. The positions of species and site scores showed a strong influence of the hydrological factors on the species composition of each site. The most important predicators of species distribution, as indicated in their significant correlation with the first CCA axis were pH (r^2=0.99, p<0.001) and temperature (r^2=0.83, p<0.001). Although the largest amount of variation among the three sites was attributed to the first CCA axis, the second CCA axis also exhibited strong correlations with TP (r^2=0.82, p<0.001) (Table 10).

Figure 25 shows the position of site 1 (KB) in the upper left quadrant of the bi-plot corresponding to TP and DO. Site KB had the highest TP concentrations during the study (0.20mg/L) in this sampling period 4. The dissolved oxygen levels did not vary significantly between sites (one way ANOVA). Species which corresponded strongly with KB included unknown protozoa species.

Site 2 (C10) was located in the lower left quadrant of the CCA bi-plot (Figure 25). The Cyanobacteria species *Merismopedia glauca* was exclusively seen at site C10. The species *Euglena* spp. was observed in all three sites, but in higher densities at site C10 (Figure 25).

Figure 25. CCA bi-plots of dominant non diatom species composition with corresponding environmental factors for sampling period 4 by sites and species. Eigenvalues along the first and second axes were 0.3110 and 0.1991 respectively. This corresponds to 51% of the total variation. The species names have been abbreviated to fit the bi-plot.



Site 3 (LC) was situated at the top lower right quadrant and was associated with pH (Figure 24). *Pediastrum tetra* was observed exclusively at this site. Species such as unknown testate amoeba (TA) and *Staurastrum* sp., were observed in higher densities at site LC (Figure 24). The species, *Charopsis* spp. located at the top of the CCA bi-plot was observed at sites KB and LC, but in higher density at LC. The species positioned in the centre of the bi-plot, such as *Scenedesmus quadricauda*, *Pediastrum simplex*, *Colpidinium* spp., and *Trinema lineare* were observed at all sites. The species *Cosmarium* spp., corresponded with temperature and was observed in high density at LC (Table 8b).

Discussion

Variation in diatom assemblages and individual species abundances provided reliable indicators of water quality changes at the three sampling sites in the northern Lake Simcoe. A multivariate ordination analysis in this study showed that dissolved oxygen, total phosphorus (TP), conductivity, and spatial variation in sampling locations were the most significant factors influencing the periphyton community structure.

The interaction of these abiotic determinants and their influence on the periphyton natural succession process produced highly variable species assemblages, though many periphyton community members such as *Cocconeis placentula*, *Cymbella tumida*, *Gomphonema truncatum*, *Navicula* spp., and *Synedra* spp. appeared in all sampling locations and sampling periods. However, variation in the periphyton assemblages was observed between sampling periods and sites. Some of these species were observed in previous studies from northern part of Lake Simcoe by Kanavillil et al (2012 & 2013).

Environmental variables influencing the periphyton community.

The results of the Canonical correspondence analysis (CCA) revealed seasonal variations in species density and environmental factors. The distribution of certain species highly corresponded to the measured hydrological parameters and in turn the study sites during various sampling periods (Table 10). Although, some of the variations could be attributed to seasonality, potential water quality indicator species were detected in site comparison analysis in a given sampling period.

Many of the hydrological parameters tested in this study significantly correlated with first CCA axis but less often with the second CCA axis. CCA analysis for the diatom group showed a significant negative correlation between the first CCA axis and TP during all sampling periods. The non diatom group did not show the same relationship which might have been due to their lower density values and number of species. TP values significantly correlated with the second CCA axis during sampling periods 1, 2 and 4 which coincided with fall and spring turnover processes and additional internal loading of P into the water column (Winter et al., 2011).

Dissolved oxygen correlated strongly with the first CCA axis with a significant negative relationship in sampling periods 2 and 4 for both diatom and non diatom groups. Conductivity negatively correlated with the first CCA axis for all sampling periods for the diatom group. However, the non diatom group in sampling period 2 showed a significant positive relationship with the first CCA axis. The main drivers of the periphyton community composition therefore were dissolved oxygen and total phosphorus in addition to various autogenic successional processes that were discussed in chapter 2.

Species diversity is considered as a good indicator of water quality (Tilman et al., 1997; Sondergaard & Jeppesen 2007; Leira et al., 2008). Varying degrees of anthropogenic activities at the three sampling locations had varying degrees of impacts on the periphyton species composition and development. Site KB with the highest anthropogenic disturbance among the three sampling locations showed lowest species diversity however, regression analysis showed a significant positive relationship with TP. The organic pollution tolerant diatoms such as *Cymatopluera elliptica* and *Synedra cyclopium* (Kelly et al., 2005) were observed at KB (Table 10). Literature suggests that water quality decreases as nutrient concentration increases (Tilman et al., 1997; Winter & Duthie 2000; Leira et al., 2008). The decrease in species richness as observed in KB corresponds to the increase in nutrient concentration (Jeppesen et al., 2000; Biggs et al., 1998).

Site KB is a well known point source of phosphorus to Lake Simcoe and is located on a public beach, and near a park and boat launch at Kempenfelt Bay, Barrie, ON. This area does not have a natural shoreline and there is weekly scheduled application of fertilizers by the city for flower beds, grass and non native trees during summer. Additionally, the sampling location is adjacent to the waste water treatment plant of the city of Barrie. The treated waste water goes through a de-nitrification process which includes addition of various salts before the effluent is released into the lake. The water here recorded very high conductivity values; double that of the other two locations as a result of this effluent discharge. LC is another site that received effluent discharge from a waste water treatment plant. Species such as *Navicula slevencisus* was observed at KB while the diatom *Entomoneis paludosa* was present at KB and LC. Both these species indicate high electrolyte water (Potapova et al., 2013) (Table 9). Furthermore, the highly motile diatom species *Entomoneis paludosa* has the tolerance for high concentration of salts and

sulfates, which may be the case in these locations, often encountered near the waste water treatment plants (Kelly et al., 2005) (Table 9).

Sites KB and LC experienced moderate to high degrees of anthropogenic disturbances and also shared similar taxa. The diatom species *Fragilaria crotensis* contributed almost 50% of the periphyton species density at LC during the first half of the sampling period while it was common in other two sites. Previous studies reported a shift from *Stephanodiscus* spp. abundance to *Fragilaria* spp. specifically *Fragilaria crotonensis* in Lake Simcoe during post *Dreissenid* invasion (Winter et al., 2011). According to Winter et al (2011) this shift coincided with an increase in silica to phosphorus ratio (Si:P) in Lake Simcoe, which *Fragilaria* spp. can endure well (Winter et al., 2011).

Nitzschia sigmoidea was recorded at higher densities at LC. The TP concentrations at LC were consistently above the Provincial guidelines of 0.02mg/L. Previous research suggests that water quality decreases as nutrient levels increase (Tilman et al., 1997; Winter & Duthie 2000; Leira et al., 2008). The diatom *Nitzschia sigmoidea* is tolerant of heavy organic pollutants and high suspended loads (Kelly et al., 2005; Wu et al., 2011) (Table 9). Site LC is located in the main water channel of Lagoon city that drains water to Lake Simcoe. The water channels in Lagoon City are known for excessive TP loads and the occurrence of toxic Cyanobacteria blooms during summer months (Simcoe Muskoka District Health Unit 2014). Though the main channel did not observe high concentrations of Cyanobacteria like in the inner channels, the presence of organic pollutant tolerant species such as *Nitzschia sigmoidea* can be considered as a sign of deteriorating water quality at this location (Schindler, 1977; Wetzel, 1983; Stevenson et al., 1996).

Overall, the high abundance of Chlorophyceae such as *Pediastrum* spp., *Cosmarium* spp. and *Scendesmus quadricauda* observed at LC were all indicative of high nutrient concentrations and possible impairment of water quality. *Scenedesmus quadricauda* and *Dictyosphaerium pulchellum* were observed to correspond well with TP in the CCA analysis. Schneider et al (2009) developed an index for water quality based on the relationship of TP with Chlorophyceae.

The least disturbed site among the three was C10. Even though a bloom was not detected at this site, Cyanobacteria species such as *Chooroccocus* spp. and *Merismopedia glauca* were recorded in high densities during summer. As Cyanobacteria members can fix nitrogen it will be interesting to investigate this site further with respect to nitrogen concentration and its impacts. The C10 also showed an abundance of *Epithemia turgida* which was associated with moderate nutrient conditions (Kelly et al., 2005). Species distribution such as *Tabellaria flocculosa* and *Navicula* spp. showed correspondence to dissolved oxygen (DO) in the sampling locations. The diatom species *Tabellaria flocculosa* was found at site C10 only while *Navicula* spp. was observed in all sites. The DO values were lower at C10 compared to the other two locations. The species composition at C10 also showed a presence of the diatoms *Gomphone*is spp. and *Eutonia* spp. indicating the possibility of ecological impairment at this site. *Eutonia* spp. are noted for their tolerance to acidity and low nutrient conditions (Kelly et al., 2005).

Additionally, at C10 the presence of the tube dwelling diatom such as *Gomphoneis* spp. is representative of low nutrient concentration water and is able to proliferate rapidly to form large mats on substrata such as rock (Stevenson et al., 1996). It is interesting to note that *Tabellaria flocculosa* which was only observed at C10 is indicative of a low nutrient environment since this species is sensitive to pollution (Kelly et al., 2005).

The foregoing part of discussion demonstrates significance of variation of sampling locations and their environmental factors on the periphyton species composition and dynamics. As stated before, since these littoral periphyton communities are exposed directly to the land originated point source pollution and since their tolerance vary with environmental factors, they have the potential to act as biological indicators of water quality. The study will give additional information regarding the use of periphyton as biological indicators if it can be expanded to several other locations in the lake and sampling was carried out at different depths. As suggested by Kim (2011) at a depth of 1.5m or less the general trend is a mono dominance of diatom species. However, if the depth is increased to 2.5m to 30m, a poly dominance of diatom species can be detected in a periphyton community.

Conclusion

The variation in taxonomic composition between the three sites supported the hypothesis that the periphyton community dynamics would vary with season and location of the study. Seasonal variation in periphyton composition could be due to the variation in their tolerance of major environmental parameters such as temperature, nutrient availability, duration of irradiance etc. which vary seasonally.

The periphyton species diversity decreased as a result of increased nutrient availability as observed at KB. Several other factors such as the influence of phytoplankton species composition, grazing pressure, inter specific competition etc., needs to be studied to further the understanding of periphyton community dynamics. A long term study from these locations would provide more data on various indicator species and their relationships with water quality. This would help to undertake more specific mitigation and management strategies.

Although there were substantial loadings on the first CCA axis for all 4 sampling periods and the corresponding regression results with the various hydrological parameters the unaccounted variation suggests an influence of factors other than autogenic succession processes such as grazing, competition etc. in addition to a potential influence of untested environmental drivers. Therefore it may be prudent to measure more water quality parameters to relate that to the taxonomic composition. As a result of the unknown water quality parameters and their impacts on species composition the hypothesis that periphyton species composition and their dynamics can be used as an index of water quality can only be partially accepted at this time.

Finally, as the majority of literature on Lake Simcoe seems to exclude the northern geographical area of the lake a periphyton diatom based index may assist in designing a more comprehensive monitoring strategy for this part of the lake.

Chapter 5: Conclusion

Periphyton communities are system specific reliable indicators of water quality changes in aquatic ecosystems. Autogenic processes such as colonization and succession combined with hydrological parameters and nutrient (TP) inputs are strong controlling factors in the development of periphyton. Other factors, such as seasonality, substratum surface etc. can also strongly influence the abundance and taxonomic composition of periphyton community.

This study attempted to fill the gap in knowledge on periphyton community dynamics vis-a-vis variation of hydrological parameters and location of study from the northern part of Lake Simcoe. The data generated from this study thus will help to design a periphyton based water quality index for this part of Lake Simcoe

In order to understand the periphyton community dynamics with season, location of study and duration of substratum exposure (30 days), field exposure studies were carried out in three different locations in the northern part of Lake Simcoe. The study was repeated four times to represent different seasons of a year. The hypotheses tested were:

1. The periphyton community dynamics (biofilm thickness, biomass, species density, species diversity, species richness) vary with season and location (degree of exposure to anthropogenic activities) of study.

2. The periphyton species diversity decreases as a result of increased nutrient availability.

3. The periphyton community dynamics are influenced by autogenic processes.

4. Diatom abundance and species composition will increase in spring and fall seasons as a result of lake turnover processes.

The result of the tested hypotheses through the experimental design of this study has led to the following conclusions.

The periphyton community dynamics did vary with season and location (and therefore with the degree of anthropogenic influence) of study. The variation of taxonomic composition between the three sites supported the hypothesis that the periphyton community dynamics would vary with season and location of the study. Certain species were present only during certain seasons. Seasonality also had an effect on the periphyton density, as well as the taxonomic composition. The summer season gave rise to a high abundance of non-diatom groups, such as Chlorophyceae and Cyanophyceae. The spring and fall sampling periods recorded high density of Bacillariophyceae as a result of lake turnover processes and the availability of nutrients. Therefore the hypothesis that periphyton community dynamics vary between seasons is fully accepted.

It was hypothesized that periphyton species diversity would decrease as a result of increased nutrient availability. However, as a result of a multi-proxy approach, including Bacillariophyceae, Chlorophyceae, Cyanophyceae, Chrysophyceae, protozoa and other groups the expected decrease in diversity as a result of higher nutrient concentration (TP in this case) was confounded by other factors that resulted in higher diversity in periphyton. This suggests that as the diversity of Bacillariophyceae decreases (as a result of higher nutrient inputs, such as TP), the non-diatom groups increase. However, it is important to note that overall species diversity is also influenced by seasons, as temperature and light availability play influencing roles in periphyton growth. This hypothesis seems to be true only with respect to Bacillariophyceae whose diversity is decreased with an increase in TP. Therefore, this hypothesis is not fully accepted.

The general trend of periphyton development was highly influenced by natural autogenic succession processes. The overall growth trend was an increase during the early phase; a climax

during the mid phase; a sloughing off period, and finally an increase in growth in the late growth phase as re-colonization occurred. Certain species were only present during certain phases of periphyton development. This study agreed with previous studies in that early diatom colonizers generally possess a rapid reproduction strategy and therefore colonize the substratum quickly. The diatom species arriving at the mid-successional phase generally possess a morphological advantage of longer mucilaginous stalk with which they grow vertically from the basal attachment disc (e.g. *Cymbella* spp.). This will help them to obtain higher levels of irradiance. The diatom species arriving at the late successional phase are generally highly motile with morphological features such as keels (i.e *Nitzschia* sp) and are able to maintain a high growth rate at a lower irradiance.

Though the spring season did not bring the expected diatom abundances in the periphyton community, the high chlorophyll *a* concentration from the water column, observed in spring may represent the spring turnover process resulting in an increase in diatom abundance. This would suggest a relationship between the phytoplankton and periphyton communities with the former acting as a propagule supply ground to the latter. The maximum value of periphyton species density was observed during fall. Therefore the hypothesis that diatom abundance and species composition increases in spring and fall seasons as a result of lake turnover processes was partly accepted.

Periphyton species composition and their dynamics can be used as an index of water quality. The dynamic relationship between water chemistry and the taxonomic composition of periphyton, showed good correspondence with the variations in water quality parameters studied.

However, the relationship also revealed a potential influence of untested environmental drivers such as nutrients other than TP. Therefore, it may be prudent to include more water

chemistry data to correlate with the periphyton taxonomic composition. There was evidence that various species reasonably indicated variations in certain hydrological parameter such as DO, temperature, conductivity, TP and location of study. The data thus provided significant indications of using periphyton community dynamics as indicators of water quality from the northern part of Lake Simcoe.

Future research

This study was able to shed some light on the various roles that species composition play in natural autogenic processes in addition to being good indicators of water quality. However, a comparison of the periphyton assemblages and phytoplankton communities would be a promising future research area to be explored. Additionally, a long term study on periphyton community dynamics would be useful to come up with a periphyton based water quality index that would help in better management strategies for freshwater systems.

This study revealed that factors such as competition and grazing may contribute to the processes of growth dynamics of periphyton. Therefore, it may be prudent to include more biological factors to study dynamics of periphyton community to better understand the processes.

Bibliography

1. American Public Health Association (APHA). 2005. *Standard methods for the examination of water and wastewater.* American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 19th edition, Washington, D.C.

2. Artigas, J., Fund, K., Kirchen, S., Morin, S., Obst, U., Romani, A., Sabater, S., Schwartz, T. 2012. Patterns of biofilm formation in two streams from different bioclimatic regions: analysis of microbial community structure and metabolism. *Hydrobiologia*, 695: 83-96.

3. Azim, M.E., *Verdegem, M.C.J., van Dam, A.A., Beveridge,* M.C.M. 2005. *Periphyton: Ecology, Exploitation and Management.* Cabi Publishing.

4. Bernhardt, E., Likens, G.E. 2004. Controls on periphyton biomass in heterotrophic stream. *Freshwater Biology*, 49(1): 14-27.

5. Biggs, B.J., Kilroy, C., Lowe, R.L. 1998. Periphyton development in three valley segments of a New Zealand grassland river: test of a habitat matrix conceptual model within a catchment. *Archives of Hydrobiology*, 143(2): 147-177.

6. Blinn, D., Herbst, D. 2003. Use of diatoms and soft algae as indicators of environmental determinants in the Lahontan Basin, USA. *Final Report to the California Regional Water Quality Control Board and the California State Water Resources Control Board* #01-119-160-0.

7. Burgmer, T., Reiss, J., Wickham, S., Hillebrand, H., 2010. Effects of snail grazers and light on the benthic microbial food web in periphyton communities. *Aquatic Microbial Ecology*, 61: 163-178.

8. Butterwick, C., Heaney, S. I. and Talling, J. F., 2005. Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. *Freshwater Biology*, 50: 291– 300.

9. Cecala, R.K., Mayer, C.M., Schulz, K.L., Mills, E.L. 2008. Increased Benthic Algal primary production in response to the invasive zebra mussel (Dreissena polymorpha) in a Productive ecosystem, Oneida, New York. *Journal of Integrative Plant Biology*, 50 (11) 1452-1466.

10. Cardinale, B.J. 2011. Biodiversity improves water quality through niche partitioning. *Nature*, 472: 86-91.

11. Chislock, M. F., Doster, E., Zitomer, R. A. & Wilson, A. E. 2013. Eutrophication: Causes, Consequences, and Controls in Aquatic Ecosystems. *Nature Education Knowledge,* 4(4):10.

12. Cook, L.J., Francoeur, S.F. 2013. Effects of simulated short-term road salt exposure on lotic periphyton function. *Journal of Freshwater Ecology*, 28 (2): 211- 223.

13. DeNicola, D.M., de Eyto, E., Wemaere, A., Irvine, K., 2003. Production and respiration of epilithic algal communities in Irish lakes of different trophic status. *Fundamental and Applied Limnology*, 157 (1): 67- 87.

14. Dixit, S., Smog, J.P., Kingston, J.C. 1992. Diatoms: Powerful indicators of environmental change. *Environmental Science and Technology,* 26 (1): 23-32.

15. Dodds, W. K., Gudder, A., 1992. The ecology of Cladophora. *Journal of Phycology*, 28:415–427.

16. Duong, T.T., Feurtet-Mazel, A., Coste, M., Dang, D.K., Boudou. 2007. Dynamics of diatom colonization process in some rivers influenced by urban pollution (Hanoi, Vietnam). *Ecological Indicators*,7: 839- 851.

17. Environmental Protection Agency, USA (EPA). 2014. *Water Quality Criteria.* http://water.epa.gov/scitech/swguidance/standards/criteria/ [cite 04 Apr 2014].

18. European Commission. European Framework Directive. 2014. *The EU Water Framework Directive - integrated river basin management for Europe.* http://ec.europa.eu/environment/water/water-framework/index_en.html[cite 06 Apr 2014].

19. Ferragut, C., de Campos Bicudo, D. 2010. Periphytic algal community adaptive strategies in N and P enriched experiments in a tropical oligotrophic reservoir. *Journal of Hydrobiologia* 646: 295-309.

20. Fitter, A., Hillebrand, H., 2009. Microbial food web structure affects bottom-up effects and elemental stoichiometry in periphyton assemblages. *Association for the Sciences of Limnology and Oceanography*, 54 (6): 2183- 2200.

21. Gaiser, E., Childers, D.L., Jones, R.D., Richards, J.H., Scinto, L.J., Trexler, J.C. 2006. Periphyton Responses to Eutrophication in the Florida Everglades: Cross-System Patterns of Structural and Compositional Change. *American Society of Limnology and Oceanography,* 51(1): 617- 630.

22. Geoffroy ,L., Couderchet, M., Vernet, G. 2000. Catalyse activity of *Scenedesmus obliquus* as a biomarker of environmental pollution by herbicides and copper. Mededelingen, Rijksuniversiteit Gent. Belgium. 65(2B) 843:852.

23. Gottlieb, A.D, Richards, J.H., Gaiser, E. 2006. Comparative study of periphyton community structure in long and short- hydro period Everglade marshes. *Hydrobiologia,* 569: 195-207

24. Gustina, G., 1996. Periphyton community dynamics in a mountain stream during winter. *Thesis*. The Faculty of Graduate College. The University of Vermont.

25. Hadwen, W.L., Bunn, S.E. 2005. Food Web Responses to Low-Level Nutrient and ^{15}N-Tracer Additions in the Littoral Zone of an Oligotrophic Dune Lake. *Limnology and Oceanology*, 50(4): 1096-1105.

26. Hampton, S.E., Fradkin, S.C., Leavitt, P., Rosenberger. 2011. Disproportionate importance of nearshore habitat for the food web of a deep Oligotrophic lake. *Marine and Freshwater Research* 62: 350-358.

27. Hill, B.H., Herlithy, A.T., Kaufmann, P.R., Stevenson, R.J., McCormick, H., F.H., Johnson, C.B. 2000. Use of Periphyton assemblage data as an index of biotic integrity. *Journal of the North American Benthological Society*, 19(1): 50 -67.

28. Hillebrand, H., Sommer, U. 2000. Diversity of benthic microalgae in response to colonization time and eutrophication. *Journal of Aquatic Botany,* 67: 221-236.

29. Hillebrand, H., Sommer, U. 2000. Effect of continuous nutrient enrichment on microalgae colonizing hard substrates. *Journal of Hydrobiologia,* 426: 185-192

30. Iwaniec, D.M., Childers, D.L., Rondeau, D., Madden, C.J., Saunders, C., 2006. Effects of hydrologic and water quality drivers on periphyton dynamics in the southern Everglades. *Journal of Hydrobiologia*, 569(1): 223-235.

31. Izagirre, O., Serra, A., Guasch, H., Elosegi. 2009. Effects of Sediment deposition on periphytic biomass, photosynthetic activity and algal community structure. *Science of the Total Environment*, 407- 5694-5700.

32. Jeppesen, E., Jensen, J.P., Sondergaard, M., Lauridsen, T., Landkildehus, F. 2000. Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. *Freshwater Biology*, 45: 201–218.

33. Kanavillil, N., Thorn, M., Kurissery, S., 2012. Characterization of natural biofilms in temperate inland waters. *Journal of Great Lakes Research*, 38: 429- 438.

34. Kanavillil, N., Kurissery, S., 2013. Temporal variation of periphyton communities: a 3 year study from northwest Lake Simcoe, Ontario, Canada. *Journal of the International Society of Limnology,* 3(4): 473- 486.

35. Kanavillil, N., Chantler, C., Balika, D., Kurissery, S. 2014. (submitted) Influence of timing of substratum initiation on the development of natural biofilm communities. *Journal of Great Lakes Research.*

36. Kelly, M.G., Penny, C.J., Whitton, B.A. 1995. Comparative performance of benthic diatom indices used to assess river quality. *Hydrobiologia*, 302: 179-188.

37. Kelly, M.G., Bennion, H., Cox, E.K., Goldsmith, B., Jamieson, J., Juggins, S., Mann, D.J., Telford, R.J. 2005. *Common freshwater diatoms of Britain and Ireland: an interactive key. Environment Agency, Bristol.*

38. Kelly, N.E., Young, J.D., Winter, J.G., Yan, N.D. 2013. Dynamics of the invasive spiny water flea, *Bythotrephes longimanus*, in Lake Simcoe, Ontario, Canada. *Inland Waters*, 3(1):75–92.

39. Kilroy, C., Biggs, B. J. F., Vyverman, W., Broady, P.A. 2006. Benthic diatom communities in subalpine pools in New Zealand: relationships to environmental variables. *Hydrobiologia,* 561: 95–110.

40. Kim., G.V.2011. Algae of Periphyton of Lake Teletskoye. *Contemporary Problems of Ecology*, 4(1): 21–27.

41. King, L., Barker, P., Jones, R.I. 2000 Epilithic algal communities and their relationship to environmental variables in lakes of the English Lake District. *Freshwater Biology,* 45:425-442.

42. Kitner, M., Poulickova, A. 2003. A. Littoral diatoms as indicators for the eutrophication of shallow lakes. *Journal of Hydrobiologia*, 506-509: 519-524.

43. Komulaynen, S. 2002. Use of phytoperiphyton to assess water quality in north-western Russia rivers. 2002. *Journal of applied Phycology*, 14:57-62.

44. Korte, V. L., Blinn, D. W. 1983, Diatom colonization on artificial substrata in pool and riffle zones studied by light and scanning electron microscopy. *Journal of Phycology,* 19: 332–341.

45. Kwandrens, J., Eloranta, P., Kawecka, B., Wojtan, K. 1998. Use of benthic diatom communities to evaluate water quality in rivers of southern Poland. *Journal of Applied Phycology*, 10: 193-201.

46. Lake Simcoe Regional Conservation Authority. *http://www.lsrca.on.ca/.*[cite 06 Apr 2014].

47. Lagoon City Community Association (LCCA). *http://www.lcca.ca/* [cited 06 Apr 2014].

48. Lamberti, G. A., Resh, V. 1985. Comparability of introduced tiles and natural substrates for sampling lotic bacteria, algae and macro invertebrates. *Freshwater biology*, 15(1): 21-30.

49. Lane, C.R, Brown, M.T. 2007 Diatoms as indicators of isolated herbaceous wetland condition in Florida, USA. *Ecological Indicators,* 7:521-540.

50. Larned, S. 2010. A prospectus for periphyton: recent and future ecological research. *Journal of North America Benthological Society*, 29(1): 182-206.

51. Lavoie, I., Campeau, S., Grenier, M., Dillon, P.J., 2006. A diatom based index for the biological assessment of Eastern Canadian rivers: An Application of correspondence analysis. *Canadian Journal of Fisheries and Aquatic Sciences,* 63(8): 1793:1811.

52. Lavoie, I., Campeau, S., Sigic- Drakulic, N., Winter, J.G., Fortin, C. 2014. Using diatoms to monitor stream biological integrity in Eastern Canada: an overview of 10 years of index development and ongoing challenges. *Science of The Total Environment*, 15(475): 187-200.

53. Leira, M., Chen, G., Dalton, C., Irvine, K., Taylor, D.(2009). Patterns in freshwater diatom taxonomic distinctness along an eutrophication gradient. *Freshwater Biology,* 54: 1-14.

54. Lewis, M.A., Weber, Moore, J.C. 2001. An Evaluation of the Use of Colonized Periphyton as an Indicator of Wastewater in Near Coastal Areas of the Gulf of Mexico. *Archives of Environmental Contamination and Toxicology,* 43:11-18.

55. Liboriussen, L., Jeppesen, E., 2006. Structure, biomass, production and depth distribution of periphyton on artificial substratum in shallow lakes with contrasting nutrient concentrations. *Freshwater Biology*, 51: 95–109.

56. MacArthur, R. H., and E. O. Wilson. 1967. *The Theory of Island Biogeography*. Princeton, NJ: Princeton University Press.

57. MacIntyre, S. & Melack, J. 1995 Vertical and Horizontal transport in lakes: linking littoral, benthic and pelagic habitats. *Journal of North American Benthological Society*, 14, 599–615.

58. Majdi, N., Traunspurger, W., Boyer, S., Mialet, B., Tackx, M., Fernandez, R., Gehner, S., Ten-Hage, L., Buffan-Dubau, E., 2011. Response of biofilm dwelling nematodes to habitat changes in the Garonne River, France: influence of hydrodynamics and micro algal availability. *Hydrobiologia*, 673(1): 229-244.

59. Masseret, E., Amblard, C., Bourdier, G., 1998. Changes in the structure and metabolic activities of periphytic communities in a stream receiving treated sewage from a waste stabilization pond. *Water Research*, 32(8): 2299–2314.

60. Mathieu, M., J. Leflaive, J., Ten-Hage, L., De wit, R., Buffan-Dubau, E. 2007. Free-living nematodes affect oxygen turnover of artificial diatom biofilms. *Aquatic Microbial Ecology* 49: 281–291.

61. McCormick, P.V., Cairns, J, Jr. 1994. Algae as indicators of environmental change. *Journal of Applied Phycology*, 6: 509-526.

62. Microsoft. 2007. Microsoft Excel [computer software]. Redmond, Washington: Microsoft.

63. Mieczan, T., Malgorzata, A., Nawrot, D. 2013. Effect of water chemistry on the planktonic communities and relationships among food web components across a freshwater ecotone. *Archive of Biological Sciences*, 65(4): 1491-1504.

64. Mihaljevic, M., Spoljaric, D., Stevic, F., Pfeiffer, T.Z., 2013. Assessment of flood-induced changes of phytoplankton along a river–floodplain system using the morpho-functional approach. *Environmental Monitoring and Assessment*, 185(10): 8601-8619.

65. Moss, B. 1981. The composition and ecology of periphyton communities in freshwaters. II. Inter-relationships between water chemistry, phytoplankton populations and periphyton populations in a shallow lake and associated experimental reservoirs ('Lund tubes'). *British Phycological Journal*, 16(1): 59-76.

66. Mulholland, P.J. 1996. Role in nutrient cycling in streams. *In* Stevenson, R.J., Bothwell, M.L. & Lowe, R.L. (eds.) *Algal ecology: freshwater benthic ecosystems.* Academic Press, San Diego. 609-640.

67. Mulholland, P.J., and J.R. Webster. 2010. Nutrient dynamics in streams and the role of J-NABS. *Journal of the North American Benthological Society,* 29:100-117.

68. National Institute of Water and Atmospheric Research (NIWA). 2014. National River Water Quality Network (NRWQN). *https://www.niwa.co.nz/freshwater/water-quality-monitoring-and-advice/national-river-water-quality-network-nrwqn* [cite 04 Apr 06].

69. National Oceanic and Atmospheric Administration (NOAA). Great Lakes Environmental Research Laboratory. 2014. *http://www.glerl.noaa.gov/pr/ourlakes/* [cite 04 Apr 2014].

70. Nietch, C., Quinlan, E.L., Lazorchak, J.M., Impellitteri, C.A., Raikow, Walters, D. 2013. Effects of a chronic lower range of Triclosan exposure on a stream mesocosm community. *Environmental Toxicology and Chemistry*, 32(12): 2874: 2887.

71. North, R., Barton, D., Crowe, A.S., Dillon, P.J., Dolson, R., Evans, D.O., Ginn, B., Hawyrshyn, H., Jarjanzi, H., King, J.W., La Rose, J.K.L., Leon, L., Lewis, C.F.M., Liddle, G., Lin, Z.H., Longstaffe, F.J., Macdonald, R.A., Molot, L., Ozersky, T., Palmer, M., Quinlan, R., Rennie, M.D., Robillard, M., Ruhland, K.M., Schwalb, A., Smol, J.P., Stainsby, E., Trumpickas, J.J., Winter, J.G., Young, J.D. 2013. The state of Lake Simcoe (Ontario, Canada): the effects of multiple stressors on phosphorus and oxygen dynamics. *Inland Waters Research*, 3: 51-74.

72. Nuernburg, G., Molot, L. O'Connor, E. 2013. Evidence for internal phosphorus loading, hypoxia and effects on phytoplankton in partially polymictic Lake Simcoe. *Journal of Great Lakes Research*, 39(2): 259-270.

73. Oemke, M.P., Burton, T.M. 1986. Diatom colonization dynamics in a lotic system. *Hydrobiologia*, 139: 153-166.

74. Ozersky, T., Barton, D.R., Hecky, R.E., Guilford, S.J. *Dreissenid* mussels enhance nutrient efflux, periphyton quantity and production in the shallow littoral zone of a large lake. *Biological Invasions*, 15: 2799: 2810.

75. Palmer, M.E., Winter, J.G., Young, J.D., Dillon, P.J., Guildford, S.J. 2011. Introduction and summary of research on Lake Simcoe: Research, monitoring, and restoration of a large lake and its watershed. *Journal of Great Lakes Research*, 37:1–6.

76. Palmer, M.E., Hiriart-Baer, V.P., North, R.L., Rennie, M.D. Summary of Lake Simcoe's past, present, and future. *Inland Waters Research*, 3: 51-74.

77. Palmer, M. 1993. Putting Things in Even Better Order: The Advantages of Canonical Correspondence Analysis. *Ecological Society of America*, 74(8): 2215-2230.

78. Pan, Y., Stevenson, R.J., Hill, B.H., Herlihy, A.T., Collins, G.B. 1996. Using diatoms as indicators of ecological conditions on lotic systems: a regional assessment. *Journal of North American Benthological Society*, 15(4): 481-495.

79. Passy, S., Pan, Y., Lowe, R. 1999. Ecology of the Major Periphytic Diatom Communities from the Mesta River, Bulgaria. *Hydrobiologia*, 84(2): 129-174.

80. Passy, S. I. & Blanchet, F. G. 2007. Algal communities in human-impacted stream ecosystems suffer beta-diversity decline. *Diversity and Distributions*, 13: 670-679.

81. Peterson, C.G. Effects of Discharge Reduction on Diatom Colonization below a Large Hydroelectric Dam. 1986. *Journal of the North American Benthological Society,* 5 (4): 278-289.

82. Peterson, C.G. Mechanisms of lotic micro-algal colonization following space clearing disturbances acting at different spatial scales. 1996. *Acta Oecologica Scandinavica*, 77: 417-435.

83. Porter-Goff, E., 2010. Stream Periphyton community dynamics under varying natural and anthropogenic factors. *Thesis*. Rensselaer Polytechnic Institute. Troy, NY.

84. Potapova, M. G. and D. F. Charles. 2002. Benthic diatoms in USA rivers: distributions along spatial and environmental gradients. *Journal of Biogeography*, 29(2): 167-187.

85. Potapova, M., Charles, D., 2003. Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. *Freshwater Biology*, 48: 1311- 1328.

86. Poulickova, A., Duchoslav, M., Dokulil. 2004. Littoral diatom assemblages as bio-indicators of lake trophic status: A case study from peri-alpine lakes in Austria. *European Journal of Phycology,* 39: 143- 152.

87. Potapova, M., Bahls, L. (2011). *Navicula slesvicensis* . In Diatoms of the United States. *westerndiatoms.colorado.edu/taxa/species/navicula_slesvicensis* [01 Mar 2014]

88. Prescott, G.W. 1978. *How to Know Freshwater Algae*. W.C. Brown, Dubuque, Iowa.

89. Prosser, R.S., Brain, R.A., Hosmer, A.J., Soloman, K.R., Hansen, M.L. 2013. Assessing sensitivity and recovery of field collected periphyton acutely exposed to atrazine using PSII inhibition under laboratory conditions. *Journal of Ecotoxicology*, 22: 1367- 1383.

90. Public Health England. 2014. *http://www.phe- culturecollections. org.uk/technical/ ccp/ cellcounting.aspx* [cited 06 Apr 2014].

91. Reavie, E., Jicha, T.M., Angradi, T.R., Bolgrien, D.W., Hill, B.H. 2010. Algal assemblages for large river monitoring: Comparison among biovolume, absolute and relative abundance metrics. *Ecological Indicators*, 10: 167-177.

92. Ricklefs, R.E., 2001. *The Economy of Nature*. W.H. Freeman and Company, New York.

93. Rosemond, A.D., Mulholland, P.J., Elwood, J.W. 1993. Top- Down and Bottom-Up Control of Stream Periphyton: Effects of Nutrients and Herbivores. *Ecological Society of America*, 74 (4:) 1264-1280.

94. Rotter, S., Heilmeier, H., Altenburger, R., Schmitt-Jansen, M. 2013. Multiple stressors in periphyton – comparison of observed and predicted tolerance responses to high ionic loads and herbicide exposure. *Journal of Applied Ecology*, 50: 1459- 1468.

95. Round, F.E., Crawford, R.M., Mann, D.G. (1990). *The diatoms. Biology & morphology of the genera*. Cambridge University Press: New York.

96. Schneider, S., Lindstrom, E.A., 2009. Bio-indication in Norwegian rivers using non-diatomaceous benthic algae: the acidification index periphyton (AIP). *Ecological Indicators*, 9 (6):1206–1211.

97. Schneider, S., Lindstrom, E., 2011. The periphyton index of trophic status PIT: a new eutrophication metric based on non-diatomaceous benthic algae in Nordic rivers. *Hydrobiologia*, 665(1): 143- 155.

98. Schindler, David W. 1977. Evolution of phosphorus limitation in lakes: Natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. *Science.*195: 260-262.

99. Sekar, R., Nandakumar,K., Venugopalan, V.P., Nair, K.V.K., Rao, V.N.R. 1998. Spatial Variation in micro-algal colonization on hard surfaces in a lentic freshwater environment. *Biofouling*, 13(3): 177-195.

100. Sekar, R., Venugopalan, V.P., Satpathy, K.K., Nair, K.V.K., Rao, V.N.R. 2004. Laboratory studies on adhesion of microalgae to hard substrates. *Hydrobiologia*, 512: 109-116.
Simcoe District Health Unit. 2014. *http://www.simcoemuskokahealth.org/Home.aspx.*[cited 06 Apr 2014].

101. Smucker, N.J. & Vis, M.L. 2013. Can pollution severity affect diatom succession in streams, and could it matter for stream assessment? *Journal of Freshwater Ecology*, 28(3): 329-338

102. Sondergaard, M., Jeppesen, E. 2007. Anthropogenic impacts on lake and stream ecosystems, and approaches to restoration. *Journal of Applied Ecology*, 44: 1089–1094.

103. Spaulding, S.A., Lubinski, D.J. and Potapova, M. 2010. Diatoms of the United States. *http://westerndiatoms.colorado.edu*,[cite 05 Oct 2012].

104. Statistics Canada. 2014.Barrie, Ontario Census Profile. CANSIM, table 051-0046. 2012. *http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/demo05a-eng.htm*,[cited 06 Apr 2014].

105. Stevenson, J., 1990. Benthic Algal community dynamics in a stream during and after a spate. *Journal of the North American Benthological Society*, 9(3): 277-288.

106. Stevenson, J., Bothwell, M., Lowe, R. 1996. *Algal Ecology: Freshwater Benthic Ecosystems*. London: Academic Press.

107. Stević, F., Čerba, D., Čakalić, I., Turković, Pfeiffer, T.Ž., Vidaković, J., Mihaljević, M. 2013. Interrelations between *Dreissena polymorpha* colonization and autotrophic periphyton development – a field study in a temperate floodplain lake. *Fundamental and Applied Limnology*, 183(2): 107- 119.

108. Suren, A.M., Biggs, B.J.F., Duncan, M.J., Bergey, L., Lambert, P. 2003. Benthic community dynamics during summer low-flows in two rivers of contrasting enrichment New Zealand. *Journal of Marine and Freshwater Research,* 37(1) :71-83.

109. Szabo, K., Makk, J., Kiss, K.T., Eiler, A., Acs, E., Toth, B., Kiss, A.K., Bertilsson. 2008. Sequential colonization by river periphyton analysed by microscopy and molecular fingerprinting. *Journal of Freshwater Biology*, 53: 1359-1371.

109. Taniwaki, R.H., Borghi, T.C., Magrin, A.G.E., Calijuri, Maria do Carmo, B.F, Moschini-Carlos, V. 2013. Structure and dynamics of the community of periphytic algae in a subtropical reservoir (state of São Paulo, Brazil). *Acta Botanica Brasilica*, 27(3):51-559.

110. ter Braak, C.J.E., Verdonschot, P.E.M. 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Journal of Aquatic Sciences*, 57(3): 255-289.

111. Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., Siemann, E., 1997a. The influence of functional diversity and composition on ecosystem processes. *Science,* 277: 1300–1302.

112. Township of Ramara. 2012. Environmental Services, Brechin/Lagoon City Water. *http://www.ramara.ca/index.php?option=com_content&task=view&id=54&Itemid=84*. [cited: 06 April 2014].

113. Vadeboncoeur, Y., Steinman, D., 2002. Periphyton function in lake ecosystems. *The Scientific World Journal*, 2: 1449- 1468.

114. van Dam, H., Mertens, A., Sinkeldam, J., 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Netherlands Journal of Aquatic Ecology*, 28: 117–133.

115. Vis, C., Hudon, C., Cattaneo, A., Pinel-Alloul, B. 1998. Periphyton as an indicator of water quality in the St. Lawrence River (Quebec, Canada). *Journal of Environmental Pollution*, 101 : 13-24.

116. Von Schiller, D., Marti, E., Rieria, J.L., Sabater, F. 2007. Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. *Journal of Freshwater Biology*, 52:891-906.

117. Wehr, J., Sheath, R.G., Kociolek, P. 2003. *Freshwater Algae of North America: Ecology and Classification.* Elsevier Science USA.

118. Wetzel, R.G. 1983. Limnology 2nd Edition. Philadelphia, PA: Saunders Publishing.

119. Wiklund, J.A, Bozinovski, N., Hall, R.I., Wolfe, B.B. 2010. Epiphytic diatoms as flood indicators. *Journal of Paleolimnology*, 44: 25-42.

120. Winter, J.G., Duthie, H.C. 2000. Epilithic Diatoms as Indicators of Stream Total N and Total P Concentration. *Journal of the North American Benthological Society*. 19(1): 32-49.

121. Winter, J.G., Eimers, M.C., Dillon, P.J., Scott, L.D., Scheider, W.A., Willox, C.C. 2007. Phosphorus Inputs to Lake Simcoe from 1990 to 2003: Declines in Tributary Loads and Observations on Lake Water Quality. *Journal of Great Lakes Research*, 33(2): 381-396.

122. Wu., Y., Liu ,J., Yang, L., Chen, H., Zhang, S., Zhao, H., Zhang, N. 2011. Allelopathic control of cyanobacterial blooms by periphyton biofilms. *Environmental Microbiology*, 13(3); 604-615.

123. Young, J.D., Landre, A.L., Winter, J.G., Jarjanzi, H., Kingston, J. 2010. Lake Simcoe water quality update. Toronto (ON): *Ontario Ministry of the Environment Report.*

124. Young, J.D., Winter, J.G., Molot L. 2011. A re-evaluation of the empirical relationships connecting dissolved oxygen and phosphorus loading after *Dreissenid* mussel invasion in Lake Simcoe. *Journal of Great Lakes Research*, 37: 7:14.

125. Zebek, E. 2013. Differentiation of periphyton and phytoplankton assemblages in anthropogenically transformed conditioned of the littoral zone in a shallow urban lake (Lake Jeziorak, Maly, Poland). *Journal of Applied Ecology and Environmental Research*, 11(3): 232-342.