Ecological Factors Controlling Microcystin Concentrations in the Bay of Quinte, Maumee Bay, and Three Grand River Reservoirs

by

Sarah Jane Yakobowski

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Master of Science
in
Biology

Waterloo, Ontario, Canada, 2008

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Certain types of cyanobacteria have the potential to produce toxins including microcystin, a hepatotoxin. Toxic cyanobacterial blooms are becoming increasingly common worldwide. They are a concern in the Great Lakes and surrounding waters. In this study, Lake Ontario's Bay of Quinte, Lake Erie's Maumee Bay, and three reservoirs along the Grand River were studied. Environmental variables, cyanobacterial biomass inferred from the Fluoroprobe, and microcystin concentrations were measured. In 2005 the three reservoirs, Belwood Lake, Conestogo Lake, and Guelph Lake were sampled every two weeks from July to September. Belwood Lake was also sampled in October when a cyanobacterial bloom occurred. In 2006 the Bay of Quinte was sampled twice, in July and September, and Maumee Bay was sampled twice, in June and August.

Physical variables measured included water transparency and temperature. All species of nitrogen (N) and phosphorus (P) were measured, along with extracted chlorophyll *a* and particulate carbon (C), N, and P. The distribution of chlorophyll and major algal groups throughout the water column was profiled *in situ* using a spectral fluorometer (Fluoroprobe).Variable fluorescence of phytoplankton was assessed using Pulse Amplitude Modulated (PAM) fluorometry to measure photosynthetic parameters. Phytoplankton counts were performed on selected samples from the Bay of Quinte and Maumee Bay.

Total and dissolved microcystin were measured using the protein phosphatase inhibition assay (PPIA). PPIA was chosen over alternative detection methods because it is a functional assay that measures the level of microcystin in a sample via the amount of protein phosphatase inhibition that it exerts. This yields ecologically relevant data as protein phosphatase inhibition is the main mode of microcystin toxicity. The PPIA formulation used in our lab was based on variations in the literature that use unconcentrated water samples directly in the assay. The assay was optimized to employ both a higher and lower standard curve through the use of two enzyme concentrations. The

lower enzyme concentration allowed the method detection limit to be decreased to 0.05 µg/L to accommodate our low-microcystin samples.

In the Bay of Quinte, microcystin levels were higher in July 2006 (total mean=2.25 µg/L) than in September 2006 (total mean=0.58 µg/L). In July a cyanobacterial bloom consisting of 97% *Microcystis* spp. was present. In September 83% of the cyanobacterial biomass was composed of *Anabaena spiroides* and only 8% was *Microcystis* spp. In the Bay of Quinte elevated microcystin concentrations were associated with higher soluble reactive P levels, lower seston C:P molar ratios, and lower total N. In Maumee Bay microcystin levels were higher in August 2006 (total mean= 4.45 µg/L) than they were in June 2006 (<0.05 µg/L). In August a cyanobacterial bloom consisting of 22% *Microcystis* spp. and 48% *Aphanizomenon flos-aquae* was observed. Higher microcystin concentrations in Maumee Bay were associated with decreased total N: total P molar ratios, increased total P, and decreased water transparency as measured by Secchi depth.

Belwood Lake had the highest microcystin levels of the three reservoirs but only once exceeded the recommended World Health Organization concentration of 1.0 µg/L. Belwood Lake's largest cyanobacterial bloom in October 2005 was accompanied by relatively low microcystin levels (<0.2 µg/L). Conestogo and Guelph lakes always had microcystin levels below 0.2 µg/L and 0.6 µg/L, respectively. In the Grand River reservoirs, increased microcystin concentrations were associated with higher chlorophyll *a*, higher light attenuation coefficients, lower total N, lower total N: total P molar ratios, higher C:P molar ratios, lower nitrate, higher cyanobacterial biomass, and higher total P. When data from the Bay of Quinte, Maumee Bay, and Grand River reservoirs were pooled, total microcystin had the most significant positive correlation with total P. Total microcystin and water temperature also had a significant positive correlation.

Acknowledgements

I would firstly like to thank my advisor, Dr. Stephanie Guildford for all of her guidance, expertise, support, and patience throughout my degree. It has been greatly appreciated! I thank my committee members, Dr. Ralph Smith and Dr. Kirsten Muller, for their input, and Dr. Josh Neufeld and Dr. William Taylor for kindly sitting in on my thesis defence. I am very grateful to my UWAEG labmates, especially Amanda Poste and Aline Chhun with whom I collaborated on the microcystin project. I thank Amanda Poste for organizing the Bay of Quinte and Maumee Bay field work and for performing the PAM measurements. I also thank Annie Chiavaroli whose work for her 4th year project on the GRCA reservoirs formed the basis of my fourth chapter. I am thankful to all who participated in the Bay of Quinte 2005 sampling and provided me with water to analyze, especially Kim Rattan, Greg Silsbe, Dan Hamilton, Tim Kuntz, and any others. I am grateful to those who assisted in the lab and field, especially Zing-Ying Ho, Janet Ma, Ann Balasubramaniam, Cindy Wang, and Justin Lorentz. I thank David Depew, who performed the CN analysis, Dr. Yuri Kozlov, who performed much of the chemical analyses in my fourth chapter, and Ryan Sorichetti who performed silica analysis. I also thank Dr. Luis Leon for providing data on Grand River catchment area land use.

I would like to thank my family and friends who supported me personally throughout my degree, especially my partner, Tomas Grana, and my parents, Edward and Barbara Yakobowski. Thank you for patiently listening.

Dedication

In memory of my grandparents,

Douglas and Ethel Wicks and Edward and Helen Yakobowski.

Table of Contents

List of Figures

List of Tables

Chapter 1
Introduction

1.0 Introduction to Thesis

In recent years, cyanobacterial blooms have received increasing attention worldwide due to their more frequent and severe occurrences (Falconer 2005). The ability of many bloom-forming species to produce toxins is particularly alarming to water quality managers. Genera capable of producing these cyanotoxins (Table 1.1) are important research subjects as much remains to be understood about the conditions that trigger potentially toxic blooms in a variety of water bodies. One such cyanotoxin is microcystin. In this study, two Great Lakes bays and three small reservoirs were surveyed to better understand the dynamics of microcystin within them and the environmental variables influencing its concentrations. In this introductory chapter (1), I provide a general review of the ecology of cyanobacteria, background information about microcystin, a brief review of the factors linked with microcystin occurrence, a description of the three study sites and an outline of my hypotheses to be tested. Chapter 2 describes the research I conducted to adapt a sensitive assay for measuring total and dissolved microcystin in unconcentrated natural water samples across a range of concentrations. Subsequent chapters describe the results of surveys conducted in the Bay of Quinte and Maumee Bay during early and late summer 2006 where physical and chemical variables were measured and related to microcystin concentrations (Chapter 3), and the results from similar bi-weekly surveys of the three Grand River reservoirs sampled from July through September 2005 (Chapter 4). In Chapter 5 I briefly compare the data in all three study sites and provide my overall conclusions.

Table 1.1. Summary chart of the known structural classes of cyanotoxins (Chorus and Bartram 1999; Falconer 2005).

Toxin Name	Mammalian Target Organ	Producers (Genera)
Cyclic Peptides		
Microcystins	Liver	*Microcystis* *Anabaena* *Planktothrix/ Oscillatoria* *Nostoc* *Hapalosiphon* (soil dweller) *Anabaenopsis* *Snowella* *Woronichinia*
Nodularin	Liver	*Nodularia*
Alkaloids		
Anatoxin-a	Nerve synapse	*Anabaena* *Planktothrix/ Oscillatoria* *Aphanizomenon*
Aplysiatoxins	Skin	*Lyngbya* *Schizothrix* *Planktothrix/ Oscillatoria*
Cylindrospermopsins	Liver	*Cylindrospermopsis* *Aphanizomenon* *Umezakia*
Lyngbyatoxin-a	Skin, G.I. tract	*Lyngbya*
Saxitoxins	Nerve axons	*Anabaena* *Aphanizomenon* *Lyngbya* *Cylindrospermopsis*
Lipopolysaccharides		
	Skin/ Exposed Tissue (irritant)	All

1.1 Ecology of Cyanobacteria Linked to Their Success

Cyanobacteria are intriguing organisms as they are the only known prokaryotic oxygenic photosynthesizers and have become adapted to varied habitats such as hot springs, snow and ice, the calm surface waters of stratified eutrophic lakes, and in deep dimly lit layers (Graham and Wilcox 2000). Their success in these varied niches is a testament to their ability to compete with other

photosynthesizers. A variety of characteristics can potentially give cyanobacteria a competitive advantage under certain circumstances, and these will now be discussed.

1.1.1 Buoyancy Regulation

Some cyanobacteria possess the ability to produce gas vesicles which allow them to regulate their position within the water column. When enough gas vesicles are formed and intact, individual cells, filaments, and colonies are positively buoyant and move up toward their light source. Buoyancy becomes negative and cyanobacteria sink for various reasons including gas vesicles collapse (when turgor pressure becomes too great during rapid growth) and the accumulation of dense photosynthetic products (Ibelings et al. 1991, Reynolds 2006). Buoyancy-regulating cyanobacteria may be able to out compete other phytoplankton by migrating between richer nutrient supplies in deeper waters and more abundant light in shallower waters (Ganf and Oliver 1982). However, buoyancy regulation can only occur and be advantageous if a stable water column is present, as turbulent water mixes all phytoplankton (Huisman et al. 2004). Stability is achieved when water is stratified in summer and wind energy is not sufficient to mix the epilimnion. Warm water also promotes strong stratification and is well-tolerated by cyanobacteria but not all phytoplankton (Robarts and Zohary 1987). This temperature tolerance assists them in community dominance in late summer when temperate lakes are warmest (Kalff 2003).

1.1.2 Resting Cells

The filamentous bloom-forming genera, such as *Aphanizomenon* and *Anabaena,* produce akinetes which are specialized thick-walled cells ideal for resting in the sediment (Kalff 2003) and recolonizing the water body when appropriate. Notably, *Microcystis* has no such specialized reproductive cells, but can survive well in its vegetative form in the sediments (Falconer 2005).

1.1.3 Nitrogen and Phosphorus

Cyanobacterial dominance has long been associated with high total P (Downing et al. 2001) and with a low N to P ratio (Ferber et al. 2004, Smith 1982). These observations may be explained by the fact that cyanobacteria are much better competitors for N than P and, therefore, do not dominate under low P conditions. As well, some cyanobacteria possess heterocysts which are specialized cells which fix atmospheric nitrogen under conditions of nitrogen limitation. Nitrogen is then stored within the cell as cyanophycin particles which contain N-rich arginine and asparagine (Graham and Wilcox 2000). Notably, no group of algae other than cyanobacteria can fix nitrogen so, when water has a low N to P ratio, N may limit the growth of eukaryotes while N-fixing cyanobacteria exploit the large available P pool, becoming dominant (Levine and Schindler 1999, Schindler 1977). It has also been proposed that non-N fixing cyanobacteria can become dominant if N is limiting in the epilimnion because they can access any benthic NH_4^+ source by vertical migration via buoyancy regulation (Blomqvist et al. 1994).

1.1.4 Influence of Dreissenid Mussels

Decades of reduction in point-source P inputs to the Great Lakes have successfully lowered P levels to those that would not be expected to promote high cyanobacterial biomass (Nicholls and Hopkins 1993), yet it is occurring (Nicholls et al. 2002). The introduction of invasive *Dreissena spp*. mussels may be at least partly responsible. Because dreissenids are such efficient filterers, they increase water clarity and the length of the clear water phase, which promotes phytoplankton growth, including that of cyanobacteria (MacIsaac 1996). Several characteristics of *Microcystis* explain why it may be more successful in the presence of dreissenids than other phytoplankton. Firstly, *Microcystis* colonies are sometimes so large that dreissenids cannot consume them (Vanderploeg et al. 2001) and thus *Microcystis* is able to grow while other phytoplankton are grazed down. Secondly, evidence suggests that dreissenids can differentiate between toxic and non-toxic *Microcystis* and that they selectively

reject still-viable toxic cells as pseudofeces, thereby promoting the formation of toxic blooms (Vanderploeg et al. 2001). Thirdly, research has shown that dreissenids may indirectly promote *Microcystis* by altering the ratio of available N:P. They do this during their process of nutrient regeneration by excreting much more phosphorus than nitrogen (N:P is <20) (Arnott and Vanni 1996) and by increasing the nitrate flux to the sediments while decreasing the flux of (Bykova et al. 2006). Furthermore, studies have found an interaction effect between total phosphorus concentrations and the positive affect of dreissenids on *Microcystis* (Raikow et al. 2004, Sarnelle et al. 2005). For instance, dreissenid abundance and 'low' total P (<25 ug/L) were seen to promote *Microcystis* dominance but dreissenid presence at higher total P did not have the same effect (Raikow et al. 2004).

1.1.5 Mucilage

Mucilage is a gelatinous secretion which surrounds the unicells, colonies, and filaments of certain members of various phytoplankton groups including cyanobacteria (Reynolds 2006). Although the function of mucilage is still not fully understood, several properties of it may give cyanobacteria which possess it, such as *Microcystis* colonies, a competitive advantage. Firstly, mucilage is much less dense than water and so contributes to positive buoyancy (Reynolds 2006). Secondly, a mucilaginous sheath increases the streamlining of colonies and filaments, thereby facilitating vertical movements in the water column (Reynolds 2006). Thirdly, mucilage can protect cyanobacteria from grazing by increasing the size of colonies and filaments, making them difficult or impossible to be filtered out of the water (Reynolds 2006). However, if they are consumed by grazers, a fourth function of mucilage can come into play. As cyanobacteria pass through the gut of some grazers, they can survive digestion due to their protective sheaths, and emerge as viable cells (Porter 1976, Reynolds 2006). While passing through the gut, they can even absorb some nutrients from their

would-be consumers (Porter 1976, Reynolds 2006). In certain situations these benefits of mucilage may help promote the success of sheathed cyanobacteria.

1.1.6 Pigments

Because many cyanobacteria are buoyant and can form surface blooms, photoinhibition can be problematic. Photoinhibition is a decrease in photosynthetic activity caused by over-excitation of the light-harvesting centres of photosystem II (Reynolds 2006). If several generations of cyanobacteria are exposed to high irradiace, they accumulate zeaxanthin, a type of carotenoid (Reynolds 2006). Zeaxanthin allows cyanobacteria to dissipate excess energy as heat, thereby preventing damage to the photosynthetic apparatus (Reynolds 2006). This can prove to be very advantageous to cyanobacteria under high irradiance.

1.2. Structure and Properties of Microcystin

Microcystin is a hepatotoxic cyclic peptide and is the most frequently encountered and best studied cyanotoxin (Chorus and Bartram 1999). There are currently over 70 known structural variants of microcystin (Codd et al. 2005), with the best known variant being microcystin-LR. The microcystin molecule contains seven variable amino acids and, most notably, the unusual Adda, which is involved in binding protein phosphatase and accounts for most of the toxicity (Falconer 2005). Adda, unlike the twenty standard ribosomally-translated amino acids, is produced through post-translational modifications performed by a peptide synthetase enzyme (Kaebernick and Neilan 2001).

A single cyanobacterial strain can produce multiple microcystin variants at the same time and the relative abundance of each variant produced has been shown to change throughout a culture's population growth (Lyck 2004). This has environmental implications as the different variants of microcystin elicit different degrees of toxicity. Those with more hydrophobic L-amino acids

(including microcystin-LR) are more toxic and those with more hydrophilic amino acids are less toxic (Falconer 2005).

Microcystin is mainly held within the cell until it lyses, which means that the senescence of a large microcystin-producing cyanobacterial bloom results in a strong pulse of toxins into the water. Once in the water, microcystins are stable, with the following four routes of detoxification occurring in nature: adsorption by sediments, thermal decomposition aided by low pH and high temperature, photolysis, and microbial degradation (reviewed by Harada and Tsuji 1998). Notably, the activity of enzymes of the human gut, such as trypsin, is not included in this list. In nature, microcystins may persist for weeks, although the precise length of time appears to be dependant on the numbers of degrading bacteria present (Mazur and Plinski 2001). The degradation products of microcystin do not display toxicity (Harada and Tsuji 1998).

It is generally thought that microcystin is a secondary metabolite as is not required for an organism's primary metabolism (as is a primary metabolite) (Carmichael 1992, Kaebernick and Neilan 2001a). There has been some debate on this issue since some research has identified a correlation between growth rate and microcystin production (Orr and Jones 1998). However, the fact that non-toxic strains of cyanobacteria can function as well as toxin-producing strains supports the idea that microcystin is not involved in basic metabolism.

There is no conclusive theory about the endogenous function of microcystin, but research into the topic has generated several preliminary hypotheses which follow. Microcystin has a high affinity for iron and binds Fe^{2+} so it has been proposed that the toxin may be useful in collecting iron under conditions of low availability (Lukac et al. 1993). Alternatively, microcystin may chelate Fe^{2+} when intracellular iron concentrations are high, thereby protecting the cell from free radical formation and damage (Kaebernick and Neilan 2001, Utkilen and Gjolme 1995). Furthermore, it has been asserted that microcystin may play a role in photosynthesis. Evidence to support this includes

that the observation that the 'Adda' portion of microcystin binds to the thylakoid and that genetic studies have shown increased transcription of *mcy* genes (the genes that encode microcystin production) under high light conditions (Kaebernick and Neilan 2001, Kaebernick et al. 2000). Yet another hypothesis addresses the allelopathic properties of microcystin (discussed below) and the possibility that such cyanotoxins function in aiding competition with other phytoplankton (Figueredo et al. 2007). All of these hypotheses assume that microcystin is still functional to cyanobacteria, but it is plausible that this peptide is simply an evolutionary relic which has lost its purpose to these ancient organisms but happens to be toxic.

1.3 Effects of Microcystin: from Enzyme to Ecosystem

Microcystin exerts wide-spread effects, one of which is the inhibition of protein phosphatases (PP) 1 and 2A which are important regulatory enzymes in all eukaryotes (MacKintosh et al. 1990). The Adda amino acid binds to the enzyme at the hydrophobic groove of its catalytic site thus preventing enzymatic activity (Goldberg et al. 1995). Microcystin requires a transport system to enter cells and, in vertebrates, the only suitable system is the bile acid carrier between the stomach and the liver (Falconer 1993). Microcystin then accumulates in hepatocytes and PP inhibition can lead to collapse of the hepatocyte cytoskeletons and possibly death by hemorrhaging (Wiegand and Pflugmacher 2005). Long-term low-level exposure or a strong exposure episode can result in chronic liver injury, including cancer (Chorus and Bartram 1999).

The effects of microcystin in the environment range from primary producers to top carnivores. Dissolved microcystin in the water can affect other phytoplankton including cyanobacteria (Sedmak and Elersek 2005). In a laboratory experiment, the presence of microcystins induced cell aggregation, increased cell and chloroplast volume, and resulted in an overproduction of photosynthetic pigments in *Microcystis aeruginosa* and the green alga *Scenedesmus quadricauda* (Sedmak and Elersek 2005). Cell aggregation could benefit cyanobacteria by both allowing their own

colonies to adjust their buoyancy more quickly and by increasing the sedimentation rate of competitors from other algal divisions (Sedmak and Elersek 2005). The sedimentation rate of the motile green alga *Chlamydomomas reinhardtii* was also shown to be increased by microcystin since the toxin caused paralysis of the cells (Kearns and Hunter 2001). Evidence for the allelopathic function of microcystin includes the observation that a ***M. aeruginosa*** culture grown in spent non-toxic *Planktothrix agardhii* medium produced more toxins (Engelke et al. 2003). In a study of a related toxin, cylindrospermopsin, evidence for allelopathic function was also found when phytoplankton grown in the exudates of the toxin-producer *Cylindrospermopsis raciborskii* showed inhibited photosynthesis (Figueredo et al. 2007).

Microcystin can affect vascular plants as well. The submerged plant *Ceratophyllum dermersum* showed reduced growth following microcystin exposure and impaired photosynthesis was documented in *Elodea canadensis*, *Myriophyllum spicatum*, and *Phragmites australis* (Pflugmacher 2002). Toxin present in irrigation water reduced the growth rate and chlorophyll content of *Solanum tuberosum* L. (potato), inhibited seedling growth of *Synapis alba* L. (mustard), and reduced root development in *Phaseolus vulgaris* (bean). Furthermore, microcystin was retained in these plants' tissues, which is particularly concerning in these crop species (McElhiney et al. 2001).

Negative effects of microcystin have been documented for a variety of zooplankton, including *Bosmina*, *Chaoborus*, and *Tetrahymena* (Wiegand and Pflugmacher 2005). With the common cladoceran, *Daphnia*, experiments have showed that certain *Microcystis* cells can rapidly clog the filtering apparatus and, for those that ingest them, depressed function or death can result (Nizan et al. 1986, Thostrup and Christoffersen 1999). Microcystin has been implicated in fish kills (Huisman et al. 2004) but it can have subtler effects on fish as well. For instance, it has been shown to decrease motility in *Danio rerio* and *Leucaspius delineatus* and to reverse the diurnal activity

pattern of *L. delineatus* (Baganz et al. 2004). Such changes could have a variety of consequences for behaviour-dependant processes like reproduction and predator avoidance.

1.4 Factors Linked to Toxin Production

The rate at which individual cells produce microcystin can vary greatly within a species, so factors beyond abundance of potentially toxigenic species must be investigated in order to understand microcystin production. The ability of cyanobacterial strains to produce microcystin has been traced to the *mcy* gene cluster (Meisner et al. 1996). If this gene cluster is present, it can be expressed to varying degrees or it may not be expressed at all (Meisner et al. 1996). Both laboratory and field studies have revealed some intriguing relationships between environmental factors and microcystin levels that have furthered our collective understanding of microcystin dynamics (reviewed in Zurawell et al. 2005).

Field studies have shown associations between microcystin and total P, soluble reactive P, total N, the N to P ratio, chlorophyll *a*, light, and dissolved O_2 (Billam et al. 2006, Kardinaal and Visser 2005). The literature shows much variability in these relationships, however, and they are often contradictory (Kardinaal and Visser 2005). For instance, even the relationship between total phosphorus and microcystin has been shown to be positive (Giani et al. 2005), negative (Oh et al. 2000), and almost nonexistent (Sivonen 1990). Nonetheless, some general patterns can be seen, such as microcystin production generally being higher under lower light conditions (ex.: Kotak et al. 2000).

Laboratory studies have been used to isolate the effects of individual variables on cell division rates and microcystin production in particular strains. Culture growth stage, light, temperature, major nutrients (N, P), salinity, pH, and micronutrients (example: Fe) have all been investigated. Reviews have noted that toxigenic strains generally produce the most microcystin under optimal growth conditions, which typically include elevated nutrient concentrations (Kardinaal and

Visser 2005, Sivonen and Jones 1999). The exact environmental variables found to best explain microcystin concentrations appear to be strain-specific, however (Orr and Jones 1998). This led Orr and Jones (1998) to develop their hypothesis that microcystin production is directly affected by cell division rate regardless of which environmental factor is limiting that rate at the time.

A review of culture studies showed that toxin production within a single strain can vary only by a factor of 3 to 4, even over a broad range of environmental conditions (Sivonen and Jones 1999). However, field microcystin levels can vary by over three orders of magnitude, as can the responses of different strains to similar growth conditions in the lab (Sivonen and Jones 1999). This suggests that the majority of natural microcystin variability can be explained not by environmental conditions but by the relative abundance of the toxic strains present (Giani et al. 2005, Ozawa et al. 2005). The seasonal succession of cyanobacterial species and strains is most likely very important to microcystin concentrations, but it is not well understood (Billam et al. 2006, Codd et al. 2005). To date, a reliable and universal predictor of microcystin production has yet to be identified.

1.5 Study Sites

1.5.1 Maumee Bay

Maumee Bay comprises the westernmost part of Lake Erie's Western Basin (Figure 1.1) and toxic cyanobacterial blooms have become a problem there in recent years (Bridgeman 2005). Maumee Bay is a relatively shallow eutrophic body of water (Table 1.2) and has been impacted by a variety of human activities. The bay and its major tributary, the Maumee River, are both bordered by the historically industrial city of Toledo, Ohio, USA. A major glacial wetland known as the Great Black Swamp, which was once located north of Toledo, was drained and converted to farmland in the 1800's, thereby changing the hydrology and natural filtering capacity of the area. More wetland bordering Maumee Bay was filled in the 1980's to create Maumee Bay State Park (U.S. Army

Engineer District, Buffalo 1983). Furthermore, Maumee Bay and neighbouring areas have been dredged over the years to harvest sand and concern has arisen that contaminants trapped in the sediments could be released (U.S. Army Engineer District, Buffalo 1983). The aforementioned processes have contributed to the eutrophication and disturbance of the Maumee Bay aquatic ecosystem.

The relatively high summer total phosphorus (TP) of the area (Table 1.2) and its sheltered and calm water column can promote cyanobacterial blooms. The high turbidity introduced into the bay by the Maumee River has also been associated with the presence of *Microcystis* blooms (Bridgeman 2005). Furthermore, the presence of *Microcystis*-promoting dreissenids has been documented in Maumee Bay (Fraleigh et al. 1991).

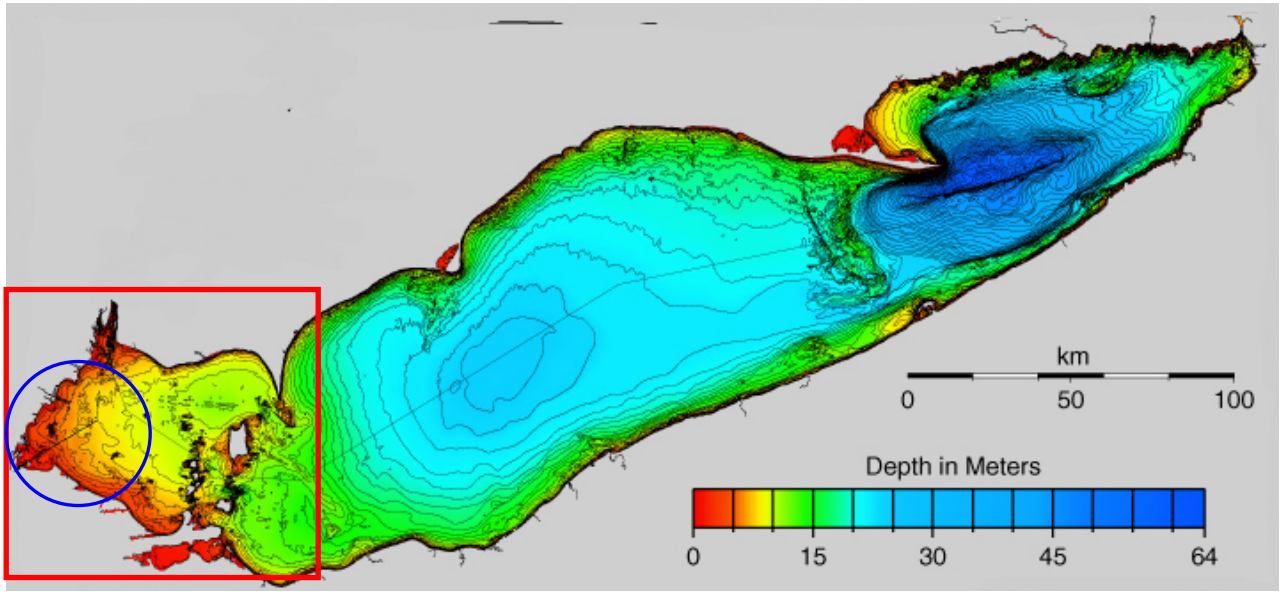


Figure 1.1. A bathymetric map of Lake Erie courtesy of the National Geophysical Data Center: National Oceanic and Atmospheric Administration (www.ngdc.noaa.gov/mgg/image/erie.jpg). The relatively shallow western basin of Lake Erie is indicated by the large square and Maumee Bay is indicated by the smaller circle.

Table 1.2. Selected characteristics of the study sites. Information sourced from the following literature: (Bailey et al. 1999, Bur et al. 2002, Grand River Conservation Authority 1980, Grand River Conservation Authority 1984, Hartman 1973, Minns 1995, Minns et al. 1986, Nicholls and Hopkins 1993, Porta et al. 2005).

Attribute	Water Body				
	Bay of Quinte	**Maumee Bay**	**Belwood Lake**	**Conestogo Lake**	**Guelph Lake**
Surface Area (km2)	257.4	~	7.77 (max.)	7.35 (max.)	3.6 (max.)
	(Minns, 1995)		*(GRCA, 1980)*	*(GRCA, 1980)*	*(GRCA, 1984)*
Catchment Area (km2)	18 182	~	799	518	~
	(Minns, 1986)		*(GRCA, 1980)*	*(GRCA, 1980)*	
Mean Depth (m)	Upper Bay: 3.5	approx. 7	varies	Varies	varies
	Middle Bay: 5.2	*(Hartman, 1973)*			
	Lower Bay: 24.4				
	(Minns, 1995)				
Max. Depth (m)	66.4	approx. 10	~	22.6	~
	(Minns, 1995)	*(Hartman, 1973)*		*(GRCA, 1980)*	
Avg. Summer TP (ug/L)	35	45	22.4	18.3	17.9
	(2005 UW data)	*(Western Basin)*	*(2005 UW data)*	*(2005 UW data)*	*(2005 UW data)*
		(Porta et al., 2005)			
Avg. Summer Secchi (m)	2.2	2.7	1.5	2.6	2.6
	(2005 UW data)	*(LEFTG, 2002)*	*(2005 UW data)*	*(2005 UW data)*	*(2005 UW data)*
Year of Damming	n/a	n/a	1942	1958	1975
			(GRCA, 1980)	*(GRCA, 1980)*	*(GRCA, 1980)*
Dreissenids Present?	Yes, since 1993	Yes, since 1989	No	No	No
	(Bailey et al., 1999)	*(Nicholls & G., 1993)*			

1.5.2 Bay of Quinte

The Bay of Quinte is a well-studied area of Lake Ontario which is readily recognized by its characteristic z-shaped border (Figures 1.3 and 1.4). Like Maumee Bay, the Bay of Quinte has been strongly influenced by colonial human settlement. After colonization in the 1800's, watershed deforestation, mining, and agricultural practices all resulted in increased nutrient and fine particle inputs to the bay (Minns 1995). Mining and industrial development resulted in the discharge of chemicals into the water body while natural hydrology was disturbed by damming (Minns 1995). In

the 1900's, a growing urban population furthered eutrophication and algal blooms had already been reported twice in the Bay of Quinte by the 1930's (Minns 1995). Phosphorus inputs from detergents and sewage severely altered the Bay of Quinte ecosystem. The system was pushed from a clearwater state to a turbid one in which macrophyte abundance and fish stocks were significantly reduced (Minns 1995). As in the Maumee Bay area, the draining of wetlands adjacent to the Bay of Quinte exacerbated the decline in water quality.

Point-source phosphorus loading controls and the use of upgraded waste water treatment plants with tertiary treatment were implemented in the 1970's. Nonetheless, the Bay of Quinte was defined as one of 42 areas of concern in the Great Lakes in 1985 by the International Joint Commission and a Remedial Action Plan was formed to deal with eutrophication, undesirable algae, and degraded phytoplankton and zooplankton communities (Nicholls et al. 2004).

P-loading reduction resulted in a dramatic decrease in total phytoplankton abundance (Nicholls et al. 2004), however the invasion of dreissenids between 1993 and 1994 quickly resulted in an undesirable species shift (Bailey et al. 1999). In 1995, *Microcystis* showed a sudden 13-fold increase that occurred while all other algal taxa abundances remained the same or decreased (Nicholls et al. 2002). Microcystin has since become a concern in the bay and studies of its scope were soon underway.

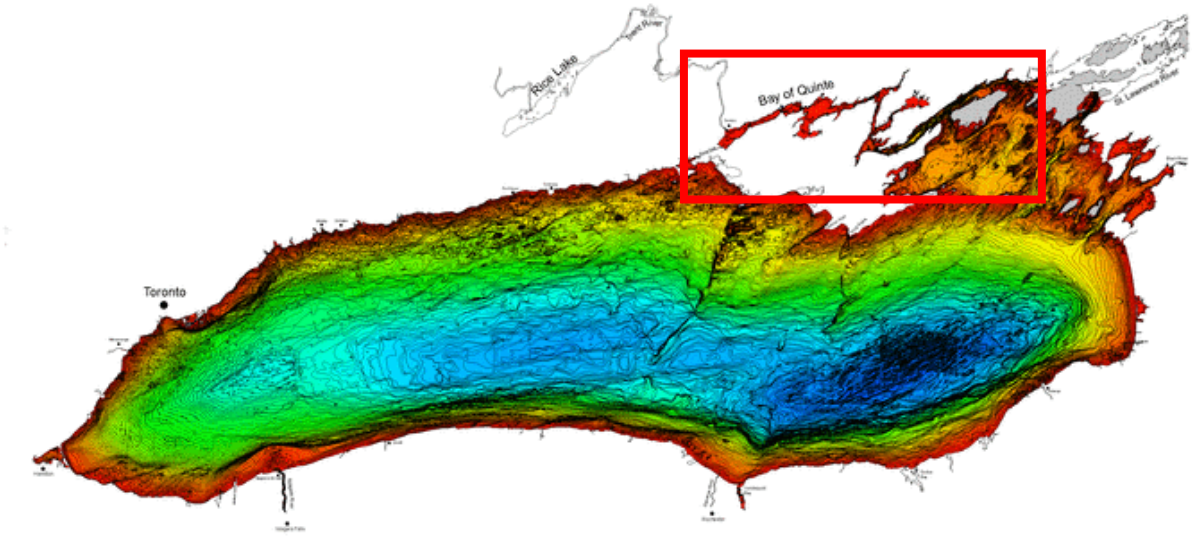


Figure 1.2. A bathymetric map of Lake Ontario courtesy of the National Geophysical Data Center: National Oceanic and Atmospheric Administration (http://www.ngdc.noaa.gov/mgg/image/ontario512.jpg). The Bay of Quinte is indicated and labelled.

Figure 1.3. A map of the Bay of Quinte showing the relatively small geographical area surveyed from Deseronto, Ontario.

1.5.3 Grand River Reservoirs

The Grand River is an important tributary to Lake Erie which originates just south of Georgian Bay (Rott et al. 1998). The entire Grand River catchment area (Figure 1.4) has been largely converted for agricultural and urban uses. The watershed is home to much livestock, including approximately 500,000 cattle, 750,000 pigs, and 9,000,000 hens and chickens (Dorner et al. 2004), which results in substantial nutrient input to the river. Belwood Lake, Conestogo Lake, and Guelph Lake (Figure 1.5) are all reservoirs created by damming which occurred in 1942, 1958, and 1975, respectively (Grand River Conservation Authority 1980). Belwood Lake resulted from the building of the Shand Dam, which was constructed on the Grand River near Fergus, Ontario. The Conestogo Dam was built on the mid-reaches of the Conestogo River, while a main dam and two smaller ones were constructed on the Speed River near Guelph, Ontario. All dams were constructed as a way to limit downstream flow following the spring melt and to provide more constant river flow throughout the year (Grand River Conservation Authority 1980). Discharge from the dams is carefully regulated by the GRCA in anticipation of flow regimes to prevent flooding. As a result, the depth of the reservoirs varies dramatically throughout the year (Grand River Conservation Authority 1980). Since their inception, the reservoirs have become popular recreational sites as well (Grand River Conservation Authority 1980).

All three reservoirs were reported as being eutrophic and having hypolimnetic oxygen depletion problems by 1980 (Grand River Conservation Authority 1980). Their warm, stratified, calm waters are ideal conditions for excess algal growth (Grand River Conservation Authority 1980). A massive cyanobacterial bloom on Belwood Lake in the late summer of 2004 alarmed the public and water quality managers and created the impetus for this study.

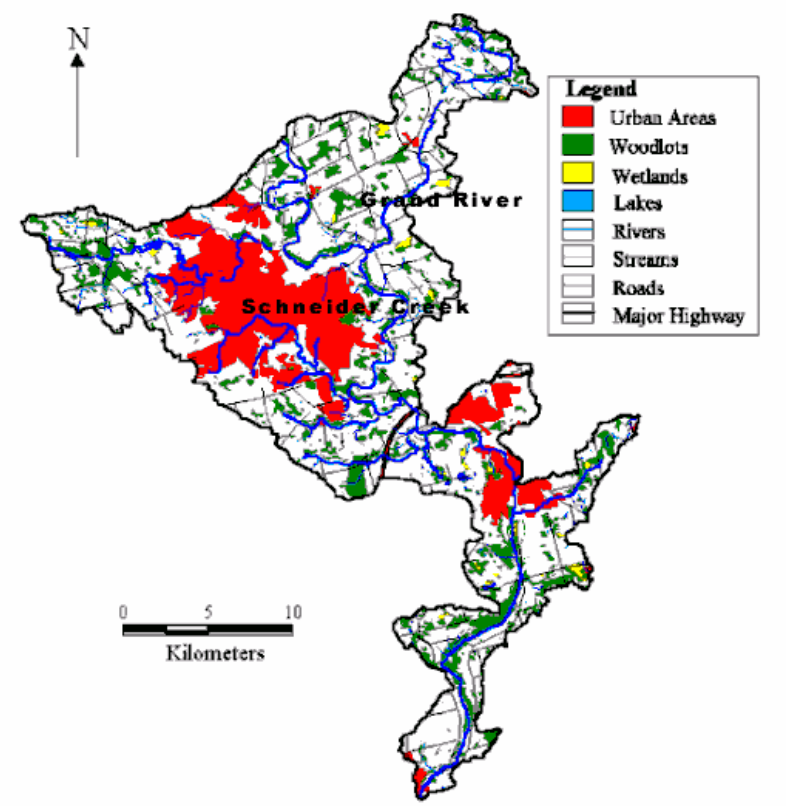


Figure 1.4. A map showing land use in the Grand River watershed. Areas not coloured are rural/agricultural. This image is credited to Dr. Bob Sharpe and Sonya Chittick and was sourced from: http://info.wlu.ca/~wwwgeog/thesis/tour2.html. Reproduced with permission.

Figure 1.5. The Grand River watershed with Conestogo, Belwood, and Guelph lakes circled from left to right. Image courtesty of GRCA: http://library.mcmaster.ca/maps/images/GRCAMap.gif.

1.6 Hypotheses

Following the review of the literature outlined above, several hypotheses were formed and were tested in this study. Those hypotheses are as follows:

1) If nutrient status affects microcystin concentrations and favourable growth conditions result in more microcystin production, then indicators of greater nutrient deficiency will be negatively associated with microcystin levels.

2) If the abundance of different cyanobacterial groups contributes to microcystin concentrations, then dominance by particular potentially toxic species will be associated with higher microcystin concentrations.

3) Greater water column stability, a low N to P ratio, higher soluble reactive P and total P, the presence of dreissenids, and decreased water transparency all promote the production of microcystin and will be positively associated with microcystin concentrations.

Chapter 2
Microcystin Detection

2.1 Introduction to Methods for Microcystin Detection

Over the past decades, microcystin detection methods have evolved to currently offer several effective alternatives. Early microcystin detection used the mouse bioassay in which mortality was the main indication of toxicity. Three more accurate and humane methods have now replaced the mouse bioassay. These techniques are liquid chromatography followed by mass spectrometry, the enzyme-linked immunosorbant assay (ELISA), and the protein phosphatase inhibition assay (PPIA) (Table 2.1). This study employed PPIA for microcystin detection.

Table 2.1. Comparison of liquid chromatography, ELISA, and PPIA methods (Mountfort et al. 2005, Bouaicha et al. 2002, Rapala et al. 2002, Neissan and van der Greef 1992, Kemeny and Challacombe 1988).

	Liquid Chromatography	**ELISA**	**PPIA**
Typical Detection Limit		0.160-0.175 µg/L with regular kit, 0.05 µg.L with high sensitivity kit	Often 0.1 µg/L, 0.05 µg/L in this study
Type of Sample Used	Concentrated water or filter extract with clean-up step	Filtered or whole water, filter extract	Filtered or whole water, filter extract
Preconcentration of Samples?	Yes, typically with C18 columns	Unnecessary	Unnecessary
Characteristic of Toxin Detected	Molecular structure as compared to multiple standards, molecular mass also if followed by mass spectrometry	Molecular structure only	Toxicity to enzyme
Major Equipment Required	HPLC System and potentially a mass spectrometer	Microplate spectrophotometer	Microplate fluorometer, incubator
Cost per Sample	~$125 if sent away, undetermined if performed in lab	$9 when duplicates performed only	<$2 when quadriplicates performed

2.1.1 Liquid Chromatography

Liquid chromatography (LC) is regarded as the most analytical and precise detection method. Generally, this type of analysis involves injecting a sample into a liquid mobile phase of organic solvents which is forced through a column packed with a stationary phase such as modified silica gel (Niessan and van der Greef 1992). Compounds in the mobile phase are sorbed to the stationary phase and are 'sorted' as they are eluted from the column. They are detected, represented on a chromatograph, and compared to various standards of microcystin variants (Niessan and van der Greef 1992). High performance liquid chromatography (HPLC) is a well-known variant of liquid chromatography that employs high pressure to force the mobile phase through the column (Niessan and van der Greef 1992). Following their passage through the columns, compounds may be analyzed by mass spectrometry. In microcystin analysis, this allows for the quantification of each structural variant and confirms their identities. The mass spectrometer detects the molecular weight and structure of compounds and measures their abundance relative to others in a sample (Niessan and van der Greef 1992).

Liquid chromatography and mass spectrometry require expensive equipment and extensive training that are inaccessible in many labs. Although samples may be sent away for analysis, this would also be costly for the testing of many samples. Liquid chromatography also requires the time-consuming concentration and cleanup of water samples prior to their analysis on C18 solid phase extraction cartridges or by ultrafiltration (Heresztyn and Nicholson 2001). C18 cartridges contain silica with chains of 18 carbon atoms that bind dissolved compounds from the water. Potentially interfering impurities may be washed out of the column ('sample clean-up') and then toxin can be eluted from the column with the use of an appropriate solvent, thereby concentrating it. Not only is this step time consuming, but it requires a larger volume of sample water (~ 1 L) than the protein

phosphatase inhibition assay. The above drawbacks reduce the utility of this well-established technique for screening of a large number of samples in most labs.

2.1.2 Enzyme-Linked Immunosorbent Assay

The commercially-available ELISA kits for cyanobacterial toxin detection employ competitive capture of microcystin (Carmichael and An 1999). This means that 'capture' antibodies are fixed to the wells of a microplate and microcystins in a sample must compete for the limited number of binding sites with enzyme-bound microcystin that is supplied as an assay reagent. After a period of time the wells are washed to remove unbound microcystin. Next, a colorimetric substrate is added which binds to the enzyme attached to the microcystins that were not present in the sample. Colour generation is then measured so that greater activity indicates lower levels of microcystin in the sample. A downfall of ELISA is that microcystins are bound based on their shape so, due to structural differences, some microcystin variants are more readily detected than others. Variants that are more readily bound may not necessarily be the most toxic or prevelant variants in the sample, so this can be problematic when the toxic effect of microcystin is of interest. ELISA kits are extremely user-friendly, however this feature is reflected in the cost of this method (the 2007 quote for one 96-well plate for cyanobacterial toxin detection from Envirologix was $396 USD).

2.1.3. Protein Phosphatase Inhibition Assay

PPIA is most distinct from LC and ELISA in that it is a functional assay. Microcystin is quantified not by its physical properties but by the way it actually affects the biological activity of organisms in nature. Microcystin's main mode of toxicity is through the inhibition of protein phosphatases (PP) 1 and 2A, which are important regulatory enzymes in all eukaryotes (MacKintosh, et al 1990). Microcystin present in a microplate sample well will irreversibly bind to the catalytic subunits of extracted PP1 and 2A (Carmichael and An 1999) thus inhibiting them. Enzyme activity is measured

by the fluorescence, colour generation, or radioactivity that occurs when the enzyme cleaves phosphate from a substrate, and more microcystin present is indicated by lower levels of phosphate cleavage. The strength of the PPIA method for ecological studies lies in the fact that the enzymatic effect measured is the one actually experienced by aquatic organisms. Data generated are estimates of microcystin-LR equivalents present, as microcystin-LR (the microcystin variant with leucine and arginine) is the standard most commonly used.

The cost of PPIA is much less than that of LC or ELISA. Although a fluorometer capable of reading microplates at the appropriate wavelengths is required, this is a multipurpose piece of equipment that can easily be shared between labs and projects. Once this investment has been made, the materials required for one assay performed in a 96-well plate are relatively modest in cost. This assay is an appropriate choice for projects which require the screening of many samples and research questions which are mainly interested in ecological effects rather than toxin variant quantification. Budget-conscious labs will also find this method appealing, however substantial time may be needed to establish the assay at the forefront of a project. Familiarity with molecular and biochemical techniques will likely be required during troubleshooting and this may not be readily available in an ecology lab.

The use of PPIA for microcystin detection has evolved and improved over the years due to optimization of the assay. The earliest versions of PPIA were radiometric (Jones and Orr 1994, Lam et al. 1995, Lambert et al. 1994, Sim and Mudge 1993). However, the increased ease and safety of using a colorimetric substrate, rather than a radiolabelled one, gave rise to the use of the PP1 enzyme and *p*-nitrophenol phosphate as a colorigenic substrate (ex.: An and Carmichael 1994). Mountfort et al. (1999) then made a useful comparison between the colorigenic substrate *p*-nitrophenol phosphate and the fluorogenic substrate methylumbelliferyl phosphate (MUP) in PPIA for the detection of okadaic acid, a dinoflagellate toxin, and found that the fluorometric form of the assay was more

accurate. Although the colorometric version of the assay is still used today, this finding incited interest in the fluorometric version. Fontal et al. (1999) further investigated fluorogenic substrates and found that, with the substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP), use of the PP2A enzyme resulted in less noise and, therefore, greater sensitivity than did use of the PP1 enzyme.

Furthermore, Honkenen et al. (1990) described that PP2A was much more sensitive (50 times) to microcystin than was PP1 so it could be used to detect smaller quantities of toxin. As a result, the use of PP2A is now much more prevalent in the literature than is the use of PP1. Bouaicha et al. (2002) built on these results by comparing the use of DiFMUP and MUP substrates with the PP2A enzyme and found lower variation in test results using the MUP substrate than the DiFMUP substrate. The above improvements to PPIA led our research group to choose the fluorometric assay using the PP2A enzyme and the MUP substrate for our analyses.

Both Heresztyn and Nicholson (2001) and Bouaicha et al. (2002) demonstrated another key improvement to the PPIA method: the ability to use unconcentrated water samples directly in the assay. Many other formulations of the assay used concentrated samples of microcystin, such as toxin extracted from algal or invertebrate tissue. However, if water with low levels of dissolved toxin is going to be tested, the sample would have to be concentrated by running a significant volume (ex.: 1 L) through a C18 solid phase extraction cartridge. With these concentrated samples a relatively small sample volume can be used. For example, Fontal et al. (1999) used 10 µL of microcystin-LR standard in a total well volume of 200 µL. This setup would not be appropriate when unconcentrated lake water, which presumably has a lower total amount of toxin per well, is tested. Heresztyn and Nicholson (2001) used untreated water samples in their assays, however they focused on their ability to use water that has not been cleaned by C18 or other means. They kept the typical small amount of sample found in other assay formulations (20 µL sample: 240 µL total volume) and, therefore, had a

lower detection limit of 0.2 µg/L microcystin. In order to use unconcentrated water samples and to have a more sensitive assay, Bouaicha et al. (2002) used more concentrated solutions of buffer and substrate so that 200 µL of sample could be present in a well with 300 µL total volume. This innovation allows filtered lake water and sonicated whole lake water to be tested directly with a documented method detection limit of 0.1 µg/L. Because of this improvement, the method outlined in Bouaicha et al. (2002) was employed to test samples for this study.

2.2 Assay Troubleshooting and Optimization

The establishment of the PPIA method in our lab was not as smooth as was hoped and required lengthy trouble-shooting. The results from Bouaicha et al. (2002) that we were attempting to imitate may be seen in Figure 2.1. The fluorescence activity is measured as a percentage of the positive control which contains no microcystin. When the assay was first attempted, results were typically as seen in Figure 2.2, which are obviously contrasted with those of Figure 2.1. As one can see, in Bouaicha et al. (2002)'s work, at a level of 1 µg/L (1000 pg/mL) the fluorescence activity was approximately 0% of its level when 0 µg/L microcystin was present. Therefore, effectively all enzyme was inactivated at the 1 µg/L microcystin level. However, as may be seen in Figure 2.2, the percent of fluorescence activity for the same 0-1 µg/L microcystin range decreased from 126.5% to only 83.9%, indicating that very little inactivation of the enzyme was occurring. It was evident that some component of the assay was malfunctioning and so extensive trouble-shooting was undertaken to uncover the culprit.

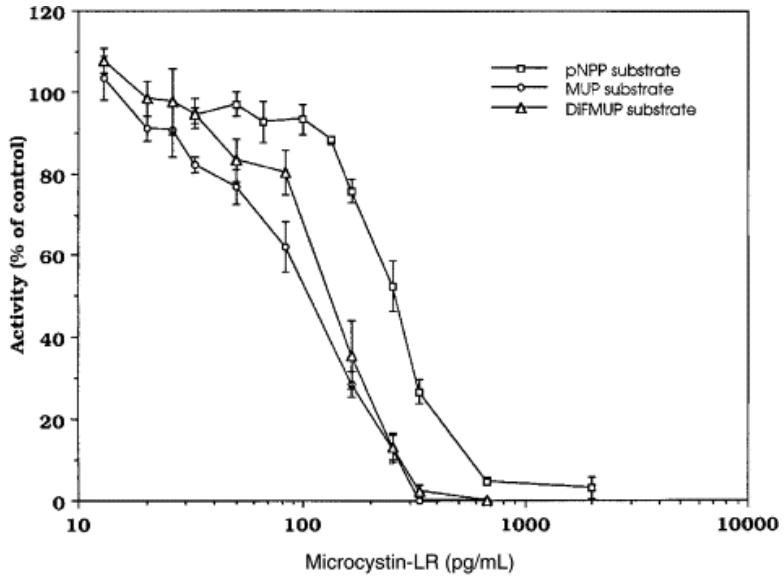


Figure 2.1. "Dose-response inhibitory activity of microcystin-LR on PP2A using colorimetric (p-NPP) and fluorogemic (MUP and DiFMUP) substrates. Each value represents the mean of three experiments +/- the standard deviation." Bouaicha et al., 2002. Copyright Elsevier, reproduced with permission.

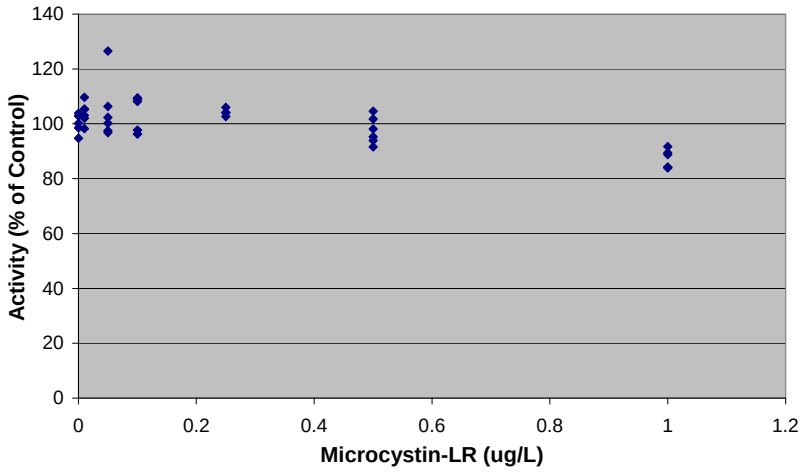


Figure 2.2. An example of results from an early assay attempt using the Upstate 1 enzyme (see Table 2.2). The variation in replicates for each microcystin standard is shown. The amount of microcystin present in the well has a nearly insignificant impact on fluorescence.

2.2.1 Microcystin

It was suspected that a lack of toxic potency of the microcystin-LR standards may have been causing the lack of noticeable difference between standards. Standard solutions used in our assays were initially prepared from powdered microcystin that had been dissolved in sterile Milli-Q water. The highly concentrated microcystin stock was aliquoted to polypropylene microcentrifuge tubes and frozen. When it was discovered that microcystin can sorb to polypropylene pipette tips (Hyenstrand et al. 2001), concern arose that microcystin loss to the microcentrifuge tubes could be reducing the amount of toxin delivered to the assay. A new Ridel-de Haën Oekanal® microcystin-LR standard was purchased from Sigma-Aldrich at a concentration of 10 µg/mL in methanol. As dissolving a powder was now unnecessary, a step which may have introduced error into the process was eliminated and the microcystin in methanol was deemed simpler to work with. A comparison of the microcystin-LR in methanol and the powdered microcystin-LR and their effects on PP2A inhibition can be seen in Figure 2.3. Although there is slightly more inhibition of the enzyme by the new microcystin than the old one, the inhibition did not reach the desired levels, so another assay component was still the problem.

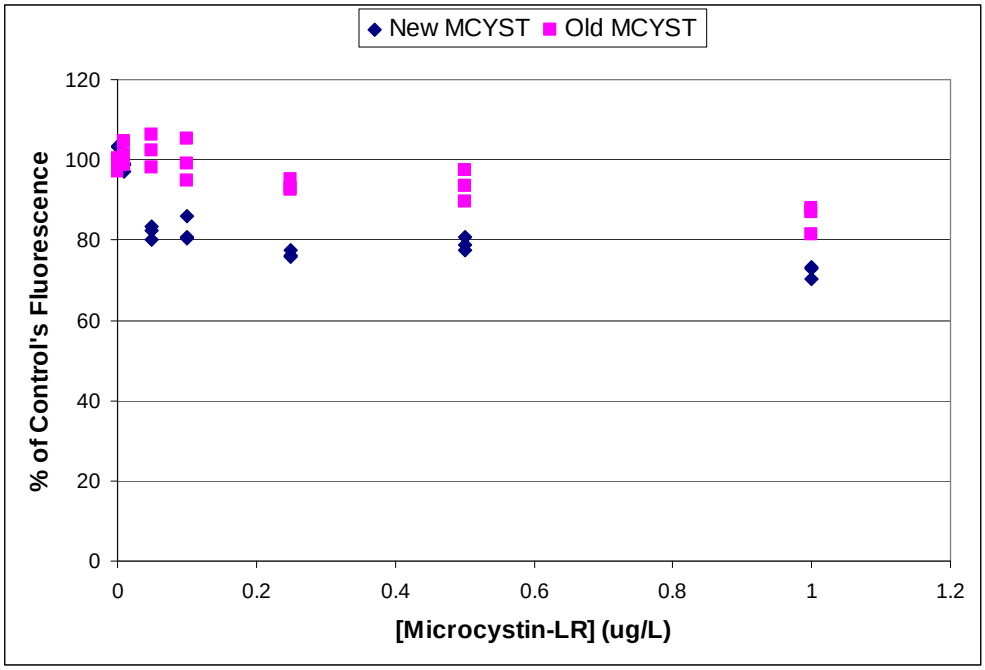


Figure 2.3. May 31, 2006 comparison of 'New' Ridel-de Haen microcystin-LR standard supplied dissolved in methanol and 'Old' microcystin-LR standard supplied as a powder. 1x Promega 1 PP2A (Table 2.2) was used to test inhibition caused by the toxin.

2.2.2 PP2A Inactivity

The first recognized issue with PP2A was the matrix in which it was supplied to us. The first enzyme received was supplied by Upstate (a subsidiary of Millipore) and was that which was used by Bouaicha et al. (2002). This was provided in 10% glycerol solution. This glycerol concentration resulted in the enzyme solution being solid when frozen and the repeated thaw-freeze cycles required to use a fraction of the enzyme at a time were thought to have possibly caused enzyme inactivation. It was believed that the enzyme problem would be solved when PP2A was purchased from Promega which was supplied in 50% glycerol solution and was a viscous liquid at -20^{o}C. This enzyme initially worked and showed vastly greater activity than the Upstate PP2A that we had been using (Figure 2.4). However, promptly after its first use the Promega enzyme also apparently became inactivated.

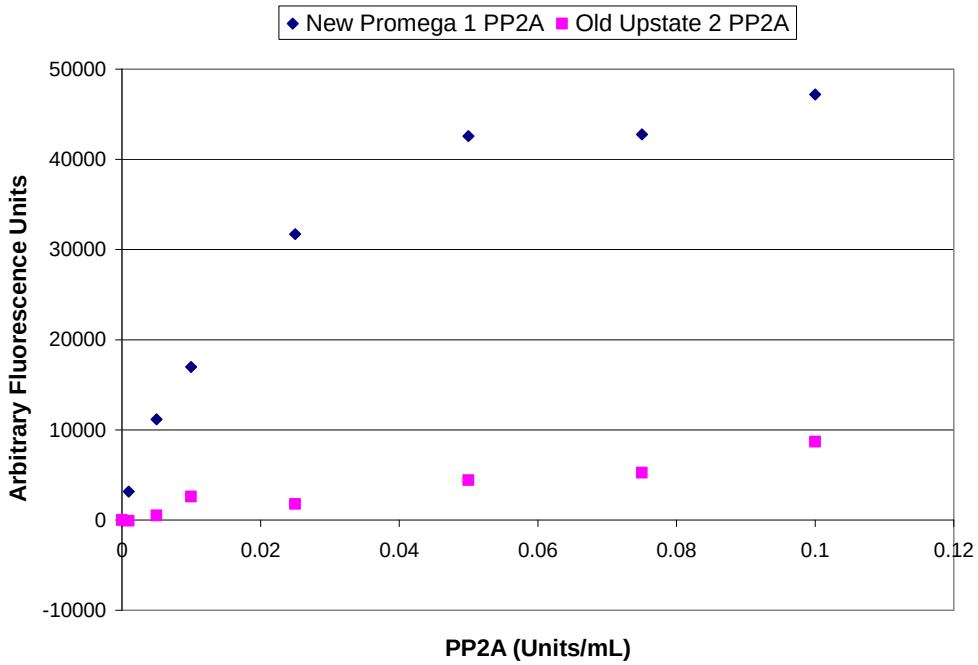


Figure 2.4. Comparison of newly purchased Promega 1 PP2A and older, relatively inactive Upstate 2 PP2A (Table 2.2) performed on May 29, 2006.

The actual problem, which was later identified by Dr. Kirsten Muller, was inappropriate storage of the PP2A enzyme. Although the enzyme was housed in a -20^{o}C freezer as per package instructions, the freezer was frost-free and so underwent cycles of thawing during which the temperature was raised sufficiently to inactivate the enzyme. After this realization, the enzyme was stored in a freezer with constant -20^{o}C temperature within a lab-top cooler that maintained the enzyme at -20^{o}C when it was removed from the freezer for brief use. The main assay problem had been solved.

2.2.3 Enzyme Supply

Enzyme supply interruptions caused further complications even after the enzyme storage issues were resolved. We were unable to immediately re-order Promega PP2A when it was needed as the

company was having supply problems and forecasted a year-long delay. Although we had yet to be successful with the Upstate enzyme, it was the only other North American supplier and so it had to be resorted to. Upon receipt of the new enzyme it was aliquoted into small portions in separate microcentrifuge tubes so that each volume would only have to be thawed twice. Once the assay was working well with the Upstate enzyme, Upstate's supply was interrupted for one year. This problem was disconcerting due to the possibility that supply interruptions could converge and PP2A would not be available commercially in North America. PP2A can be isolated from animal tissues by researchers (Heresztyn and Nicholson 2001), however this is not desirable or feasible in our lab. Both Upstate and Promega PP2A are isolated from human red blood cells and the reasons for supplier delay are unknown. To date, seven batches of enzyme have been received from these two companies (Table 2.2). When switching to a new batch of enzyme, initial optimization of the assay is required to account for varying levels of enzyme activity.

Table 2.2. PP2A enzyme batches received from Upstate and Promega suppliers over the course of the study.

Enzyme Batch	Approximate Date Received
Upstate 1	July 2005
Upstate 2	April 3, 2006
Promega 1	May 29, 2006
Upstate 3	September 1, 2006
Promega 2	October 2006
Upstate 4	February 2007
Promega 3	July 12, 2007

2.2.4 PP2A Level Optimization

Both Upstate and Promega state the number of enzyme units supplied per µL, where 10 units of enzyme are approximately equivalent to 5 µg of protein, and where 1 unit of PP2A should liberate 1

nmol of phosphate from p-NPP per minute at 30^{o}C. However, the enzyme's actual activity did not always match its reported activity. Bouaicha et al. (2002)'s PP2A level in the enzyme solution used in the assay was 200 mU/mL and this study is referred to as '1x PP2A'. After difficulty in duplicating the curve in Bouaicha et al. (2002), different enzyme concentrations were tried. For simplicity, these concentrations were called 0.18x, 3x, etc. with reference to the level used in Bouaicha et al. (2002) and assuming the enzyme concentration stated for that batch was valid. An example of one such enzyme level trial is represented in Figure 2.5. It can be seen that, with 3x PP2A 0.5 µg/L and 1 µg/L microcystin can be differentiated between, however 0-0.1 µg/L cannot. It appeared that autofluorescence of the additional enzyme masked subtle differences between these low standards. On the contrary, the 1x PP2A showed better sensitivity to differences between lower microcystin concentration but cannot reliably distinguish between 0.5 and 1 µg/L. Heresztyn and Nicholson (2001) noted that assay sensitivity increases as enzyme concentration decreases, but also that precision can be compromised by enzyme concentrations that are too low. Carmichael and An (1999) noted the plasticity of the PPIA standard curve in that the linear section of the curve used for quantification may be shifted by changing the amount of protein phosphatase enzyme.

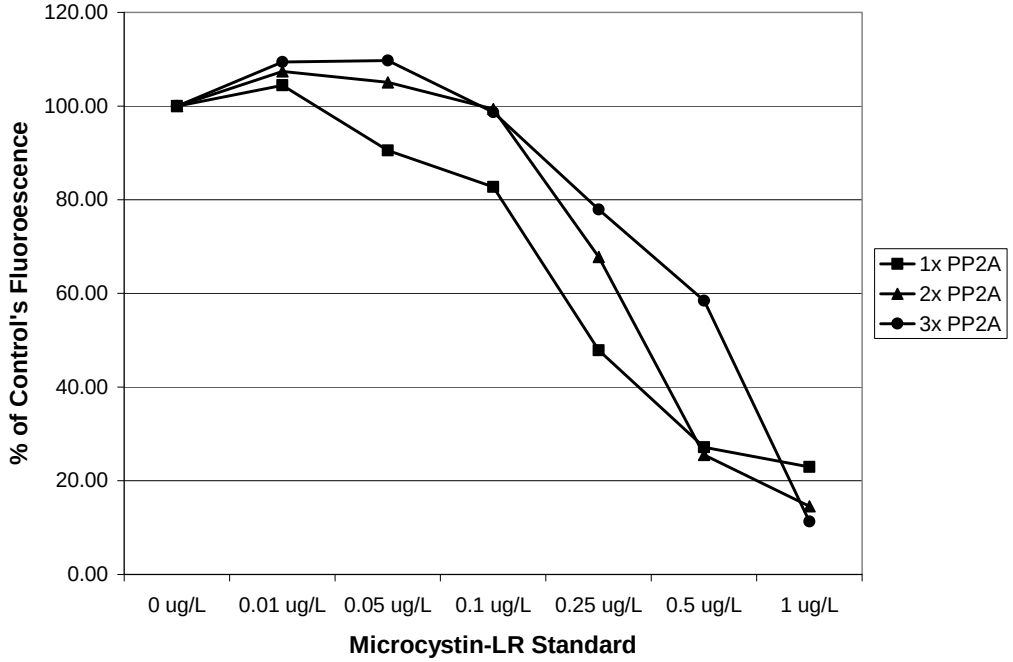


Figure 2.5. September 5, 2006 comparison of three enzyme concentrations using the Upstate 3 enzyme batch (Table 2.2).

The standard curves that I produced were more like the curve shown in Heresztyn and Nicholson (2001) (Figure 2.6) than that in Bouaicha et al. (2002) (Figure 2.1). The linear portion of Heresztyn and Nicholson (2001)'s curve occurs between 80% and 20% of control, which makes the method detection limit 0.2 µg/L microcystin. Many of the samples in this study contained less than 0.2 µg/L microcystin, so this detection limit would not have yielded sufficiently low results. The solution to this problem was to reduce the concentration of enzyme in order to shift the linear portion of the curve to the left thereby detecting the differences between lower microcystin concentrations. In order to achieve sufficient resolution for samples containing 0.05 µg/L, so little enzyme had to be used that there was not enough to tease apart higher samples, a complication which can be seen in Figure 2.7A. However, good resolution of samples between 0.05 and 0.1 µg/L was obtained through

interpolation (Figure 2.7B). This led us to develop multiple standard curves with different enzyme concentrations in order to maximize resolution along different areas of the standard curve. The curve used for sample concentrations between 0.1 and 0.25 µg/L microcystin is illustrated in Figure 2.8. Although earlier in sample testing some curves with higher enzyme concentrations were used for samples with more than 0.25 µg/L, concern over enzyme supply encouraged more frugal use. Samples with higher microcystin levels were then diluted to within 0.1-0.25 µg/L. Any samples that tested near 0.1 µg/L were tested on both curves to ensure a valid reading. Samples that approached 0.25 µg/L were diluted in case they were actually over this limit. This precaution proved to be necessary based on multiple testings of each sample.

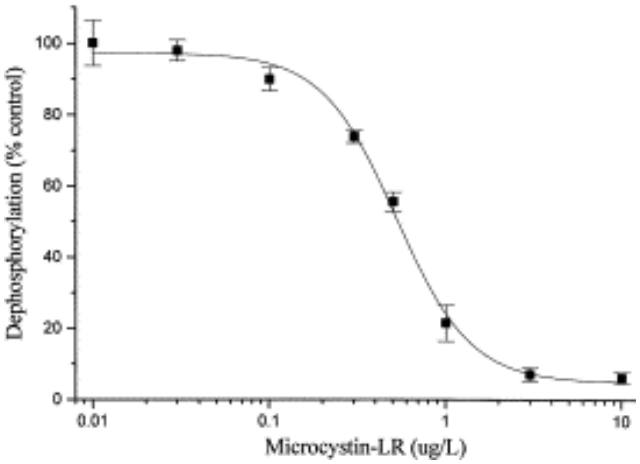


Figure 2.6. "Inhibition curve for microcystin-LR standards analysed in four replicates in high-purity water with error bars representing standard deviation." Heresztyn and Nicholson (2001). Copyright Elsevier, reproduced with permission.

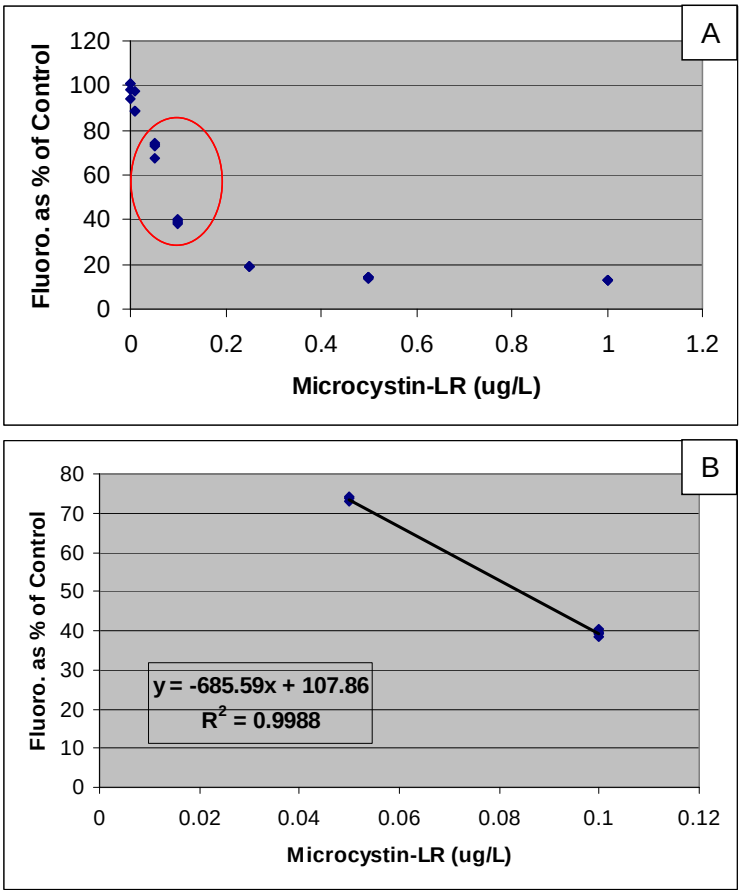


Figure 2.7. A standard curve used to isolate 'low' samples between 0.05 and 0.1 µg/L microcystin performed on July 12, 2007. Upstate 4 enzyme (Table 2.2) was used at a concentration of 0.18x. Graph 'A' shows all of the standards and that the greatest resolution occurred between the desired 0.05 and 0.1 µg/L. Standards lower and higher than those, respectively, could not be differentiated from each other. Graph 'B' shows the interpolation between 0.05 and 0.1 that was used for quantification of samples.

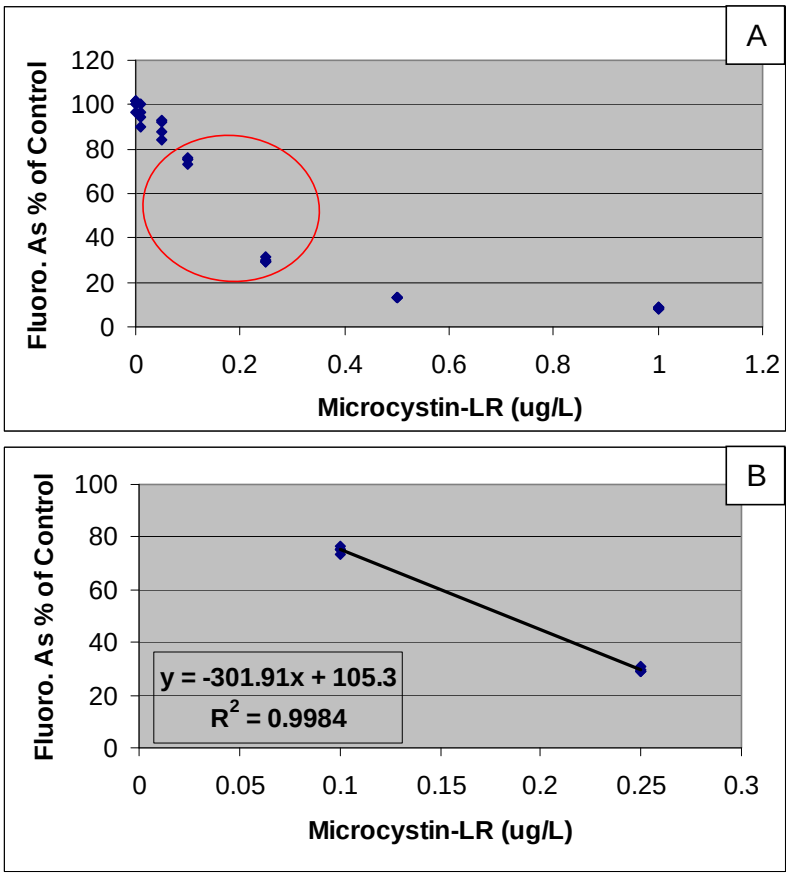


Figure 2.8. An example of a standard curve from March 30, 2007 that employed Upstate 4 enzyme (Table 2.2) at a concentration of 0.4x. This curve was used to test samples between the 0.1 and 0.25 µg/L range. Graph 'A' shows all of the standards and the obvious magnification of the 0.1-0.25 area of the curve. Graph 'B' shows the result of interpolation between those points that was used for sample quantification.

2.3 Method Validation with *Microcystis* Cultures

Table 2.3 lists the results of PPIA testing of *Microcystis aeruginosa* UTCC 299 cultures grown under different P conditions. This testing was undertaken to confirm that the method was valid and could detect expected differences in toxin concentrations between cultures. All cultures were grown under the same light conditions and in either P-limited or P-replete BG-11 media (Rippka et al. 1979). As

can be seen in Table 2.3, cultures grown in P-replete media had the highest microcystin concentrations. Dilutions were required on all samples with over 0.25 µg/L microcystin. These dilutions performed over multiple assays produced consistent results and validated the use of this technique. For example, when the replicates of P-limited total microcystin samples were diluted, read, and multiplied up, similar results were of 3.86 µg/L and 4.04 µg/L were obtained (Table 2.3)

Table 2.3. Results of testing of *Microcystis* cultures maintained by Cindy Wang under different P conditions. Non-microcystin results used with permission. 'Lim.' = limited, 'Rep.'= replete, 'Diss.'= dissolved, and 'Mcyst'= microcystin.

Code	A	B	C	D	P-Lim. 1	P-Lim. 2	P-Rep. 1	P-Rep. 2
Date	18-May-07	18-May-07	18-May-07	18-May-07	28-Jun-07	28-Jun-07	28-Jun-07	28-Jun-07
Days into Growth	25	25			65	65	49	49
Light (uE/m/s)	50	50	50	50	50	50	50	50
P (umol/L)	2 (Lim.)	2 (Lim.)	172 (Rep.)	172 (Rep.)	2 (Lim.)	2 (Lim.)	172 (Rep.)	172 (Rep.)
Chl *a* (µg/L)	60.43	60.43	204.46	1455.86	1.43	1.43	2384.98	2384.98
Part P (umol/L)	1.97	1.97			0.56	0.56		
PAM Yield	0.17	0.17			0.32	0.32		
Total Mcyst (µg/L)	150.89	172.94	168.80	410.58	3.86	4.04	777.59	709.20
N (Total)	4	4	4	3	4	4	4	4
Total Mcyst/Chl *a*	2.50	2.86	0.83	0.28	2.70	2.83	0.33	0.30
Diss. Mcyst (µg/L)	3.71	2.26	12.69	145.48	0.85		300.53	
N (Dissolved)	4	4	4	4	3		4	
Diss./ Chl *a*	0.06	0.04	0.06	0.10	0.59		0.13	
% Diss.	2.46	1.31	7.52	35.43	21.91		38.65	

2.4 Final PPIA Formulation

2.4.1 Preparation of Buffers, Enzyme, and Substrate Solutions

The buffer and substrate concentrations used in our assay are exactly as detailed in Bouaicha et al. (2002) and can be found in Table 2.4 It is important to add the dithiothreitol (DTT) to the buffer shortly preceding each assay as the half-life of DTT with EDTA at room temperature is only 4 hours. A PP2A aliquot was transported on ice from the -20^{o}C freezer to the assay benchtop, added to the 1X

buffer with BSA, and immediately used in the assay to prevent as much enzyme inactivation due to warm temperatures as possible. Both the powdered and dissolved forms of the MUP substrate were protected from light as per product insert instructions.

Table 2.4. Details of preparation of assay solutions described in Bouaicha et al. (2002).

Assay Component	Constituents	Others Details
5x reaction buffer	200 mM Tris-HCl 170 mM $MgCl_2$ 20 mM EDTA 20 mM DTT in Milli-Q water	-pH raised to 8.3 -autoclaved then stored at room temperature -DTT preweighed in sterile tubes for quick addition of buffer immediately before use
1x buffer	Dilution of 5x buffer in sterile Milli-Q water 40 mM Tris-HCl 34 mM $MgCl_2$ 4 mM EDTA 4 mM DTT	
Enzyme solution	-1x buffer supplemented with 0.5 mg/mL BSA and kept on ice -PP2A added to chilled buffer and used immediately	-10 mg/mL BSA was prepared in Milli-Q water, filter sterilized through a 0.2 µm syringe filter, and frozen in aliquots for assay use
MUP solution	-prepared in 1x buffer to concentration of 720 µM	-MUP was preweighed in sterile tubes for quick addition of buffer immediately before use -MUP was protected from light

2.4.2 Preparation of Microcystin-LR Standards

The microcystin-LR standard in methanol (10 µg/mL) was provided in a glass vial with a rubber top and so a Hamilton Microliter™ glass syringe inserted in the top to extract standard which was then diluted for use in the assay. Typically 0.1 µL was extracted with the syringe and diluted to 10 mL with sterile Milli-Q water in a glass volumetric flask. Standards used in the assay were prepared in autoclaved glass cuvettes. These standards were 1.00 µg/L, 0.50 µg/L, 0.25 µg/L, 0.10 µg/L, 0.05

µg/L, 0.01 µg/L, and 0.00 µg/L. Before delivering microcystin solution with a pipette tip, the solution was aspirated, ejected, and discarded three times as recommended in Hyenstrand et al. (2001a). This theoretically saturates all microcystin sorption sites on the polypropylene to ensure that the microcystin solution, when delivered, contains the intended amount of toxin (Hyenstrand et al. 2001). After use, the Hamilton syringe was rinsed 3 times with methanol and stored disassembled to allow the methanol to evaporate.

2.4.3 Assay Step Sequence

During all steps of the assay and reagent preparation pipette tips were pre-coated to ensure that the volume dispensed was as accurate as possible. The microcystin standards were prepared first, then the 5x and 1x buffers were mixed in centrifuge tubes. The assays were performed in new black 96-well microplates. If enough samples were being run to warrant the use of multiple plates, standards were only placed on one of the plates. Each standard or sample was given four replicate wells. The first component added to the wells was 200 µL of standard or sample, which were delivered with a single-channel pipette. The positive control consisted of the 0 µg/L microcystin standard and the negative control contained 225 µL of Milli-Q water, no enzyme solution, but regular volumes of buffer and substrate solution. A volume of 50 µL of 5x buffer was added to each well with a multi-channel pipette through the use of a disposable reagent well. Care was taken to not touch the pipette tips to the solution or sample already in the wells.

BSA solution was then added to the 1x buffer which was placed on ice. If Upstate PP2A was being used, an enzyme aliquot in a microcentrifuge tube was transported on ice to the benchtop. Then 1x buffer was pipetted into the microcentrifuge tube, the tube was shaken, and the buffer was returned to its tube three times to dissolve the enzyme in the buffer. If Promega PP2A was being used, a quantity was pipetted from the enzyme tube to 1x buffer on ice. A volume of 25 µL of enzyme solution was then promptly delivered to each well by submerging the tips of a multi-channel

pipette into the liquid already in the wells. This was necessary as the enzyme solution was very viscous and clung to the tips otherwise. The plates being used were then covered with single-use plate sealers and placed in an incubator warmed to 37^{o}C for 5 minutes.

While the plates incubated, the MUP substrate was dissolved in 1x buffer and poured into a reagent reservoir. After the incubation period, 25 µL of substrate solution was promptly add to each well via multi-channel pipette and the wells were rapidly mixed 3-5 times by pipetting. The plate covers were reapplied and reinforced with tape to prevent evaporation during the subsequent 60 minute incubation at 37^{o}C. After 60 minutes the plates were transported to the lab of Dr. Neils Bols for reading on a SPECTRAmax GEMINI XS Dual Scanning Microplate Spectrofluorometer. The plates were mixed on the fluorometer for 2 seconds and then read on automatic cutoff mode at excitation and emission levels of 360 nm and 460 nm, respectively, after Bouaicha et al. (2002).

2.5 Data Handling

Four replicate wells were typically performed for each standard and sample. Any wells that had substantially different results were excluded from data analysis due to a probable error in pipetting. The fluorescence of a negative control, which contained buffer and enzyme only, was subtracted from all standards and samples. This negative control was chosen over a seemingly more logical choice of buffer and substrate only since PP2A appeared to quench some fluorescence and the control with just buffer and substrate often had a higher fluorescence than some of the standards. All standards and samples were then calculated as a percentage of the fluorescence of the positive control, the 0 µg/L standard. The linear portion of the standard curve was interpolated to yield the equation of the line. The percent fluorescence of each sample was then plugged into the equation and solved for the microcystin-LR equivalent concentration. Any microcystin results that were below the lower standard or above the higher standard used for the line were discarded but were used to guide

future testing of that sample. Corroborating data from at least two separate assays, which used different sets of standards, were required for each sample.

2.6 Problems and Cautionary Notes

The main problem in the application of this assay has been a lack of consistency in microcystin results for the same sample between assay runs. Each assay uses freshly prepared standards, buffers, enzyme solution, and substrate solution, but variation in the latter three components should be corrected for through the use of the negative and positive controls. It then appears that differences in the microcystin standards could be causing unacceptable variability in the results. Other than human error in reading the meniscus of the Hamilton syringe and volumetric flask used during standard preparation, it is unclear why these differences are occurring. Repeated freezing and thawing of samples as they are continually tested has been raised as a potential issue. However, if this were problematic, one would think that a common pattern in assay result variability, such as consistently decreasing or increasing values would occur, which is not the case.

The way in which this variability was dealt with was performing many assays on the same samples until two or three assays showed consistent results. This provided us with confidence that those results were a true reflection of the toxin content of the sample and not an artifact of a single assay. With all of the meticulous care that was taken in the execution of this assay it is conceivable that the problem of inconsistent readings could plague other research groups. It is, therefore, advisable that studies using PPIA publish the results of more than one assay run.

Chapter 3
Bay of Quinte and Maumee Bay

3.1 Introduction

3.1.1 Microcystin Background

Microcystin-producing cyanobacterial blooms have recently become a concern in parts of the Great Lakes, mainly due to their effects on humans and aquatic organisms. Toxic blooms are particularly worrisome when they lyse and intracellular microcystin is released. Additional information on microcystin may be found in Chapter 1 of this thesis.

Field studies have shown associations between microcystin and total P, soluble reactive P, total N, the N to P ratio, chlorophyll *a*, light, and dissolved O_2 (Billam et al. 2006, Kardinaal and Visser 2005). The literature shows much variability in these relationships, however (Kardinaal and Visser 2005). It appears that toxigenic strains generally produce the most microcystin under their optimal growth conditions, which typically include elevated nutrient concentrations (Kardinaal and Visser 2005, Sivonen and Jones 1999). However, the exact environmental variables found to best explain microcystin concentrations appear to be strain-specific (Orr and Jones 1998). It also appears that the majority of natural microcystin variability can be explained not by environmental conditions but by the relative abundance of the toxic strains present (Giani et al. 2005, Ozawa et al. 2005). The seasonal succession of cyanobacterial species and strains is most likely very important to microcystin concentrations (Billam et al. 2006, Codd et al. 2005).

3.1.2 Dreissenids in the Great Lakes

Decades of reduction in point-source P inputs to the Great Lakes have successfully lowered P levels to those that would not be expected to promote cyanobacterial blooms (Nicholls and Hopkins 1993),

yet they are occurring (ex.: Nicholls 2002). The introduction of invasive *Dreissena* spp. mussels may be partly responsible for this by promoting certain cyanobacteria. For instance, *Microcystis* colonies are sometimes so large that dreissenids cannot consume them (Vanderploeg et al. 2001) and thus *Microcystis* is able to grow while other phytoplankton are grazed down. Secondly, dreissenids may be able to differentiate between toxic and non-toxic *Microcystis* and selectively reject toxic cells as pseudofeces (Raikow et al. 2004). Thirdly, research has shown that dreissenids may indirectly promote *Microcystis* by altering the ratio of available N:P (Arnott and Vanni 1996, Bykova et al. 2006).

3.1.3 Study Sites

The Bay of Quinte (Figure 1.3) and Maumee Bay (Figure 1.1) have been sites in the Great Lakes of recent toxic cyanobacterial blooms (Bridgeman 2005, Conroy et al. 2005). Maumee Bay, which comprises the westernmost part of Lake Erie's Western Basin, and the Bay of Quinte on Lake Ontario are both relatively shallow eutrophic bodies of water (Table 1.2). Both receive nutrient inputs from agricultural lands and have had surrounding wetlands drained (Minns et al. 1986, U.S. Army Engineer District, Buffalo 1983). The high turbidity introduced into the bay by the Maumee River has been attributed with the presence of *Microcystis* blooms (Bridgeman 2005). The Bay of Quinte also exhibits elevated turbidity as its ecosystem was pushed from a clearwater state to a turbid one (Minns 1995). *Microcystis*-promoting dreissenids are present in both Maumee Bay and the Bay of Quinte (Nicholls et al. 2002, Sedmak and Kosi 2002). Problems with algal blooms in the Bay of Quinte date back to the 1930's (Minns 1995) and the presence of microcystin has been documented in both western Lake Erie (Rinta-Kanto et al. 2005) and the Bay of Quinte (Ontario Ministry of the Environment, personal communication).

3.1.4 Hypotheses

Microcystin concentrations and various biological, chemical, and physical parameters were investigated in the Bay of Quinte and Maumee Bay to better understand microcystin dynamics in those bodies of water. The hypotheses formed prior to the undertaking of this study are as follows:

1) If nutrient status affects microcystin concentrations and favourable growth conditions result in more microcystin production, then indicators of greater nutrient deficiency will be negatively associated with microcystin levels.

2) If the abundance of different cyanobacterial groups contributes to microcystin concentrations, then dominance by particular potentially toxic species will be associated with higher microcystin concentrations.

3) Greater water column stability, a low N to P ratio, higher soluble reactive P and total P, and decreased water transparency will all promote the production of microcystin and will be positively associated with microcystin concentrations.

3.2 Methods

3.2.1 Study Sites

Six stations in the Bay of Quinte, Lake Ontario were surveyed on July 4-5, 2006 and on Sept. 22, 2006. The area sampled, near Deseronto, Ontario, is circled in Figure 1.3 and information about each station may be found in Table 1.2. Three shallow stations (maximum depth 2.4 m) and three deeper stations (4.8 m to 6.4 m) were surveyed. The GPS coordinates of these stations can be found in Appendix A. Six stations were fully surveyed in Maumee Bay from Toledo, Ohio, USA on June 20, 2006 and Aug. 22, 2006 (Figure 1.2 and 3.1). A seventh station, 'Crib' was partially surveyed for

interest as it was a water intake site. Four of these stations were shallow (1.3-3.8m) and three were relatively deep (5.5-6m). The GPS coordinates of these stations can be found in Appendix B.

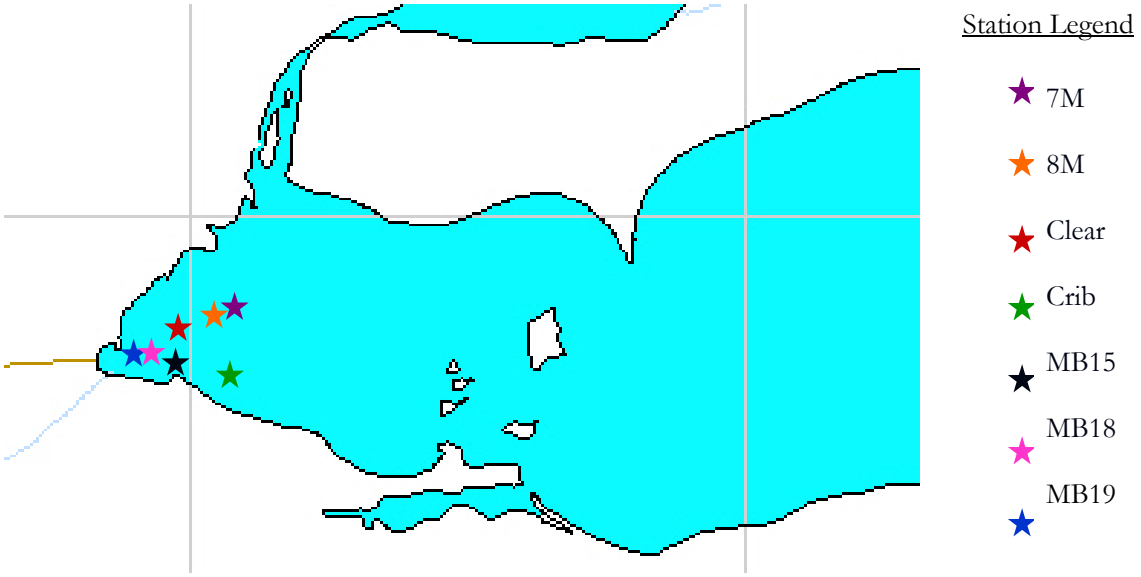


Figure 3.1. A map of western Lake Erie showing the seven stations sampled within Maumee Bay.

In 2005, the Bay of Quinte was sampled by lab associates Kim Rattan and Greg Silsbe on June 28, August 3, August 30, and October 4. Sampling took place near Napanee/Deseronto (where all of the 2006 stations were located), in Hay Bay, and in Big Bay. The latter two locations are smaller bays within the Bay of Quinte. Only results of microcystin testing will be discussed here.

3.2.2 Sampling Procedure

At each station secchi depth (an inverse measurement of water transparency: the depth at which a disk can no longer be seen from the surface) was measured and water was taken for further processing from 2, 1, or 0.5 metres depending on the station depth (see Appendix A). A Fluoroprobe was also deployed for an *in situ* measurement of total chlorophyll and the characteristic pigments of chlorophytes, cyanophytes, diatoms, and cryptophytes via fluorescence. Upon promptly returning to the lab, water was filtered with 0.7 µm glass microfibre filters (GF/F) for most analyses or with

polycarbonate filters for certain other analyses. Whole water for TN, TP, and PPIA analyses was prescreened unless phytoplankton colonies were visibly being excluded from the sample, in which case screening was not performed. Phytoplankton samples were preserved with Lugol's iodine solution. All unpreserved water and filters were frozen until analysis with the exception of the PAM filters. These were read immediately using a Walz DIVING-PAM. Variable fluorescence was measured on phytoplankton concentrated on a filter to provide a minimum fluorescence reading (F_o) of 100 relative fluorescence units that had been dark adapated for 30 minutes. Variable fluorescence is Fv/Fm where Fm is maximum fluorescence and $Fv=Fm-F_o$ (Genty et al. 1989). The maximum relative electron transport rate (ETRmax) was determined on each filter by measuring the variable fluorescence of the initially dark adapted filter at increasing light intensities. The ETR is a product of Fv/Fm and the flux of light energy absorbed by the light harvesting complex of photosystem II. Because of difficulties in quantifying the amount of energy absorbed by phytoplankton on a filter a relative ETR is often calculated instead, as the product of incident irradiance and Fv/Fm (Schulze and Caldwell 1994, Falkowski and Raven 1997).

3.2.3 Nutrient and Chlorophyll Analyses

All analyses were performed using standard operating procedures compiled by Dr. Yuri Kozlov which were based on Stainton et al. (1977) and Standard Methods for the Examination of Water and Wastewater (American Public Health Association 1992), and other references mentioned below. Total dissolved P (TDP), total P (TP), and particulate P (Part P) were measured by potassium persulfate digestion followed by the ascorbic acid method. TDP samples had been filtered through a 0.2 µm polycarbonate filter while Part P was measured on a 0.7 µm GF/F filter. SRP was also measured using the ascorbic acid method on GF/F filtrate. Particulate C and N was determined on pre-combusted GF/F filters that were packed into metal capsules and read in an Exeter CEC-440 Elemental Analyzer by David Depew after Grasshoff et al. (1983). Total N samples were digested by

alkaline oxidation, passed through cadmium reduction columns, and read on a spectrophotometer following colour generation. NH_3 samples were first run through a 0.2 µm polycarbonate filter and were measured with the OPA (orthophtaldialdehyde) method outlined in Holmes et al. (1999). Filtered samples were measured for nitrate and nitrite on an Ion Chromatograph Dionex ICS 2500. For the measurement of soluble reactive silica, unfiltered samples were acidified, colour was generated from the addition of molybdate and stannous chloride, and samples were read on a spectrophotometer. Particulate silica was collected on a polycarbonate filter, digested with sodium hydroxide, neutralized, and read as soluble reactive silica. Chlorophyll was protected from light, extracted cold from GF/F filters with acetone, and read in a Turner fluorometer.

3.2.4 Microcystin Analysis

Whole water was analyzed for total microcystin and GF/F filtrate was analyzed for dissolved microcystin. The protein phosphatase inhibition assay (PPIA) outlined in Bouaicha et al. (2002) was employed for toxin analysis except for the enzyme concentration used. For samples with 0.05-0.1 µg/L microcystin 22 mUnits of enzyme was used and for samples with 0.1-0.25 µg/L microcystin 48mUnits of enzyme was used. Samples with greater than 0.25 µg/L microcystin were diluted until they fit within these ranges. Assays were read in a SPECTRAmax GEMINI XS Dual Scanning Microplate Spectrofluorometer. In order to be confident in the microcystin data obtained from the newly established PPIA, samples were retested until multiple assay runs yielded consistent results. This applied to all samples except those from Maumee Bay in June 2006 as all of these were below the detection limit of 0.05 µg/L.

3.2.5 Data Analysis

Systat Version 9 (SPSS, 1998) was used to generate graphs and perform statistical analyses. One-way ANOVA was used with Bonferroni post-hoc tests to look for significant differences among

major variables between stations and dates within a water body. All Bay of Quinte variables were normally distributed except for Part. P, TP, NH_3, TN, TN:TP, percent dissolved microcystin, particulate microcystin per chlorophyll, and ETRmax which were all log-transformed before statistical analyses. All Maumee Bay variables were normally distributed except for TDP, extracted chlorophyll *a*, SRP, C:P, NH_3, NO_3, NO_2, and Part. N which were also log-transformed prior to analysis.

3.3 Results

3.3.1 Bay of Quinte Results

The full ANOVA results for all variables can be found in Table 3.1.

Table 3.1. All Bay of Quinte ANOVA results. Significant differences and strong trends are highlighted.

Bay of Quinte ANOVA Results				
Variable Analyzed	**Difference Tested**	**df**	***F***	***P***
Secchi	July vs. Sept.	11	50.380	<0.001
Secchi	July Deep vs. Shallow	5	2.024	0.228
Secchi	Sept. Deep vs. Shallow	5	1.000	0.374
SRP	July vs. Sept.	10	7.431	<0.05
SRP	July Deep vs. Shallow	5	5.600	0.077
SRP	Sept. Deep vs. Shallow	4	2.976	0.183
TDP	July vs. Sept.	10	199.654	<0.001
TDP	July Deep vs. Shallow	4	7.350	0.073
TDP	Sept. Deep vs. Shallow	5	0.942	0.387
log(Part P)	July vs. Sept.	9	21.668	<0.01
log(Part P)	July Deep vs. Shallow	4	0.018	0.903
log(Part P)	Sept. Deep vs. Shallow	4	0.559	0.509
log(TP)	July vs. Sept.	10	0.512	0.492
log(TP)	July Deep vs. Shallow	4	0.102	0.771
log(TP)	Sept. Deep vs. Shallow	5	0.000	0.99
SRSi	July vs. Sept.	10	5.822	<0.05
SRSi	July Deep vs. Shallow	4	0.188	0.694
SRSi	Sept. Deep vs. Shallow	5	0.077	0.795
Part Si	July vs. Sept.	11	0.010	0.921
Part Si	July Deep vs. Shallow	5	3.173	0.149
Part Si	Sept. Deep vs. Shallow	5	4.729	0.095
log(NH3)	July vs. Sept.	11	0.085	0.777

log(NH3)	July Deep vs. Shallow	5	2.601	0.182
log(NH3)	Sept. Deep vs. Shallow	5	1.492	0.289
NO2	July vs. Sept.	11	0.703	0.421
NO2	July Deep vs. Shallow	5	1.800	0.251
NO2	Sept. Deep vs. Shallow	5	10.000	<0.05
NO3	July vs. Sept.	11	1.000	0.341
NO3	July Deep vs. Shallow	5	1.072	0.359
NO3	Sept. Deep vs. Shallow	5	1.000	0.374
TN	July vs. Sept.	11	8.232	<0.05
TN	July Deep vs. Shallow	5	1.696	0.263
TN	Sept. Deep vs. Shallow	5	0.048	0.838
log(TN:TP)	July vs. Sept.	10	2.633	0.139
log(TN:TP)	July Deep vs. Shallow	4	0.309	0.617
log(TN:TP)	Sept. Deep vs. Shallow	5	0.034	0.863
Extracted Chl a	July vs. Sept.	11	18.194	<0.01
Extracted Chl a	July Deep vs. Shallow	5	10.928	<0.05
Extracted Chl a	Sept. Deep vs. Shallow	5	0.927	0.39
Part N	July vs. Sept.	11	66.334	<0.001
Part N	July Deep vs. Shallow	5	3.894	0.12
Part N	Sept. Deep vs. Shallow	5	0.029	0.874
CN	July vs. Sept.	11	4.660	0.056
CN	July Deep vs. Shallow	5	0.047	0.839
CN	Sept. Deep vs. Shallow	5	10.562	<0.05
CP	July vs. Sept.	8	5.703	<0.05
CP	July Deep vs. Shallow	3	0.398	0.593
CP	Sept. Deep vs. Shallow	4	1.250	0.345
Diss. Microcystin	July vs. Sept.	11	45.225	<0.001
Diss. Microcystin	July Deep vs. Shallow	5	0.227	0.659
Diss. Microcystin	Sept. Deep vs. Shallow	5	3.253	0.146
Total Microcystin	July vs. Sept.	11	29.432	<0.001
Total Microcystin	July Deep vs. Shallow	5	0.198	0.679
Total Microcystin	Sept. Deep vs. Shallow	5	0.065	0.812
log(% Diss. Mcyst.)	July vs. Sept.	11	45.896	<0.001
log(% Diss. Mcyst.)	July Deep vs. Shallow	5	0.750	0.435
log(% Diss. Mcyst.)	Sept. Deep vs. Shallow	5	1.757	0.256
log(Part Mcyst./ Chl)	July vs. Sept.	11	49.439	<0.001
log(Part Mcyst./ Chl)	July Deep vs. Shallow	5	1.094	0.355
log(Part Mcyst./ Chl)	Sept. Deep vs. Shallow	5	1.169	0.341
Fv/Fm	July vs. Sept.	9	4.962	0.057
Fv/Fm	July Deep vs. Shallow	4	0.292	0.626
Fv/Fm	Sept. Deep vs. Shallow	4	0.041	0.852
log(ETRmax)	July vs. Sept.	8	8.023	<0.05
log(ETRmax)	July Deep vs. Shallow	4	1.238	0.347
log(ETRmax)	Sept. Deep vs. Shallow	3	0.734	0.482

3.3.1.1 Secchi Depth, Extracted Chlorophyll, Phosphorus, and Nitrogen

Water transparency, as measured by Secchi depth, decreased significantly ($P<0.001$) between July 4, 2006 (hereafter July) and September 22, 2006 (hereafter September) as the mean Secchi depth went from 2.0m to 1.1m (Table 3.1, Figure 3.2). Secchi depth was not significantly different between shallow and deep stations within a sampling period (Table 3.1) suggesting that phytoplankton concentrations did not vary greatly with station depth. This is conditional on the assumption that Secchi depth mainly represented phytoplankton biomass and not suspended sediments. Greater phytoplankton biomass was detected in September than in July as is evidenced by significantly higher extracted chlorophyll *a* concentrations (Figure 3.3, $P<0.01$) and significantly higher particulate P (Figure 3.4, $P<0.01$) in September. In July, extracted chlorophyll *a* was found to be significantly higher at shallow stations than deeper stations (Figure 3.5, $P<0.05$), but this was not seen in September ($P=0.39$). The mean TP was found to be quite similar on both sampling dates (Figure 3.6, $P=0.492$). SRP was low in July (mean: 3.7 µg/L) and significantly lower in September (mean: 2.4 µg/L) ($P<0.05$, Figure 3.7). An even greater decrease between July and September was observed in TDP as its mean was approximately halved over that time period (Figure 3.8), a change that was very statistically significant ($P<0.001$). In July, both SRP (Figure 3.9) and TDP (Figure 3.10) showed strong trends being higher at deep stations than at shallow stations (SRP: $P=0.77$, TDP: $P=0.073$). This was not the case in September (Table 3.1).

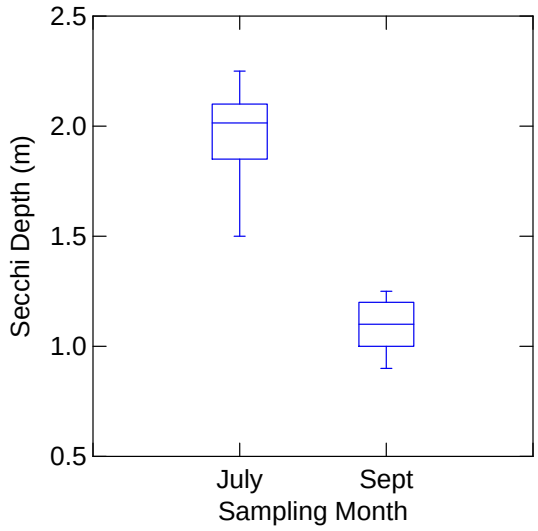


Figure 3.2. Boxplot of Secchi depth at 6 Bay of Quinte stations sampled in 2006. Variation shown within a sampling period is that between stations.

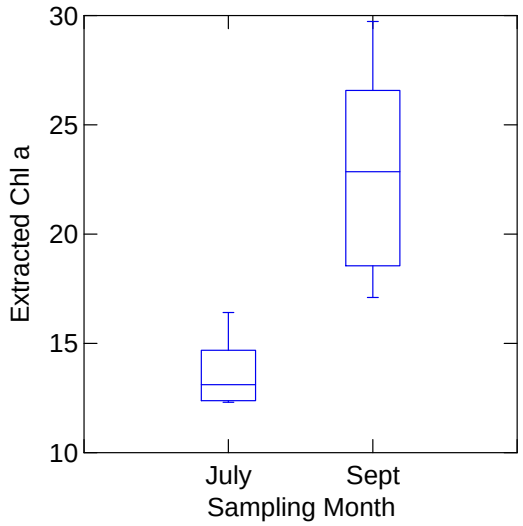


Figure 3.3. Boxplot of extracted chlorophyll *a* at six Bay of Quinte stations in 2006. The extracted chlorophyll values represent the means of duplicate extractions and readings.

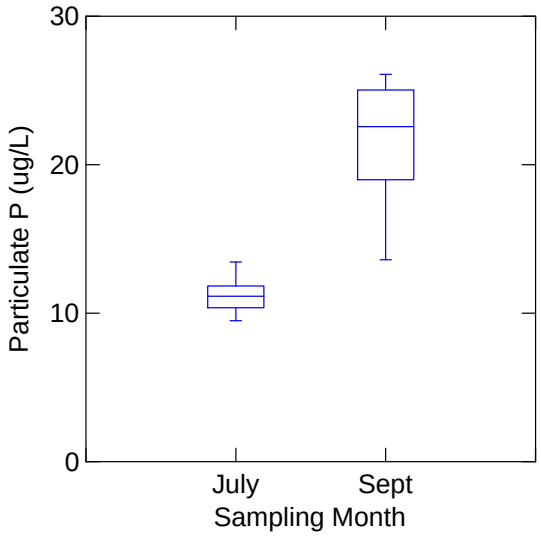


Figure 3.4. Boxplot of particulate phosphorus measured at six stations in the Bay of Quinte in 2006.

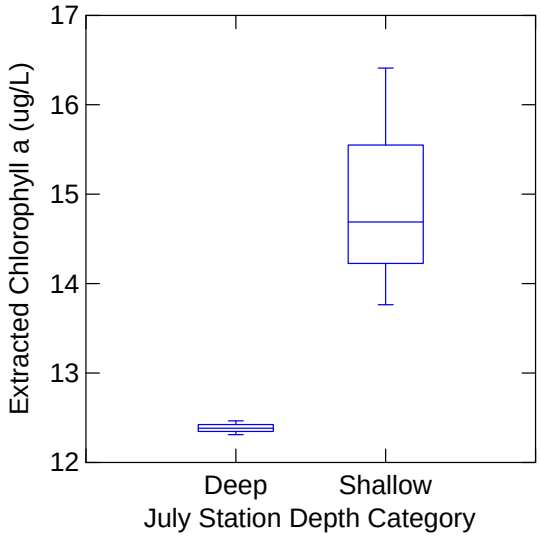


Figure 3.5. Boxplot of July 4, 2006 Bay of Quinte chlorophyll *a* levels at three deep and three shallow stations.

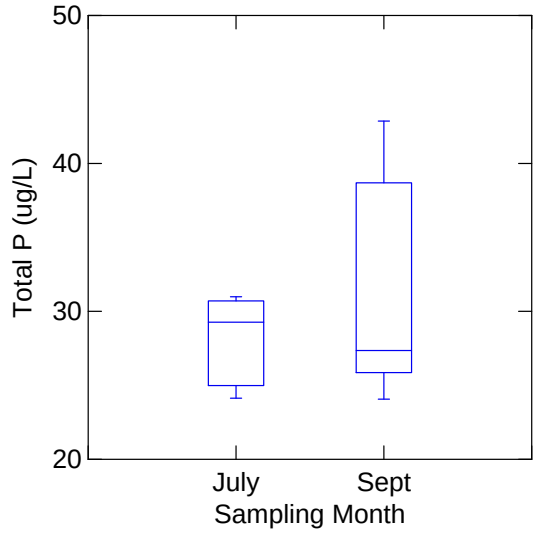


Figure 3.6. Boxplot of TP from six stations in the Bay of Quinte in 2006.

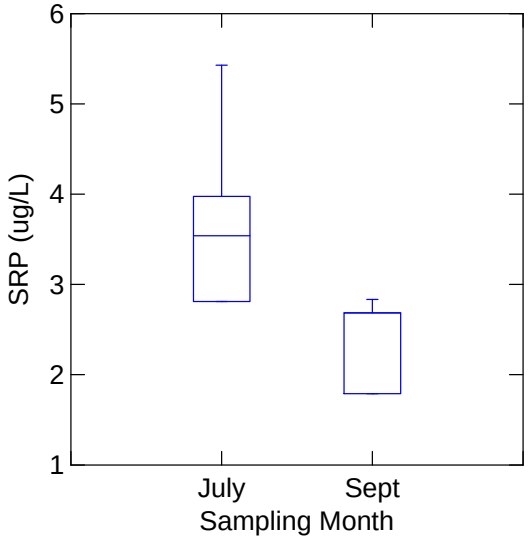


Figure 3.7. Boxplot of SRP concentration in the Bay of Quinte on July 4, 2006 and Sept. 22, 2006.

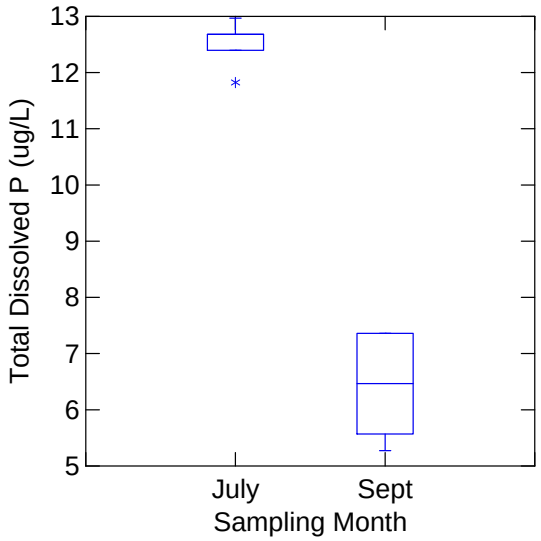


Figure 3.8. Boxplot of TDP from six Bay of Quinte stations in 2006.

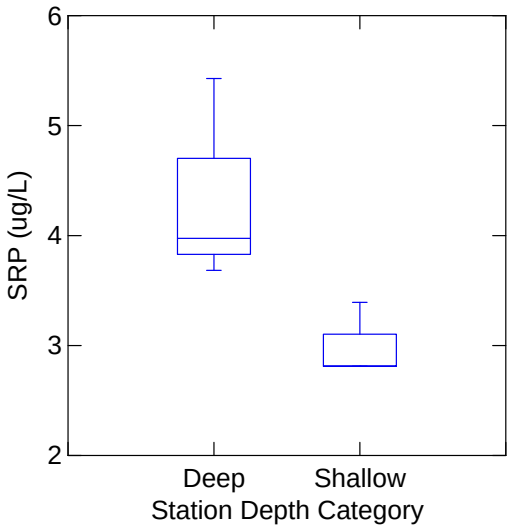


Figure 3.9. Boxplot of July 4, 2006 SRP concentrations at three deep and three shallow stations in the Bay of Quinte.

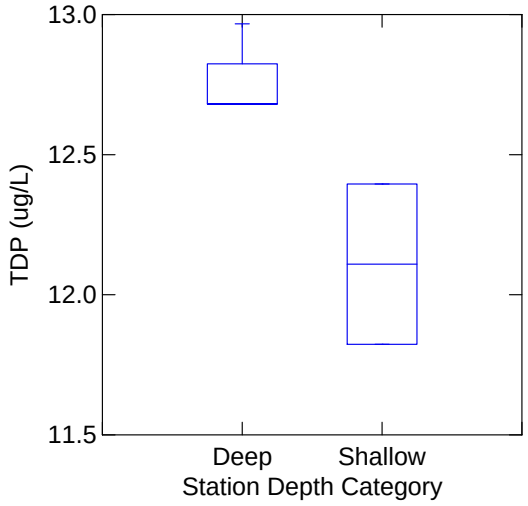


Figure 3.10. Boxplot of July 4, 2006 TDP concentrations at three deep and three shallow stations in the Bay of Quinte.

Ammonia concentrations between July and September showed no significant difference (P=0.777) (Figure 3.11). The ammonia value for station NA in September is an outlier which may have resulted from a contaminated sample and so was excluded from Figure 3.11. Nitrate concentrations in July were much lower than ammonia values for all sites with the exception of DS which was located near the Deseronto Shore. This much higher outlier may be the result of nitrate-rich runoff from, for example, fertilizer application. In September, nitrate concentrations at all stations were below the readable limit of 3.0 µg/L except for station MBO (Figure 3.12). As in July, the nitrate levels were lower than the ammonia levels at 5 of 6 stations. On both sampling dates ammonia concentrations were higher than were nitrate levels. July and September nitrate levels were not significantly different from each other (P=0.341). Nitrite levels showed no overall difference between July and September (Figure 3.13) but did show a significant difference between deep and shallow stations in September only (Figure 3.14, P<0.05).

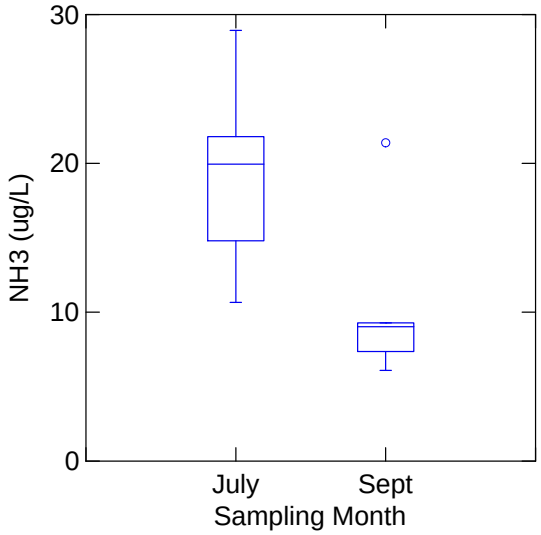


Figure 3.11. Boxplot of ammonia levels at six Bay of Quinte stations in 2006.

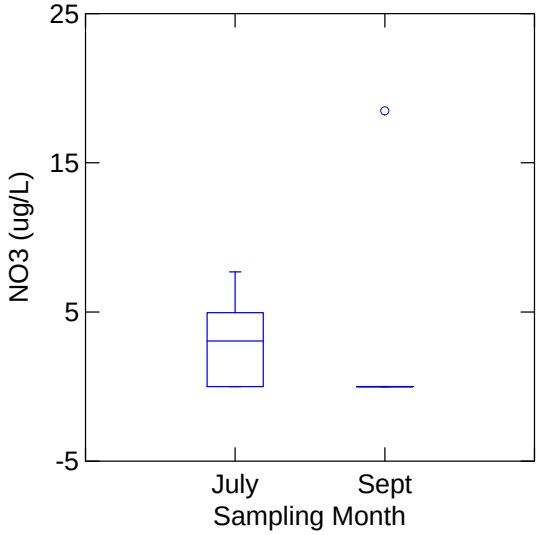


Figure 3.12. Boxplot of nitrate values for July 4, 2006 and Sept. 22, 2006 in the Bay of Quinte. The line at 0 in September represents 5 of the 6 stations which were below the detection limit.

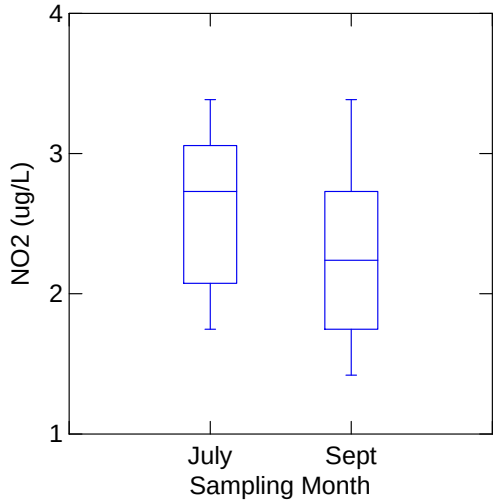


Figure 3.13. Boxplot of NO_2 concentrations at six stations in the Bay of Quinte on July 4, 2006 and Sept. 22, 2006.

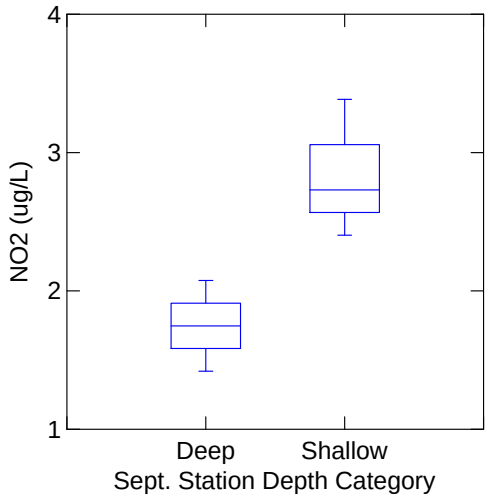


Figure 3.14. Boxplot of September 22, 2006 NO_2 at three deep and three shallow stations in the Bay of Quinte.

Particulate N was significantly higher in September than July (Figure 3.15, $P<0.001$). However, there were no trends between particulate N and depths within a sampling period (Table 3.1). Total nitrogen levels were quite high in July (mean: 1013 µg/L) and were significantly higher in

September (mean: 1460 µg/L, $P<0.05$), as can be seen in Figure 3.16. An estimate of dissolved organic N (DON) was obtained by subtracting particulate N, nitrate, nitrite, and ammonia from TN (Figure 3.16), although it should be noted that the error of these five measurements is compounded in the DON estimate. For all stations and sampling dates (except for DS July) the dissolved organic nitrogen estimate makes up more than half of TN (Appendix A). The molar TN:TP ratios were relatively high on both sampling trips (July mean: 79, September mean: 109) (Figure 3.17) but were not significantly different between months ($P=0.139$). No relationship between station depth and TN:TP was found (Table 3.1).

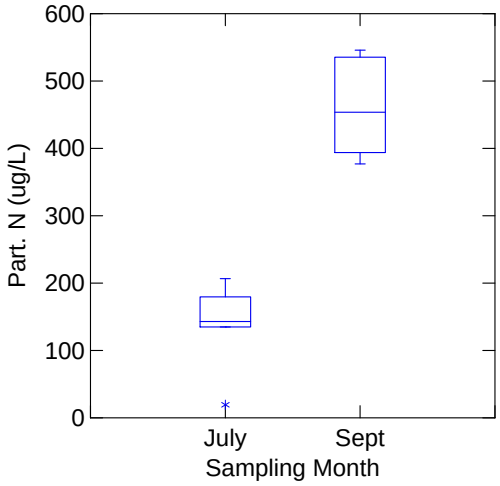


Figure 3.15. Boxplot of particulate N levels in the Bay of Quinte at six stations on July 4, 2006 and Sept. 22, 2006.

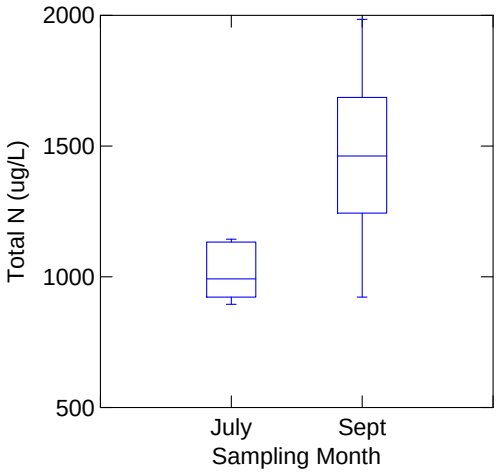


Figure 3.16. TN values for six stations in the Bay of Quinte in 2006 shown in a boxplot.

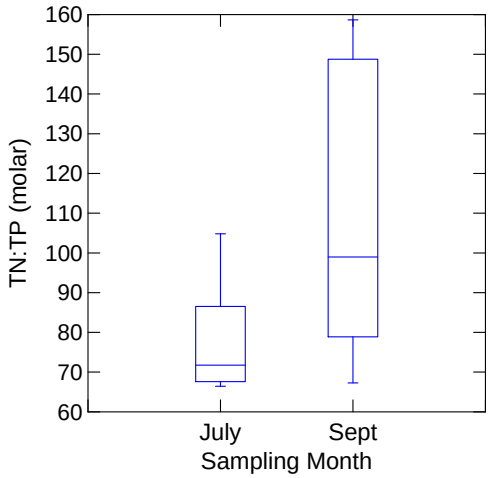


Figure 3.17. Boxplot of TN:TP (molar) from six Bay of Quinte stations in 2006.

3.3.1.2 Silica

Soluble reactive silica levels were significantly higher in July that September (P<0.05, Figure 3.18).

Particulate silica levels were not different between July and September (Figure 3.19), however a

trend with depth in September was observed (Figure 3.20). Shallow stations had higher particulate Si levels in September, although this was not statistically significant (P=0.095).

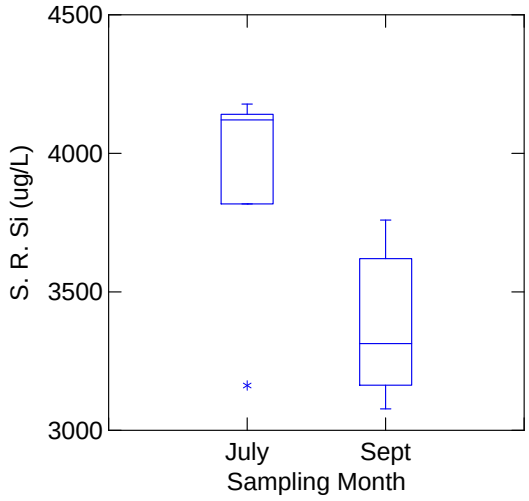


Figure 3.18. Boxplot showing soluble reactive Si concentrations at six stations in the Bay of Quinte on July 4, 2006 and Sept. 22, 2006.

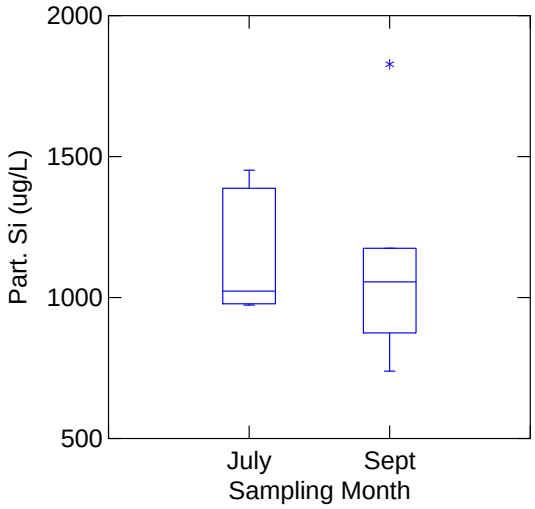


Figure 3.19. Boxplot showing particulate Si concentrations at six stations in the Bay of Quinte on July 4, 2006 and Sept. 22, 2006.

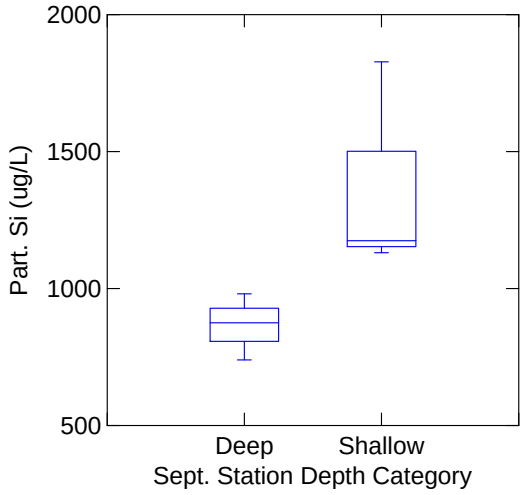


Figure 3.20. Boxplot showing particulate Si concentrations at three deep and three shallow stations in the Bay of Quinte on Sept. 22, 2006.

3.3.1.3 Nutrient Status Indicators

The C:N molar ratios of particulate matter in July were relatively low (mean: 8.1) and did not indicate N deficiency (Guildford et al. 1994). They were even lower in September (mean: 7.2) (Figure 3.21). This difference was nearly statistically significant (P=0.056). No trend between depth and C:N could be seen for July (P=0.839) but the ratios in September were significantly lower in the offshore than the nearshore (P<0.05, Figure 3.22). In July, the C:P molar ratios had a mean of 230 and in September they were significantly higher (P<0.05) with a mean of 360 (Figure 3.23). Ratios greater than 258 are indicative of severe P deficiency (Guildford et al. 1994). A trend with depth was not observed (Table 3.1). PAM results showed significantly higher ETRmax values in July than in September (P<0.05) which indicate greater photosynthetic capacity in July (Figure 3.24). The Green Point site in July was an outlier and was excluded from Figure 3.24. Dark-adapted Fv/Fm was also higher in July than in September and this difference was nearly statistically significant (P=0.057).

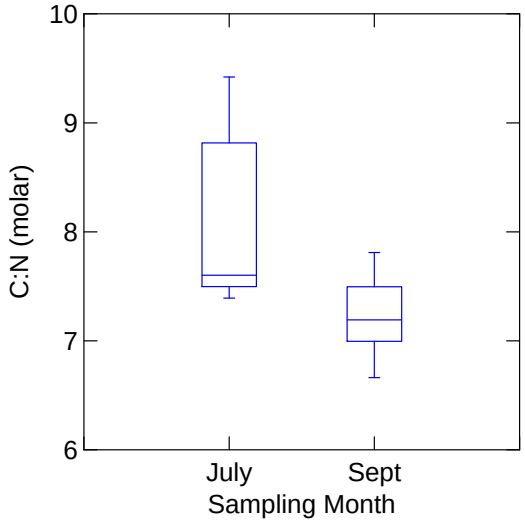


Figure 3.21. Boxplot of C:N molar ratios from six Bay of Quinte stations in 2006.

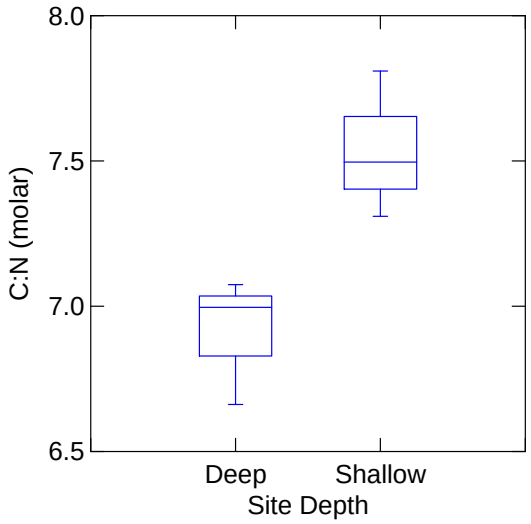


Figure 3.22. Boxplot of C to N molar ratios from the Bay of Quinte on September 22, 2006. 3 deep stations (depth range: 5.2- 6.4m) and 3 shallow stations (depth range: 1.2- 2.4m) are compared.

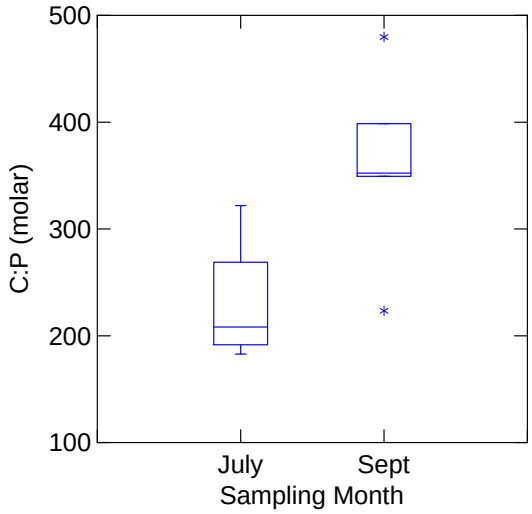


Figure 3.23. Boxplot showing C to P molar ratios for six Bay of Quinte sites in 2006.

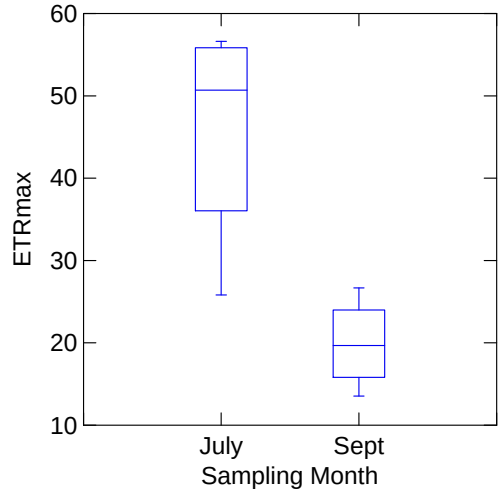


Figure 3.24. Boxplot of ETRmax measurements from the Bay of Quinte obtained via PAM fluorometry in 2006. An outlier (station GPt in Sept.) has been excluded.

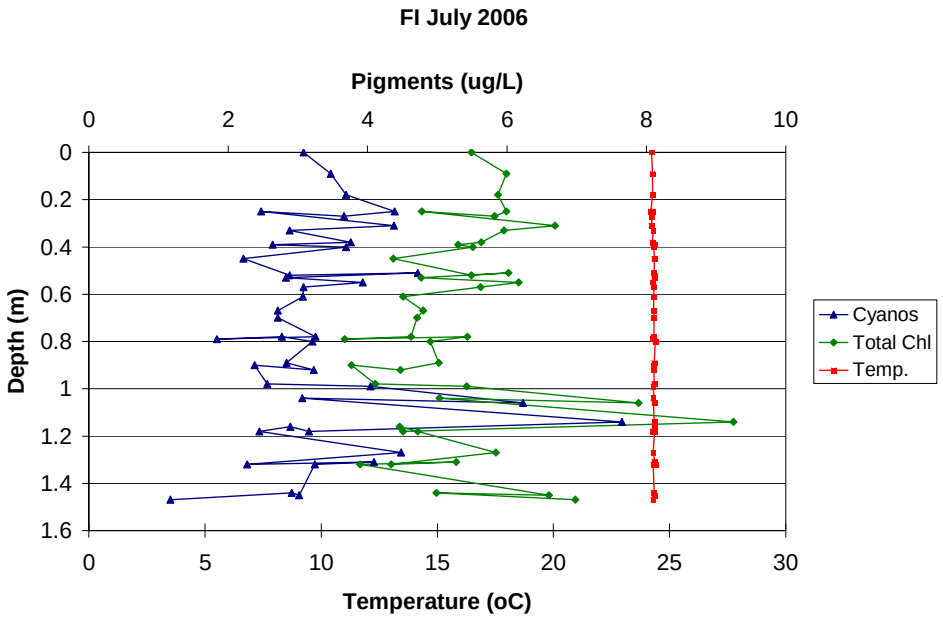
3.3.1.4 Fluoroprobe Phytoplankton Estimates

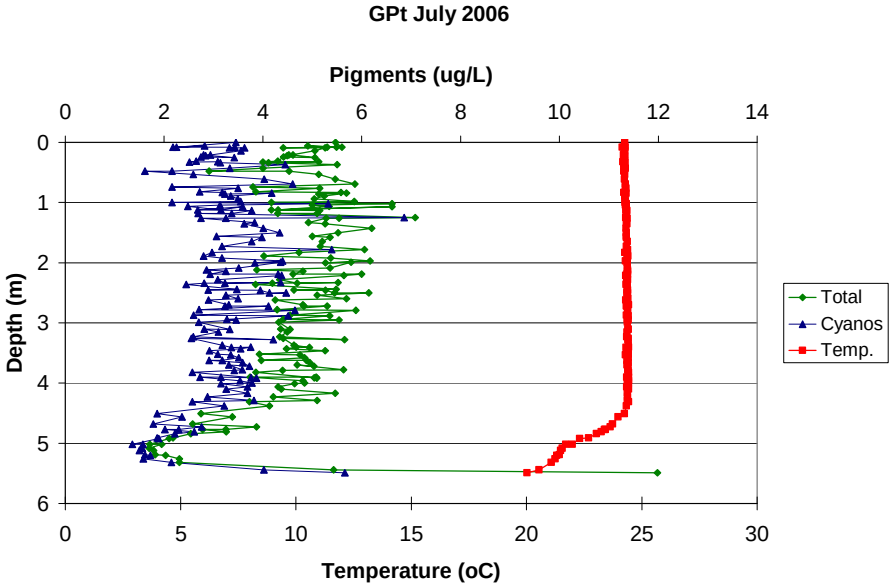
The fluoroprobe results (Figure 3.25) indicated that there were higher levels of both cyanobacterial pigments and total chlorophyll *a* in September than in July at all stations regardless of depth (Figure

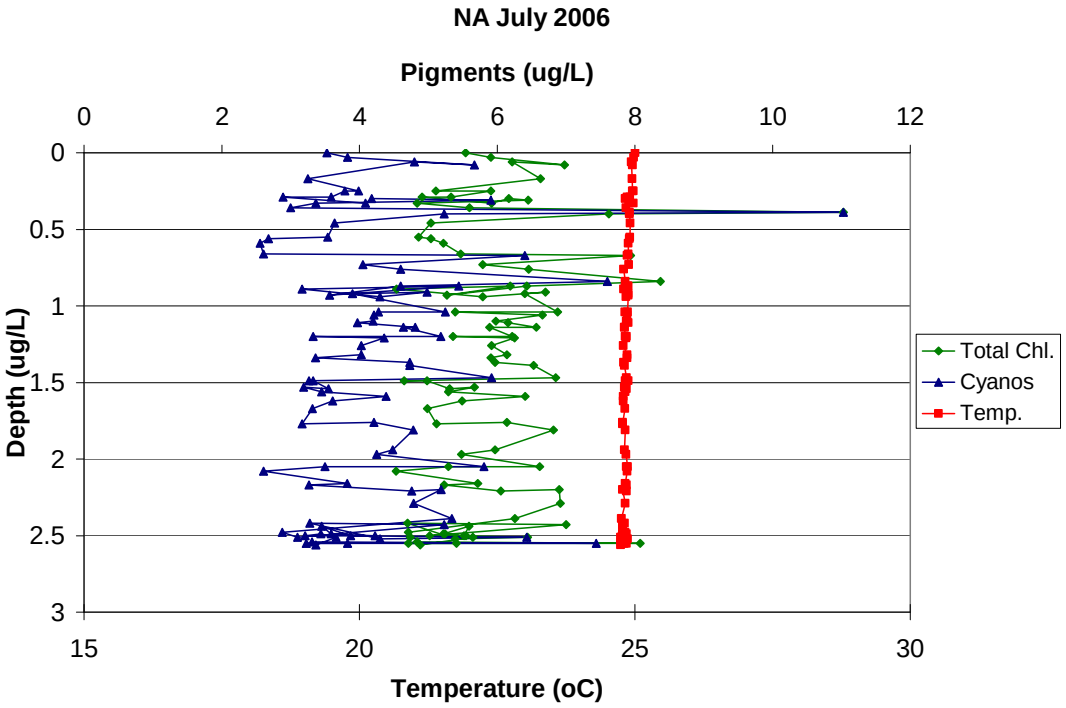
3.26). The fluoroprobe also showed that the percentage of total phytoplankton chlorophyll comprised by cyanobacteria was slightly higher in September (mean: 90.6%) than in July (mean: 83.3%).

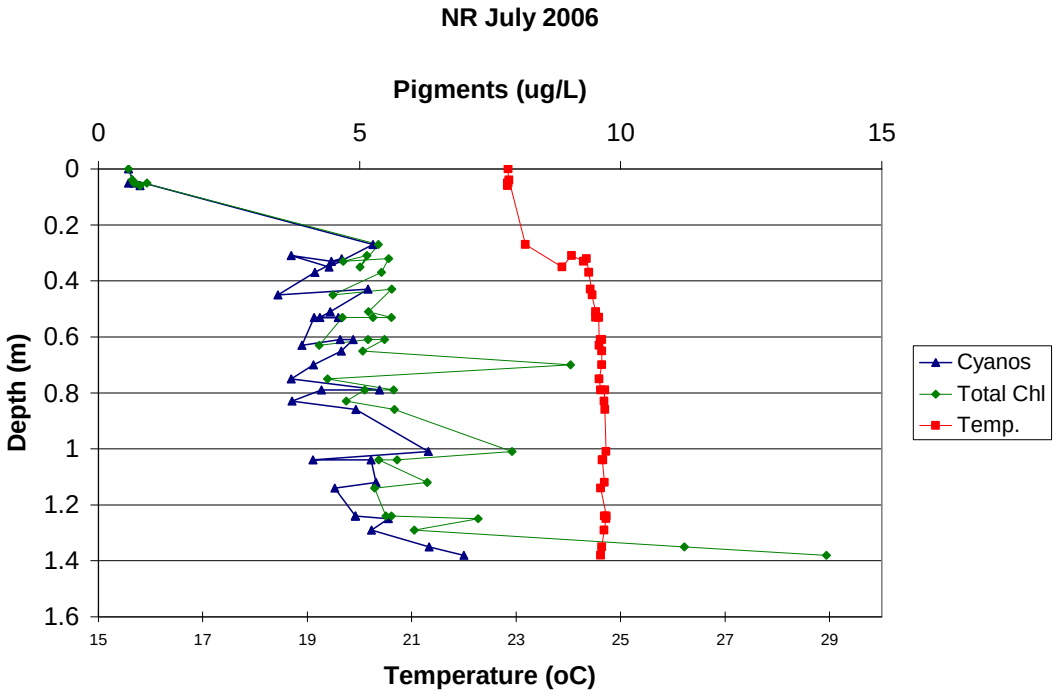
Figure 3.25. *Below* are profiles showing temperature, total chlorophyll, and cyanobacterial distributions with depth as determined by the Fluoroprobe in the Bay of Quinte in 2006.

July 2006

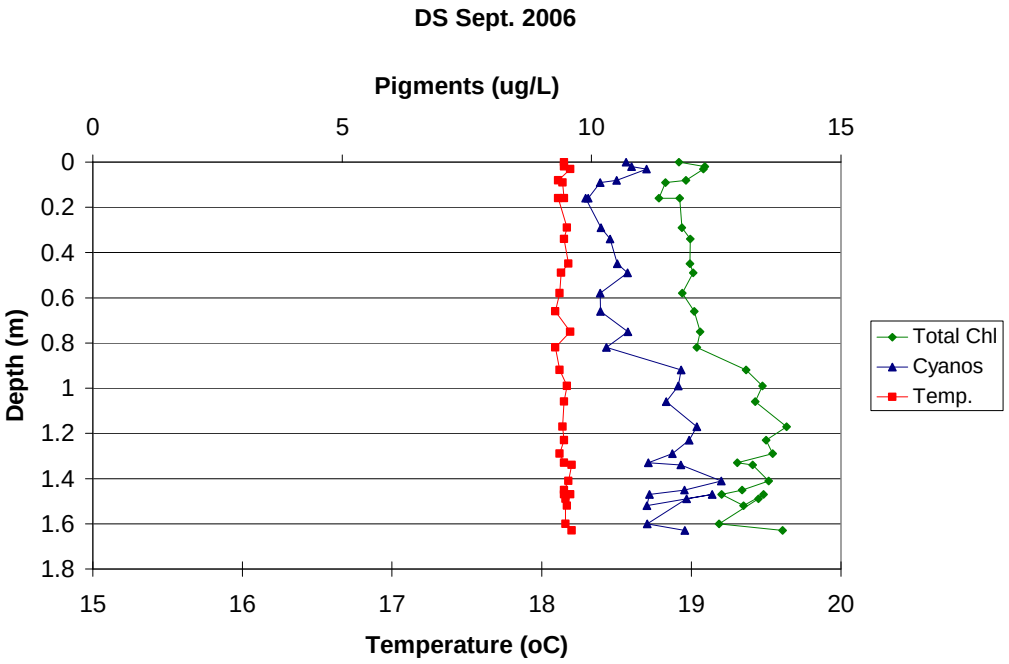


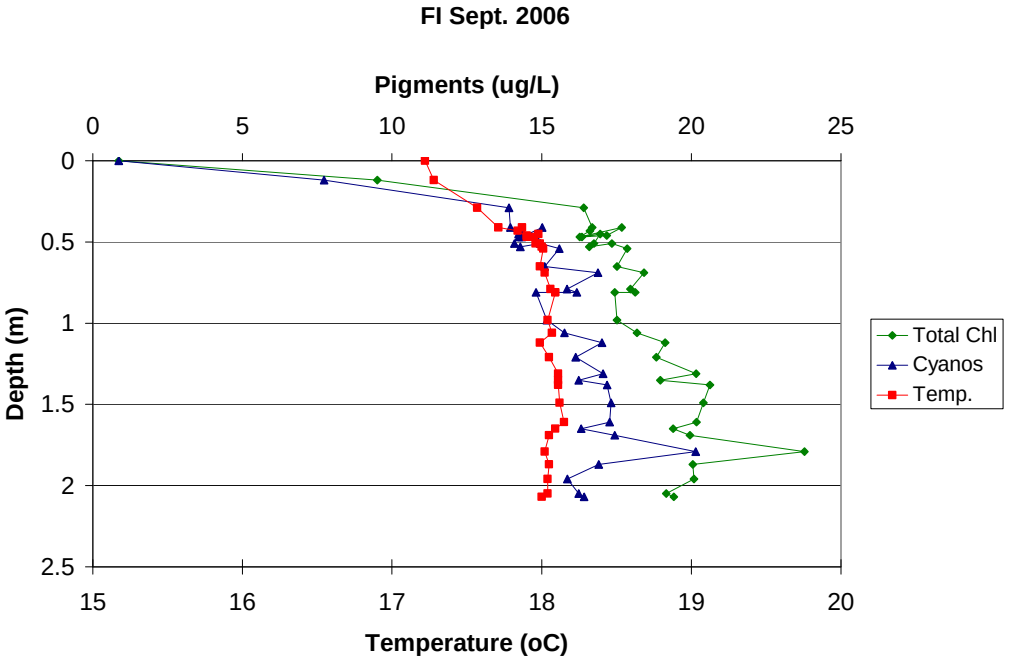


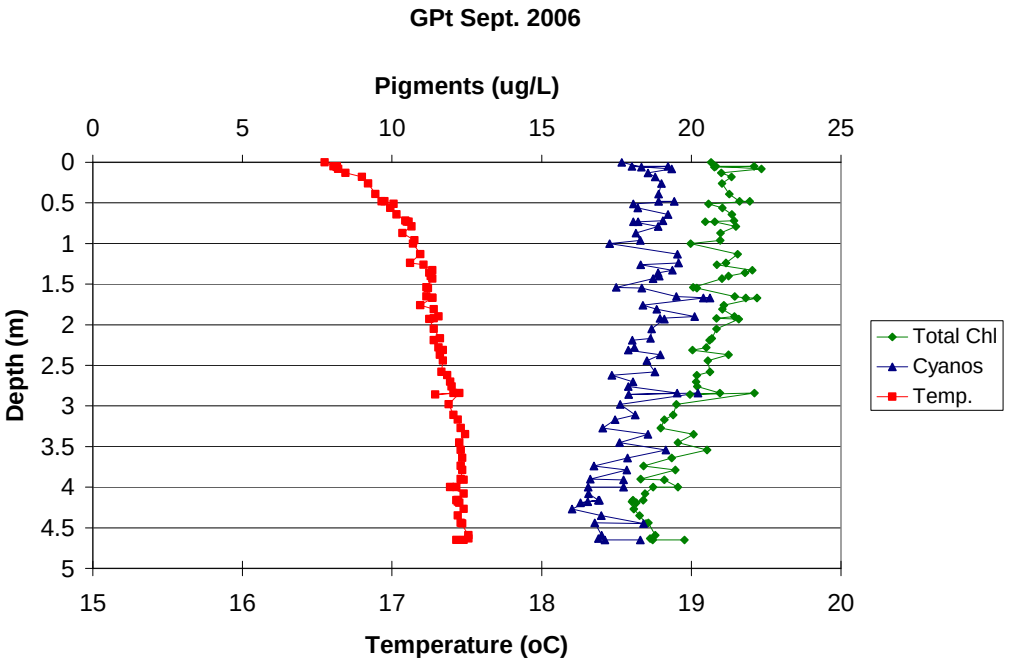

NA July 2006
Pigments (ug/L)
0
2
4
6
8
10
12
0
0.5
1
1.5
2
2.5
3
Depth (ug/L)
15
20
25
30
Temperature (oC)
Total Chl.
Cyanos
Temp.

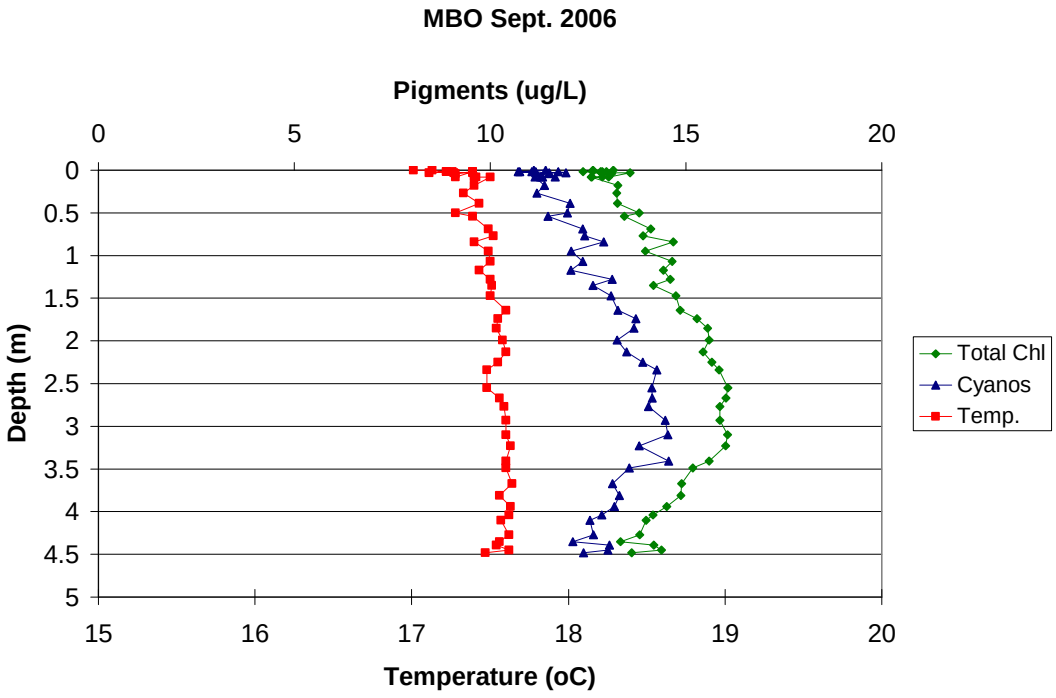

NR July 2006
Pigments (ug/L)
0
5
10
15
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Depth (m)
15
17
19
21
23
25
27
29
Temperature (oC)
Cyanos
Total Chl
Temp.

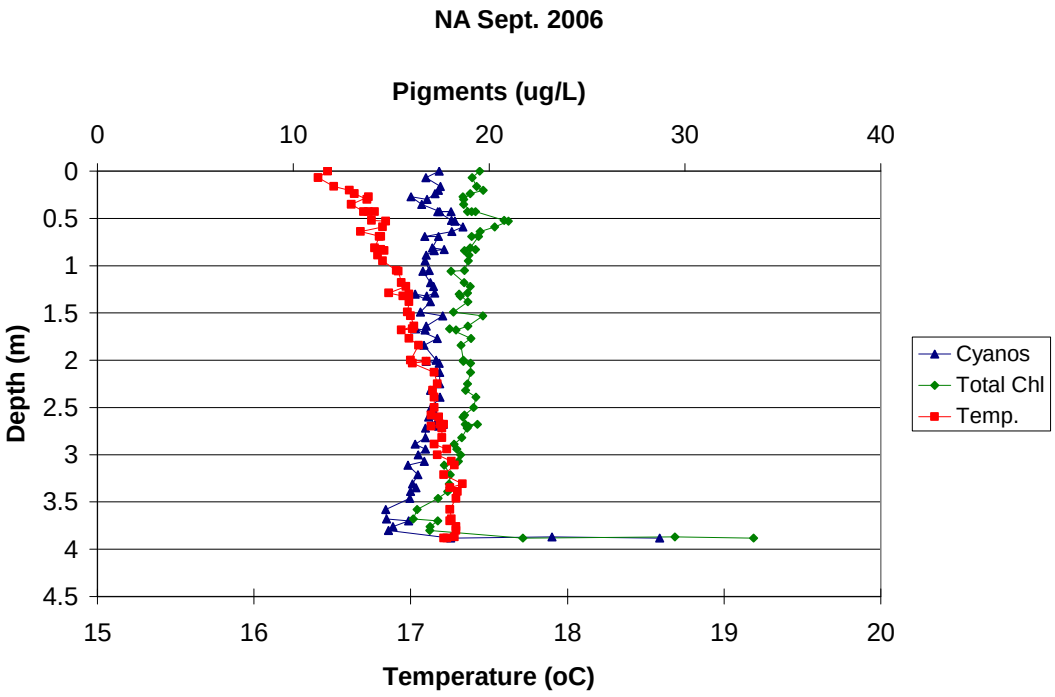
September 2006

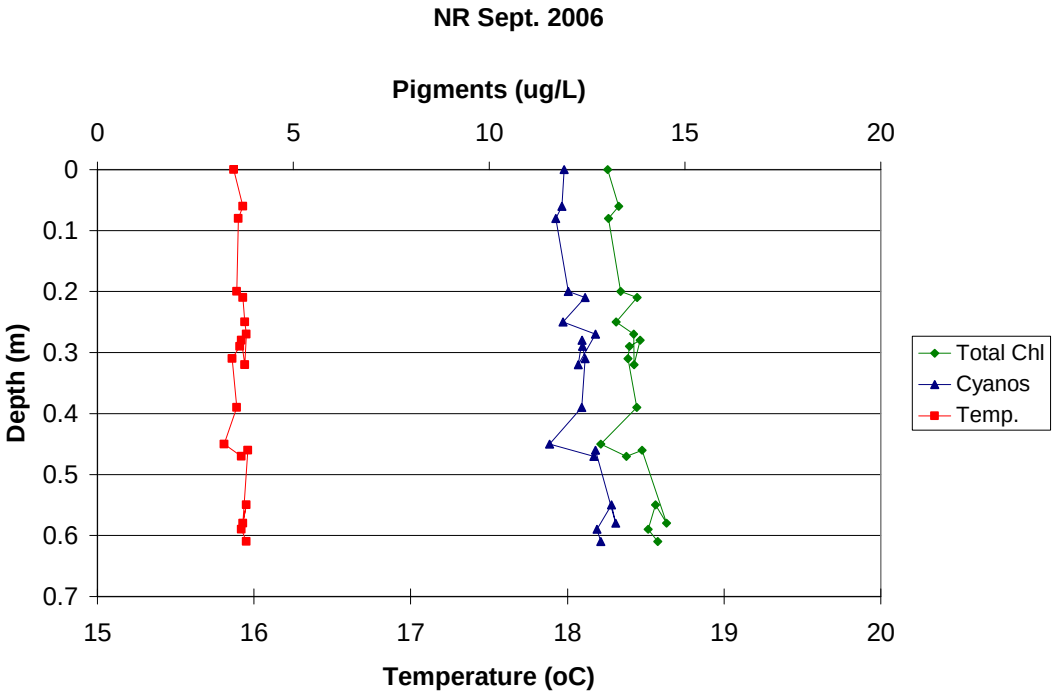





GPt Sept. 2006
Pigments (ug/L)
0
5
10
15
20
25
Depth (m)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
15
16
17
18
19
20
Temperature (oC)
Total Chl
Cyanos
Temp.


MBO Sept. 2006
Pigments (ug/L)
0
5
10
15
20
Depth (m)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
15
16
17
18
19
20
Temperature (oC)
Total Chl
Cyanos
Temp.


NA Sept. 2006
Pigments (ug/L)
0
10
20
30
40
Depth (m)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
15
16
17
18
19
20
Temperature (oC)
Cyanos
Total Chl
Temp.


NR Sept. 2006
Pigments (ug/L)
0
5
10
15
20
Depth (m)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
15
16
17
18
19
20
Temperature (oC)
Total Chl
Cyanos
Temp.

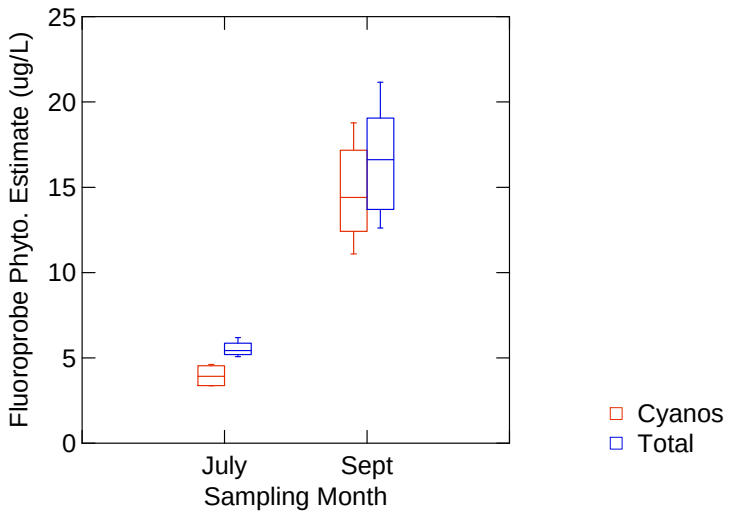


Figure 3.26. Boxplot comparing fluoroprobe results for total chlorophyll and cyanobacterial pigments from the Bay of Quinte, 2006. Each box represents six stations.

3.3.1.5 Phytoplankton Counts

The results of detailed counts to the species level on station NA (station depth: 5m) performed by phytoplankton taxonomist Hedy Kling can be found in Tables 3.2 and 3.3. In July, the phytoplankton was completely dominated by cyanobacteria as they made up 81% of the total phytoplankton biomass. Of the cyanobacterial biomass, 96.7% was composed of the genus *Microcystis* (Table 3.2). In September, cyanobacteria were responsible for even more of the total phytoplankton biomass (94%) but *Microcystis* only made up 7.9% of the cyanobacterial biomass (Table 3.3). Instead, a single species, *Anabaena spiroides*, made up 82.9% of the cyanobacterial biomass (Table 3.3). A comparison of the counts with the fluoroprobe profiles for station NA showed a similar pattern. In July the fluoroprobe estimate of cyanobacteria abundance as a percentage of total phytoplankton was 11.3% lower than the count estimate, and in September it was just 4.1% lower. Although counts were performed on samples from only one station, there is no evidence that the cyanobacterial community

varied throughout the section of the bay that was surveyed. The results of the counts on station NA station are assumed to apply to other stations since they were all in a small geographical area.

Table 3.2. Preserved phytoplankton count performed by Hedy Kling on a sample from station NA from July 4, 2006.

Bay of Quinte – July 4, 2006 – Station NA – Sampling Depth: 1m				
Phytoplankton Group	**Biomass (mg/m^3)**	**% Total Biomass**	**Cells/L**	**% of Total Cells**
Cyanophyta	1614.5	81.2	54334370	95.4
Chlorophyta	43.6	2.2	837760	1.5
Euglenophyta	0	0	0	0
Chrysophyceae	15.5	0.8	1166682	2
Haptophyta	0.3	0	29838	0.1
Diatomeae	288.3	14.5	574000	1
Cryptophyceae	11.9	0.6	11000	0
Peridineae	15.1	0.8	4000	0
Xanthophyta	0	0	0	0
========	========	========	========	========
TOTAL	1989.4		56957650	
Others	**Biomass (mg/m^3)**		**Cells/L**	
Protozoa	94.7		7000	
Rotifers	436.4		2000	
Heterocysts	0		10000	
# Heterocysts as % of Total Cyanos:				0.018
# Heterocysts as % of Producers:				1.73
Taxon	**Biomass (mg/m^3)**	**% Total Cyano Biomass**	**Cells/L**	
Microcystis sp.	**682.2**	**42.3**	30294000	
Microcystis novacekii	566.4	35.1	12775000	
Microcystis wesenbergi	155.4	9.6	2120000	
Microcystis novacekii	112.6	7.0	5000000	
Microcystis sp	43.2	2.7	745950	
Anabaena spiroides	37.4	2.3	510000	
Anabaena lemmermannii Rich.	7.8	0.5	68000	
Chroococcus minutus (Kutz) Naeg.	4.6	0.3	40000	
Microcystis smithi	2.2	0.1	96000	
Aphanocapsa minutissima	1.1	0.07	2148336	
Pseudanabaena vornichinii	1	0.06	59676	
Aphanotheca sp	0.6	0.04	477408	
All *Microcystis*	1562	96.7		
Descriptions of States of Selected Taxa				

Species	Description
Microcystis sp.	Mainly *M. aeruginosa* loose colonies
Microcystis novacekii	Bacteria and *Pseudanabaena* in mucilage
Microcystis wesenbergi	Tight colonies
Microcystis novacekii	Old tight colonies, wide mucilage w/ many bacteria, *Pseudanabaena*
Microcystis sp	Free cells
Anabaena spiroides	Some spirals
Anabaena lemmermannii Rich.	Broken colonies

Table 3.3. Phytoplankton count performed by Hedy Kling on a sample from station NA from September 22, 2006. Note: *Aphanocapsa holsatica* specifically refers to *Aphanocapsa holsatica* (Lemm) Cronb. & Kom. Data on heterocysts are not available.

Bay of Quinte – September 22, 2006 – Station NA – Sampling Depth: 2m				
Phytoplankton Group	**Biomass (mg/m^3)**	**% of Total Biomass**	**Cells/L**	**% of Total Cells/L**
Cyanophytes	15396.5	94.0	368943822	97.6
Chlorophytes	49.0	0.3	394218	0.1
Euglenophytes	0	0	0	0
Chrysophytes	93.6	0.6	6954254	1.8
Haptophytes	0	0	0	0
Bacillariophytes	704.0	4.3	262352	0.1
Cryptophytes	110.7	0.7	1553576	0.4
Dinoflagellates	27.9	0.2	32838	0
Xanthophytes	0	0	0	0
========	========	========	========	========
TOTAL	16381.7		378141060	
Others	**Biomass (mg/m^3)**		**Cells/L**	
Protozoa	133.7		45838	
Mixotrophs	11.6		268542	
Zooplankton	3455.7		2000	
Heterocysts	0		589084	
# Heterocysts as % of Total Cyanos:				0.16
# Heterocysts as % of Producers:				0.335
Taxon	**Biomass (mg/m^3)**	**% Total Cyano. Biomass**	**Cells/L**	
Anabaena spiroides	12758.2	82.9	174045054	
Microcystis viridis	1054.6	6.8	16112520	
Planktolyngbya limnetica	762.5	5.0	4953108	
Anabaena crassa	223.1	1.4	604000	
Anabaena planctonica	201.0	1.3	462704	
Microcystis novacekii	87.8	0.6	2263000	
micro bluegreens	65.0	0.4	124126080	

Microcystis wesenbergi	46.0	0.3	500000	
Aphanocapsa sp	41.7	0.3	26155048	
Aphanizomenon sp	35.1	0.2	40838	
Microcystis smithi	30.7	0.2	1790280	
Aphanizomenon issatchenkoi	28.9	0.2	59676	
Anabaena mendotae	19.5	0.1	648000	
Pseudanabaena sp	18.7	0.1	59676	
Cylindrospermopsis raciborskii	8.4	0.05	29838	
Aphanocapsa holsatica	7.7	0.05	14700000	
Aphanizomenon skujae	3.4	0.02	4000	
Aphanotheca sp	2.4	0.02	2200000	
aphanizomenon akinete	1.0	0.006	2000	
Pseudanabaena vornichinii	0.5	0.003	60000	
Coelospaerium kuetzingiana	0.5	0.003	128000	
All *Microcystis*	1219.1	7.9	20665800	

3.3.1.6 Microcystin

Total microcystin in the Bay of Quinte in July 2006 was above the World Health Organization's recommended maximum exposure level of 1 µg/L (stations had a mean of 2.25 µg/L) (Figure 3.27) and dissolved microcystin was just below 1 µg/L (mean: 0.76 µg/L) (Figure 3.28). The production and release of microcystin appears to vary substantially between July and September. In July, the mean percent dissolved was 35% but in September it was only 11% (Figure 3.29 and 3.30). All measured aspects of microcystin were statistically significantly different between July and September. Dissolved microcystin was significantly higher in July ($P<0.001$) as was total microcystin ($P<0.001$), percent dissolved microcystin ($P<0.001$), and particulate microcystin per unit chlorophyll *a* ($P<0.001$, Figure 3.31). No trends between microcystin levels and depth were evident (Table 3.1).

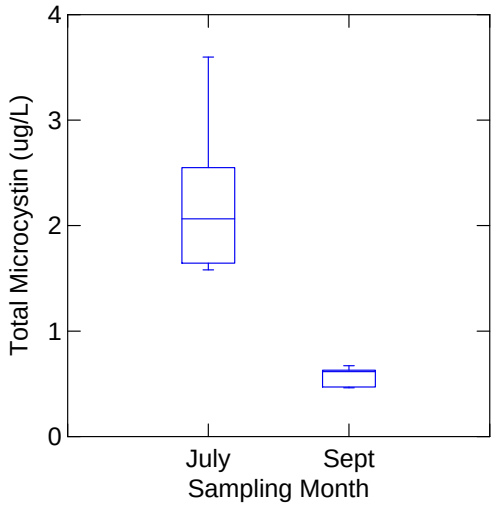


Figure 3.27. Box-plot showing total microcystin-LR equivalents (both intracellular and extracellular) for all six stations in the Bay of Quinte in 2006.

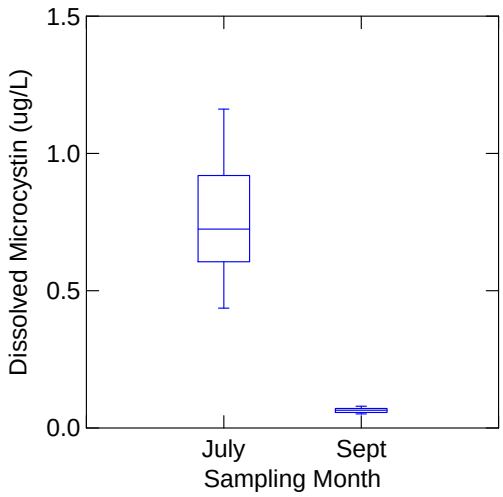


Figure 3.28. Box-plot showing dissolved microcystin-LR equivalents for six stations in the Bay of Quinte, 2006.

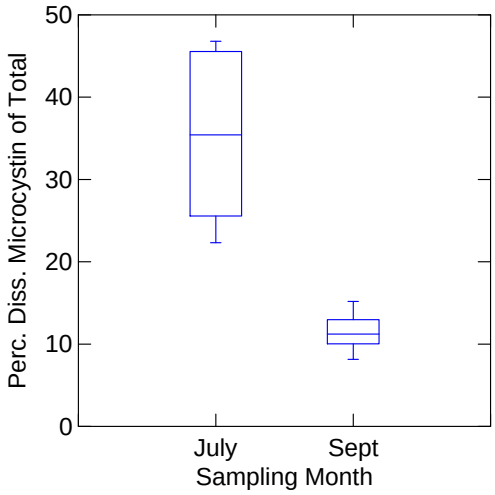


Figure 3.29. Boxplot showing the percentage of total microcystin comprised by dissolved toxin in the Bay of Quinte in 2006.

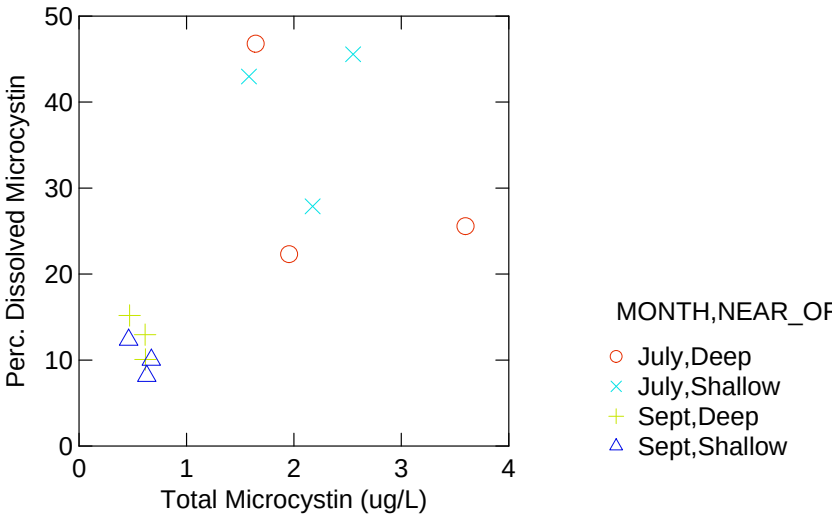


Figure 3.30. Scatterplot showing the relationship between percent dissolved microcystin and total microcystin. The deep vs. shallow and July vs. Sept. samples have been differentiated for comparison.

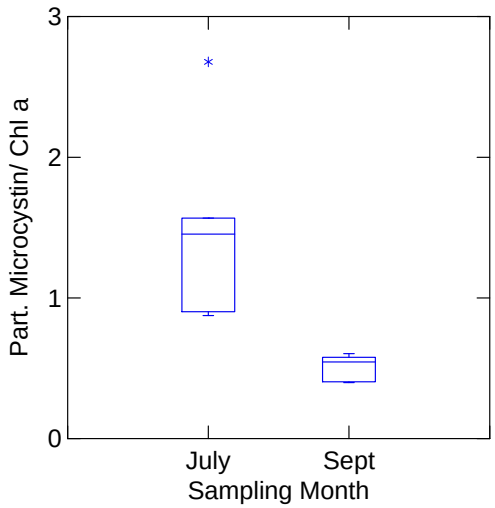


Figure 3.31. Boxplot of particulate microcystin/ chlorophyll *a* for two sampling periods in the Bay of Quinte, 2006.

3.3.2 2005 Bay of Quinte Microcystin Results

The results from microcystin testing of 2005 Bay of Quinte samples can be found in Table 3.4 along with their GPS coordinates. The results from Napanee can be best compared to those from the 2006 season since they were taken from the same approximate area. On June 28, 2005, the two Napanee stations sampled showed a range of relatively low microcystin concentrations (0.07 µg/L, 0.15 µg/L, and 0.42 µg/L). On August 3, 2005 Napanee had very low to no microcystin present (0.06 µg/L and <0.05 µg/L detected). Again on August 30, 2005, microcystin was below the detection limit of 0.05 µg/L in Napanee. By October 4, 2005, one Napanee station was still below the detection limit but another showed 0.36 µg/L microcystin. The highest microcystin level detected from the 2005 samples was in Big Bay on June 28, 2005 (0.62 µg/L).

Table 3.4. Bay of Quinte 2005 microcystin results. '# Runs' refers to the number assays from which results were averaged to yield the total microcystin number listed.

Date	Location	Samp. Depth (m)	Sample	Tot. Microcystin (ug/L)	SD	CV	# Runs
28-Jun-05	Napanee	2	Q5099	0.07	0.01	0.21	2
28-Jun-05	Napanee	2	Q5100	0.15	0.03	0.22	2
28-Jun-05	Hay Bay	4.5	Q5101	0.13	0.03	0.19	2
28-Jun-05	Big Bay	2	Q5103	0.62	0.08	0.13	2
28-Jun-05	Napanee	2	Q5104	0.42	0.25	0.59	2
03-Aug-05	Big Bay	2	Q5272	0.06	0.00	0.01	1
03-Aug-05	Napanee	2	Q5273	0.06	0.01	0.09	1
03-Aug-05	Hay Bay	2	Q5274	0.09	0.00	0.03	1
03-Aug-05	Napanee	2	Q5277	BDL			1
30-Aug-05	Big Bay	2	Q5340	0.21	0.00	0.02	1
30-Aug-05	Napanee	2	Q5341	BDL			1
04-Oct-05	Napanee	2	Q5393	BDL			2
04-Oct-05	Napanee	2	Q5395	0.36	0.03	0.08	1

Longitude & Latitude (Decimal Degrees)		
Station	Long.	Lat
Napanee	77.03993	44.18035
Hay Bay	77.07205	44.0937
Big Bay	77.25072	44.15342

3.3.3 Maumee Bay 2006 Results

The full ANOVA results for Maumee Bay can be found in Table 3.5.

Table 3.5. All Maumee Bay ANOVA results. Significant differences and strong trends are highlighted.

Maumee Bay ANOVA Results				
Variable Analyzed	**Difference Tested**	**df**	***F***	***P***
Secchi	June vs. Aug.	13	8.248	<0.05
Secchi	June Deep vs. Shallow	6	1.077	0.347
Secchi	Aug. Deep vs. Shallow	6	0.017	0.9
log(SRP)	June vs. Aug.	11	0.01	0.922
log(SRP)	June Deep vs. Shallow	5	8.271	<0.05
log(SRP)	Aug. Deep vs. Shallow	5	0	1
log(TDP)	June vs. Aug.	12	1.677	0.222
log(TDP)	June Deep vs. Shallow	6	7.276	<0.05
log(TDP)	Aug. Deep vs. Shallow	5	1.016	0.371
Part P	June vs. Aug.	12	4.361	0.061
Part P	June Deep vs. Shallow	5	0.137	0.73

Part P	Aug. Deep vs. Shallow	6	0.592	0.476
TP	June vs. Aug.	13	5.209	<0.05
TP	June Deep vs. Shallow	6	1.55	0.268
TP	Aug. Deep vs. Shallow	6	0.155	0.71
log(NH3)	Aug. Deep vs. Shallow	4	1.318	0.334
log(NO2)	June vs. Aug.	11	8.304	<0.05
log(NO2)	June Deep vs. Shallow	5	1.965	0.234
log(NO2)	Aug. Deep vs. Shallow	5	7.899	<0.05
log(NO3)	June vs. Aug.	11	11.656	<0.01
log(NO3)	June Deep vs. Shallow	5	8.738	<0.05
log(NO3)	Aug. Deep vs. Shallow	5	0.226	0.659
TN	June vs. Aug.	11	0.867	0.374
TN	June Deep vs. Shallow	4	4.28	0.13
TN	Aug. Deep vs. Shallow	6	1.115	0.339
TN:TP	June vs. Aug.	11	18.726	<0.05
TN:TP	June Deep vs. Shallow	4	0.664	0.475
TN:TP	Aug. Deep vs. Shallow	6	0.802	0.412
log(Ext. Chl a)	June vs. Aug.	12	160.443	<0.0001
log(Ext. Chl a)	June Deep vs. Shallow	5	1.231	0.329
log(Ext. Chl a)	Aug. Deep vs. Shallow	6	2.846	0.152
log(Part N)	June vs. Aug.	12	18.031	<0.005
log(Part N)	June Deep vs. Shallow	5	0.059	0.821
log(Part N)	Aug. Deep vs. Shallow	6	3.266	0.821
CN	June vs. Aug.	12	3.025	0.11
CN	June Deep vs. Shallow	5	0.549	0.5
CN	Aug. Deep vs. Shallow	6	1.715	0.247
log(CP)	June vs. Aug.	10	0.679	0.431
log(CP)	June Deep vs. Shallow	3	27.136	<0.05
log(CP)	Aug. Deep vs. Shallow	6	1.798	0.238
Diss. Microcystin	June vs. Aug.	13	17.864	<0.05
Diss. Microcystin	Aug. Deep vs. Shallow	6	1.148	0.333
Total Microcystin	June vs. Aug.	13	28.942	<0.001
Total Microcystin	Aug. Deep vs. Shallow	6	0.938	0.377
Part Mcyst./ Chl	Aug. Deep vs. Shallow	6	0.048	0.835
Fv/Fm	June vs. Aug.	9	0.704	0.426
Fv/Fm	June Deep vs. Shallow	3	1.353	0.365
Fv/Fm	Aug. Deep vs. Shallow	5	3.229	0.147
ETRmax	June vs. Aug.	9	1.675	0.232
ETRmax	June Deep vs. Shallow	3	0.979	0.427
ETRmax	Aug. Deep vs. Shallow	5	4.372	0.105

3.3.3.1 Water Transparency, Chlorophyll, and Stratification

Water transparency decreased significantly between June (mean Secchi depth: 2.4m) and August (mean Secchi depth: 1.4m) ($P<0.05$) (Figure 3.32). In both June and August, MB19 (Appendix B) had the shallowest Secchi depth and was quite similar on both sampling dates (0.55m and 0.6m, respectively). For the other three shallow stations in June Secchi depth was down to the sediment surface (Appendix B). The three deep stations had similar Secchi depths to those at shallower stations in June (deep vs. shallow: $P=0.347$). Again in August, both shallow and deep stations had similar Secchi depths ($P=0.9$) (Appendix B). Chlorophyll was extremely low in June (mean: 0.3 µg/L) but increased significantly by August ($P<0.001$) (Figure 3.33). The shallow MB19 had the highest level (0.9 µg/L) but otherwise deep and shallow stations had similar concentrations ($P=0.329$). By August, chlorophyll concentrations had risen to a mean of 17.4 µg/L although a trend with depth was not evident ($P=0.152$). In June, a stratified water column with a deep thermocline was evident in the three fluoroprobe profiles taken (Figure 3.34). However, in August, the nearshore station MB15 and offshore station Crib appear to be mixed to the bottom with a shallow layer of warm surface water that may be due to diel heating rather than stratification. The offshore station, Clear, also appears to be mixed to the bottom but with cooler surface water (Figure 3.34).

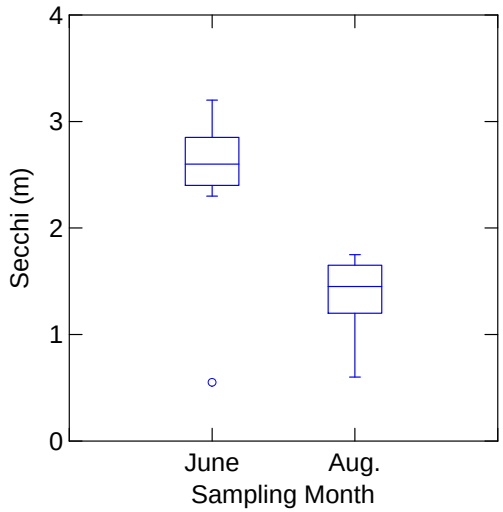


Figure 3.32. Boxplot of secchi depth from 7 stations in Maumee Bay on June 20, 2006 and Aug. 22, 2006.

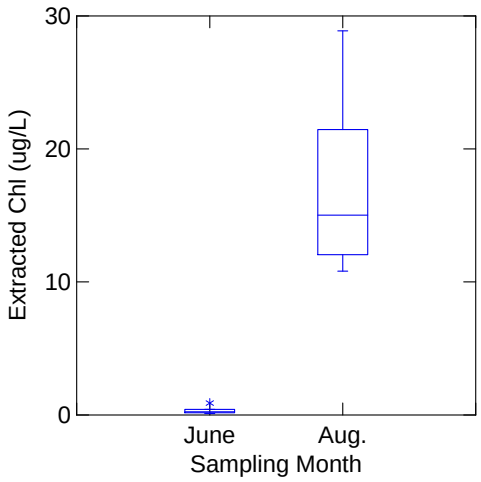
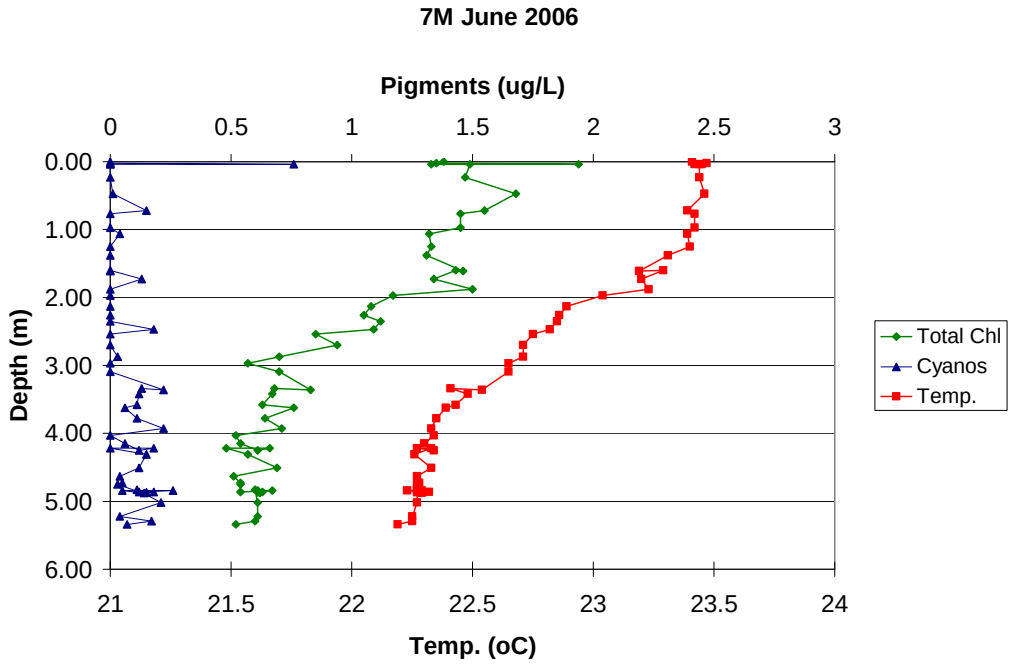
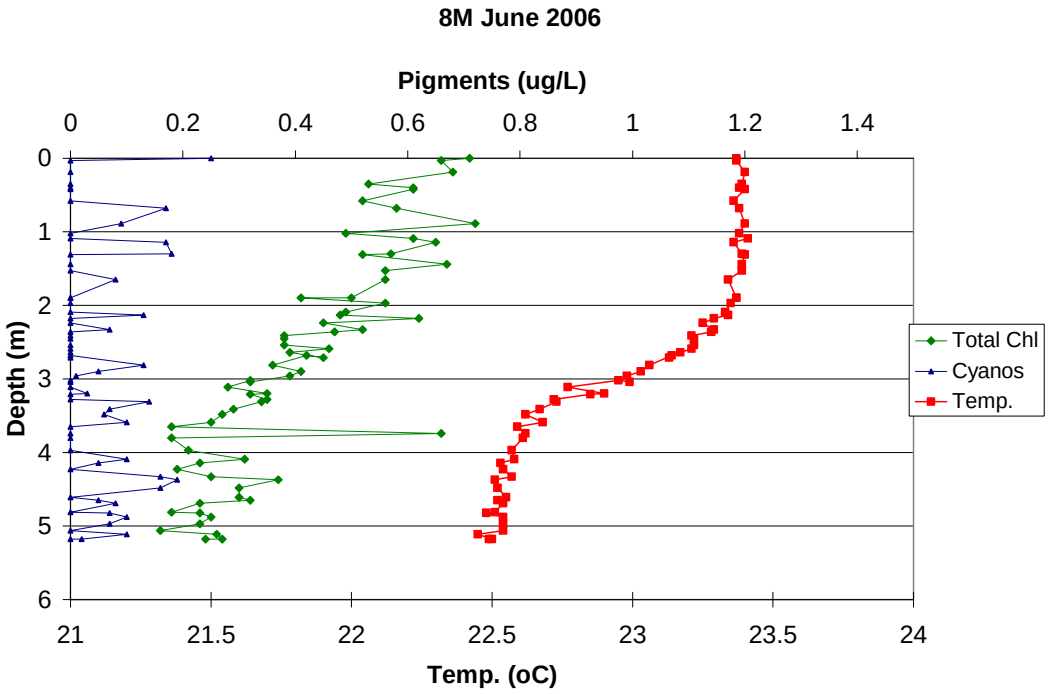


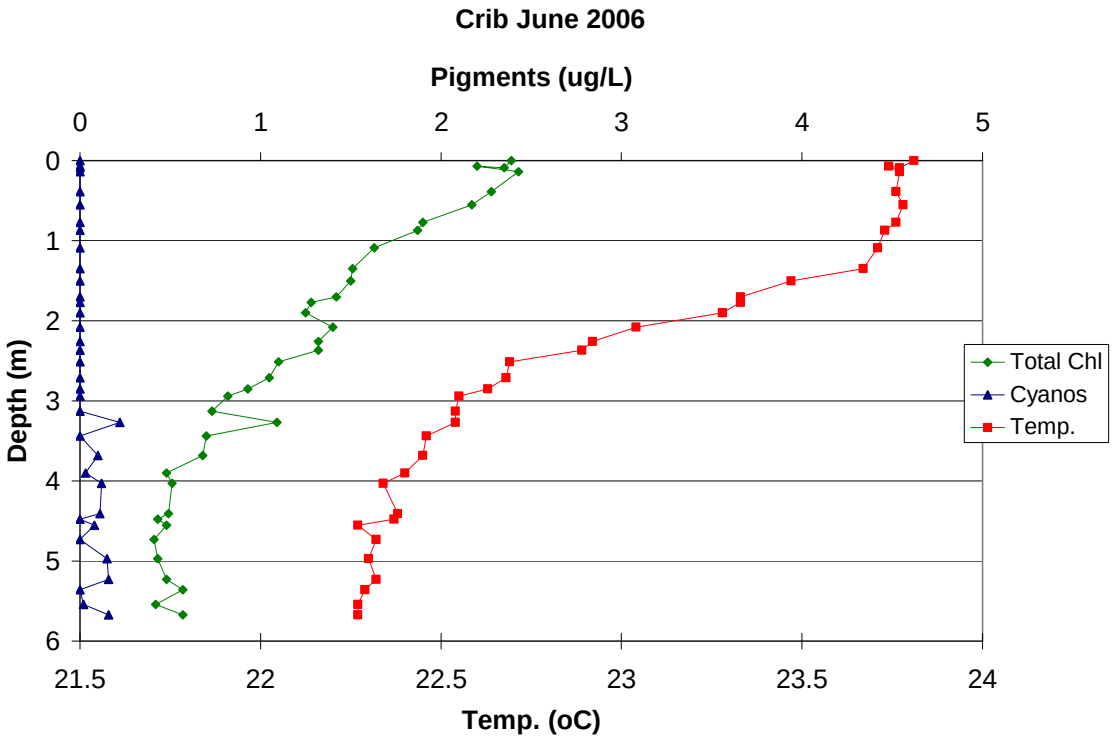
Figure 3.33. Boxplot of extracted chlorophyll *a* from 7 stations in Maumee Bay on Aug. 22, 2006 and from all stations except Crib on June 20, 2006. The Aug. data is an average of 2 extractions and analyses.

Figure 3.34. *Below* are the Maumee Bay 2006 Fluoroprobe profiles.

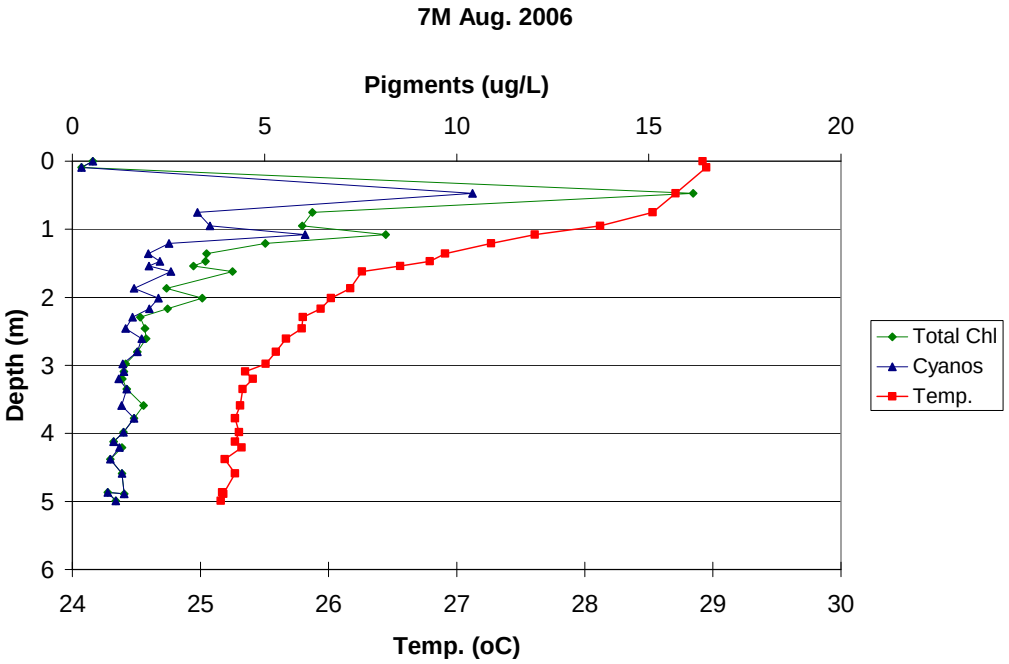
June 2006

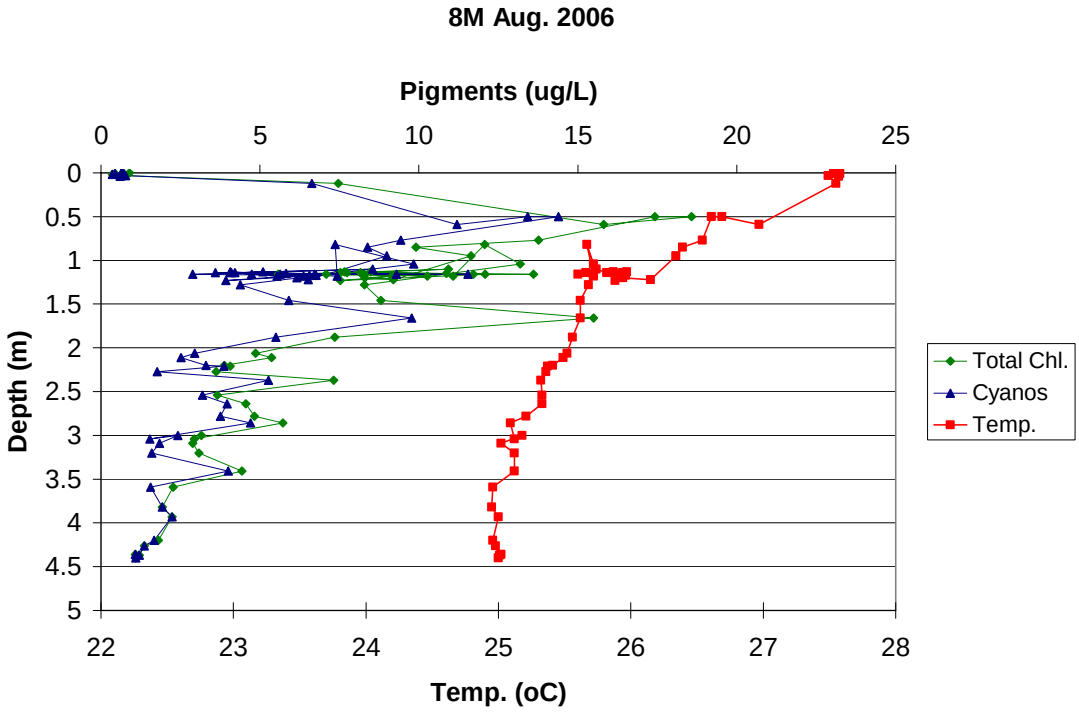




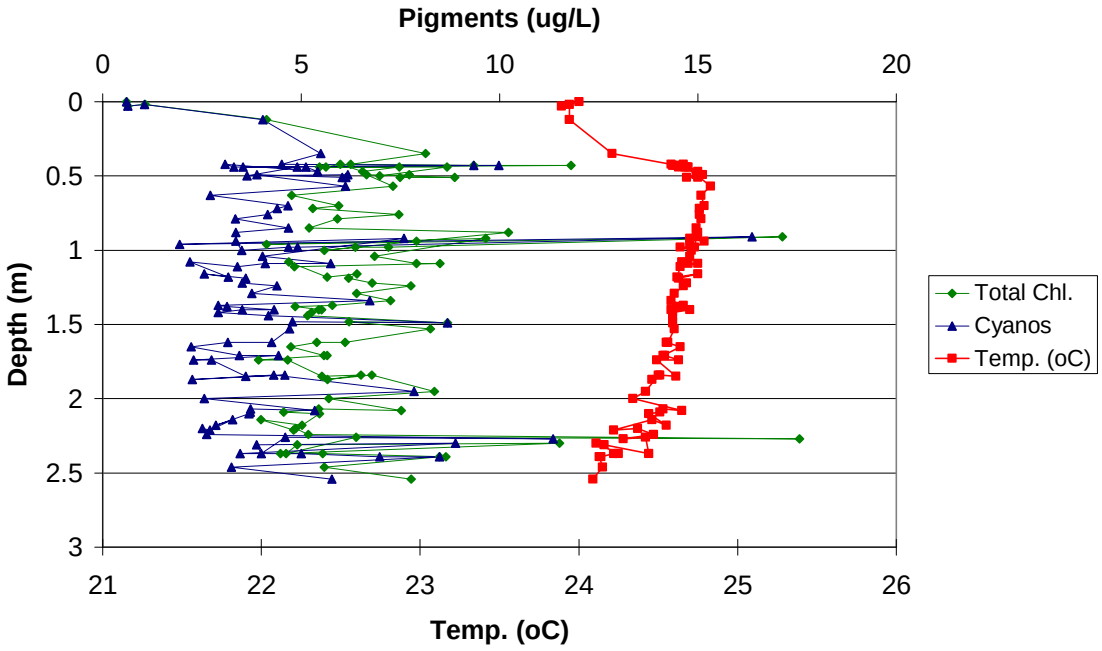

Crib June 2006
Pigments (ug/L)
0
1
2
3
4
5
Depth (m)
0
1
2
3
4
5
6
21.5
22
22.5
23
23.5
24
Temp. (oC)
Total Chl
Cyanos
Temp.

August 2006

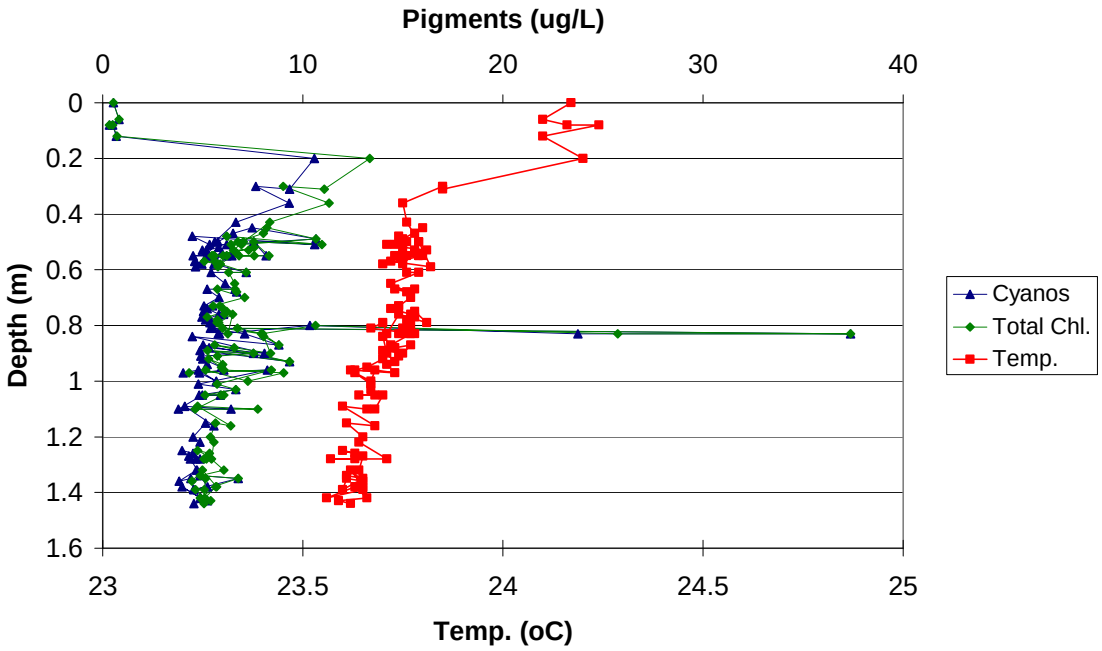

7M Aug. 2006
Pigments (ug/L)
0
5
10
15
20
Depth (m)
0
1
2
3
4
5
6
24
25
26
27
28
29
30
Temp. (oC)
Total Chl
Cyanos
Temp.


8M Aug. 2006
Pigments (ug/L)
0
5
10
15
20
25
Depth (m)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
22
23
24
25
26
27
28
Temp. (oC)
Total Chl.
Cyanos
Temp.

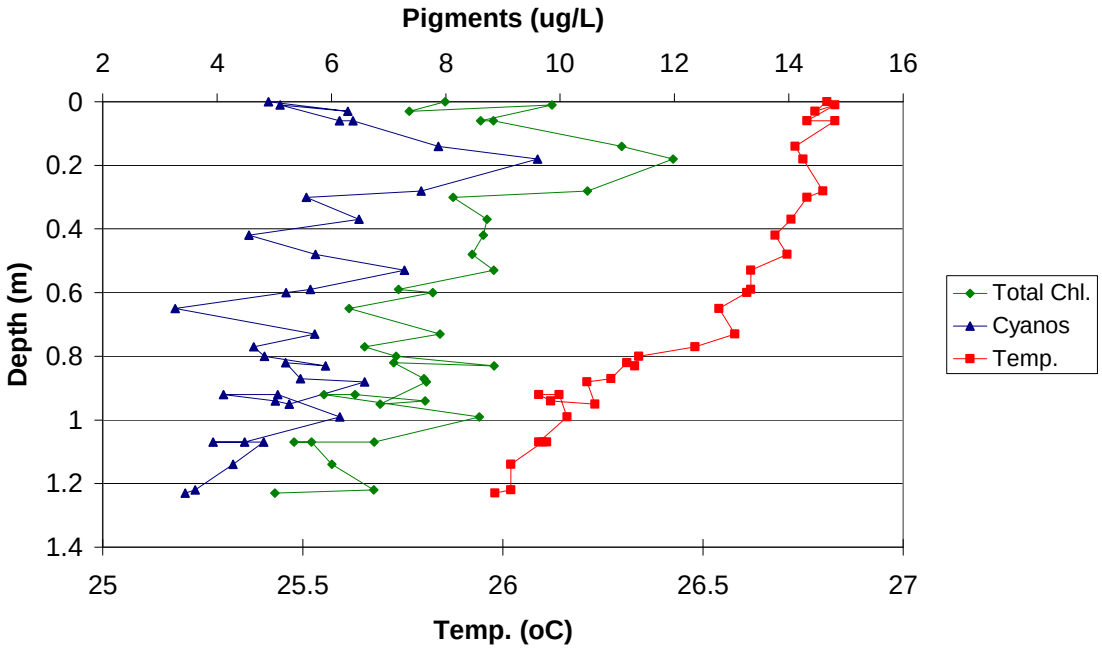
Clear Aug. 2006



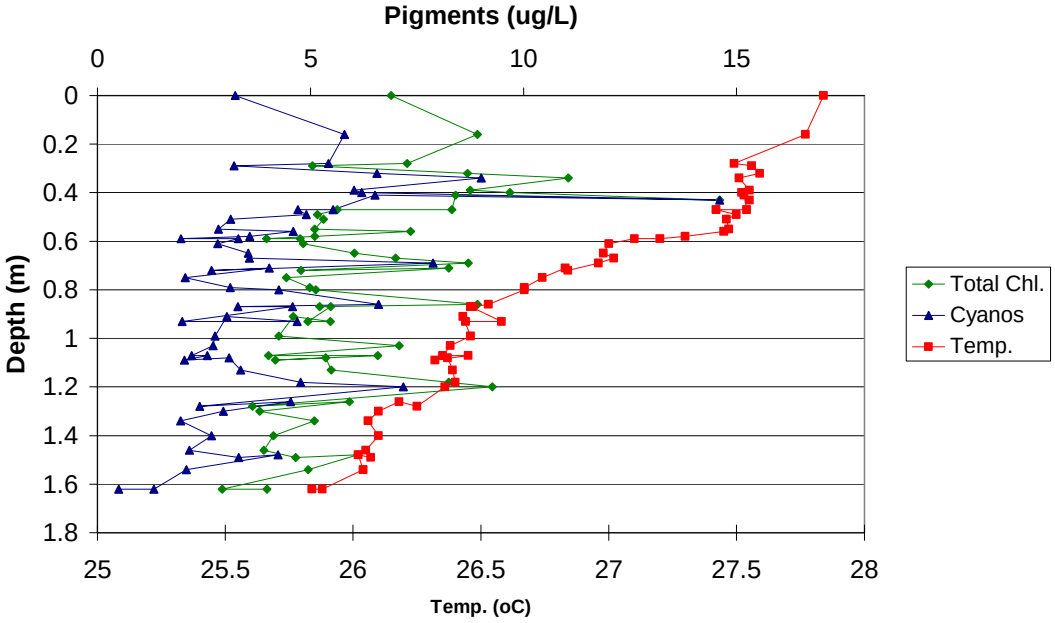
MB15 Aug. 2006



MB18 Aug. 2006



MB19 Aug. 2006



3.3.3.2 Phosphorus

Particulate P and TP both increased from June to August although only the TP increase was statistically significant (Part P: P=0.061; TP: P<0.05) (Figures 3.35 and 3.36). MB15 (a shallow nearshore station) had the lowest Part P in both June and August (2.7 and 7.2, respectively) but no other spatial patterns could be seen (Table 3.5). TP was more uniform between sites in August than it was in June although neither showed a trend with depth (Table 3.5). TDP was drawn down between June (mean: 22.4 µg/L) and August (mean: 11.9 µg/L) by half (Figure 3.37) but this difference was not statistically significant (P=0.222). In June, TDP levels were significantly lower at deeper stations (P<0.05) and had a greater range at shallow stations (Figure 3.38) while, in August, TDP was more uniform between all stations (Table 3.5). Mean SRP was very similar in August (17.4 µg/L) and June (15.8 µg/L) as can be seen in Figure 3.39 and the ANOVA p-value (P=0.922). Like TDP, SRP was significantly lower in deeper stations in June (P<0.05) but there was no difference between depths in August (Table 3.5) (Figure 3.39). Seston C to P ratios were slightly lower in June (mean molar ratio of 205) than in Aug. (mean molar ratio of 253) but this relationship was not significant (Table 3.5). At both times C:P ratios were within the 129-258 range of moderate P deficiency (Guildford et al. 1994) (Figure 3.40). June shallow stations were significantly lower than the one June deep station sampled (P<0.05).

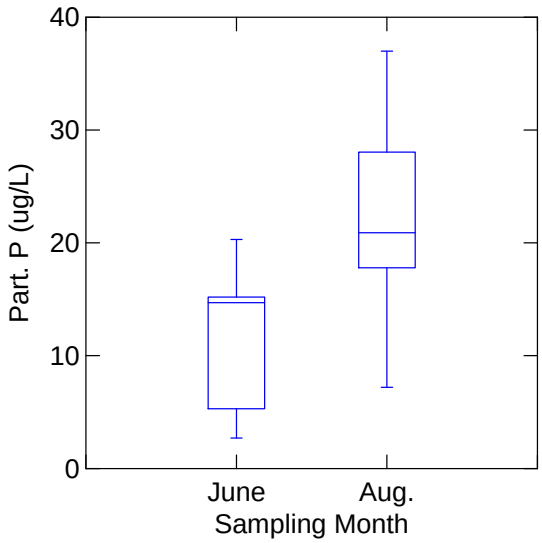


Figure 3.35. Boxplot of particulate P. No data from June for MB18 was available.

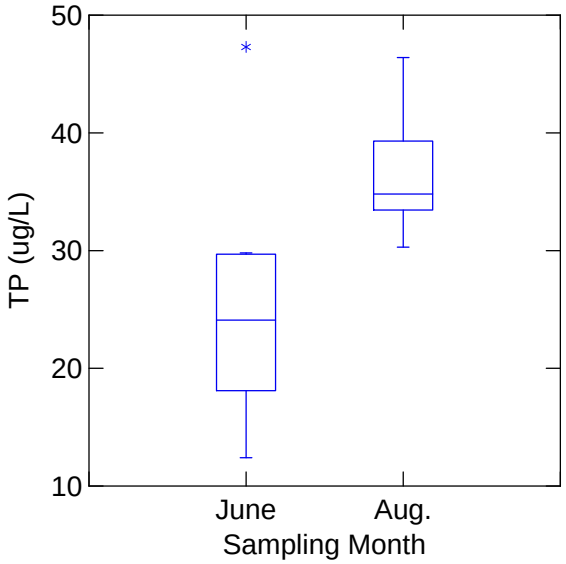


Figure 3.36. Boxplot of total P from 7 stations in Maumee Bay on June 20, 2006 and Aug. 22, 2006.

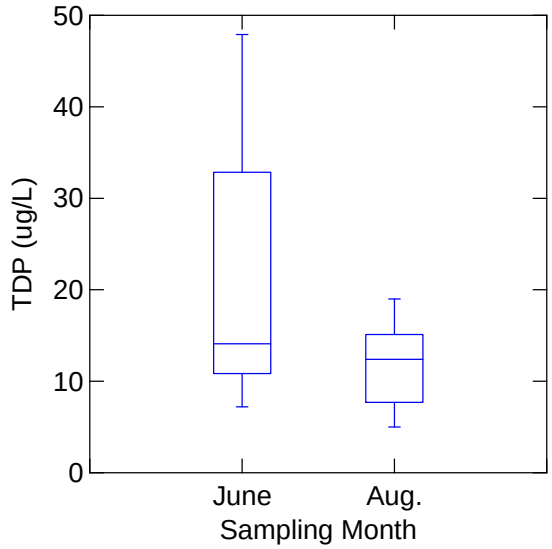


Figure 3.37. Boxplot of total dissolved P from 7 stations in Maumee Bay on June 20, 2006 and 6 on Aug. 22, 2006 (Crib not sampled then).

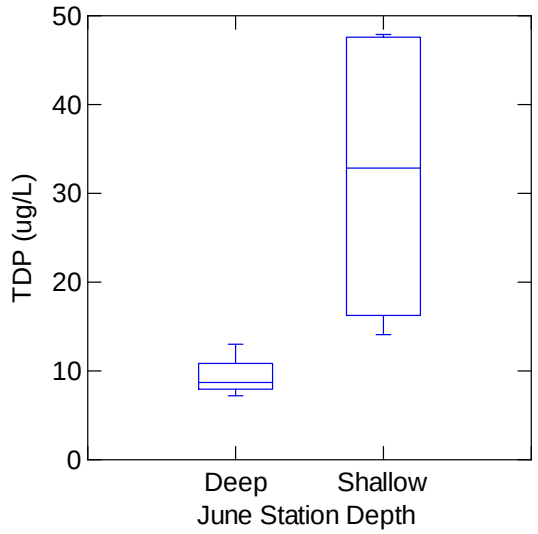


Figure 3.38. Boxplot of TDP in June, 2006 from Maumee Bay separated by station depth.

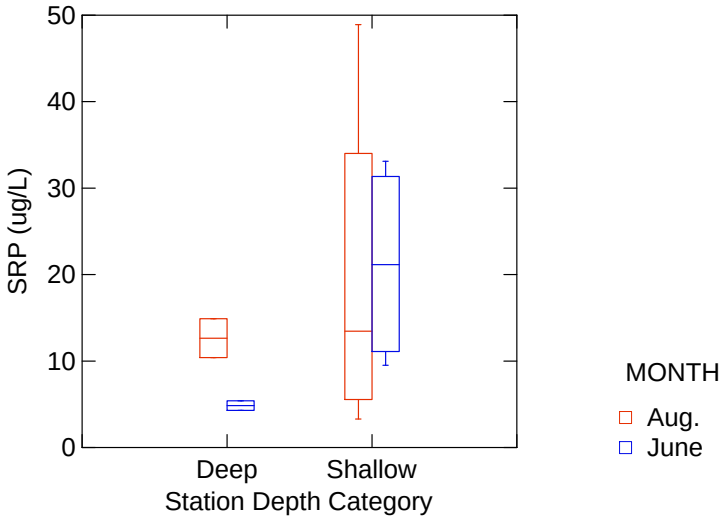


Figure 3.39. Boxplots showing distribution of SRP by depth and month in 2006 in Maumee Bay. Crib was not sampled on either date.

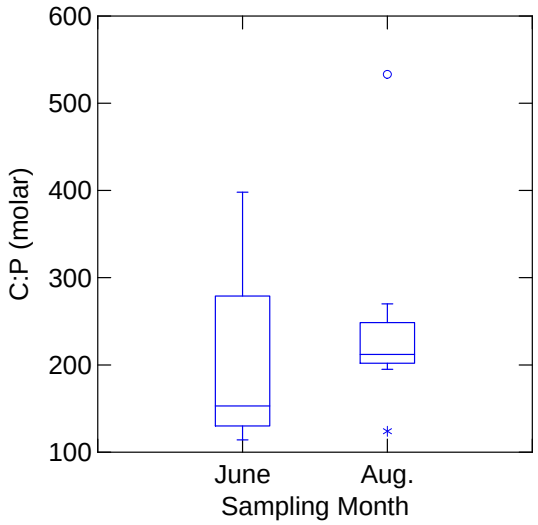


Figure 3.40. Boxplot of C to P molar ratios from 7 stations in Maumee Bay on Aug. 22, 2006 and 5 stations in June 20, 2006 (Crib and MB18 not sampled).

3.3.3.3 Nitrogen and N to P Ratios

There are no NH_3 data for June due to a laboratory error. In August, NH_3 from station 7M is the outlier that can be seen in Figure 3.41. With the outlier removed no depth-related pattern with NH_3 was found (Table 3.5). NO_3 levels were significantly drawn down between June and August ($P<0.01$) from a mean of 1616 µg/L to a mean of 277.7 µg/L (Figure 3.42). As with other variables, deep stations in June, but not August, had significantly lower NO_3 than shallow stations (Table 3.5) (Figure 3.42). NO_2 was evenly distributed among depths in June (Table 3.5) but was significantly lower in deep station in August ($P<0.05$) (Figure 3.43). NO_2 was always negligible in comparison to NO_3 levels. A highly significant increase in particulate N values in August ($P<0.01$) (Figure 3.44) corresponded to the decrease in NO_3 and the increase in phytoplankton biomass as estimated by chlorophyll *a*.

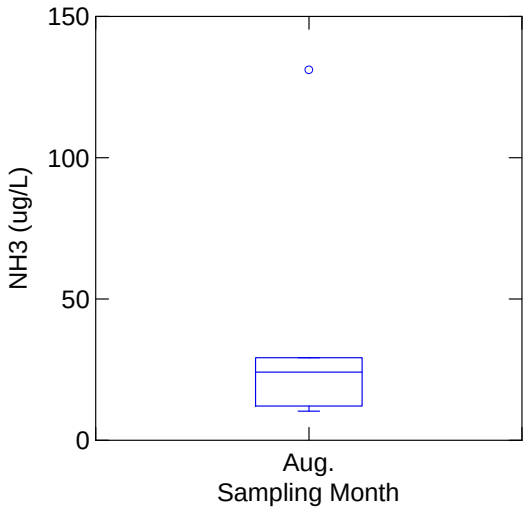


Figure 3.41. Boxplot of NH_3 measured from 6 Maumee Bay stations on Aug. 22, 2006 (Crib not sampled). No June data is available.

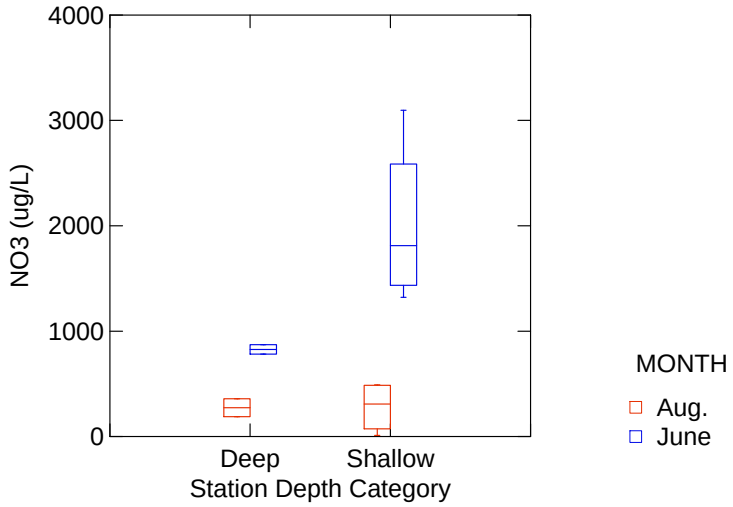


Figure 3.42. Boxplot of NO3 from 6 Maumee Bay stations on June 20, 2006 and Aug. 22, 2006 (Crib not sampled either time).

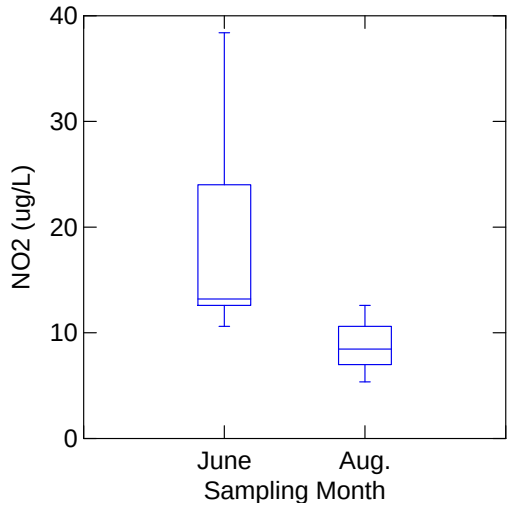


Figure 3.43. Boxplot of NO2 from 6 Maumee Bay stations on June 20, 2006 and Aug. 22, 2006 (Crib not sampled either time).

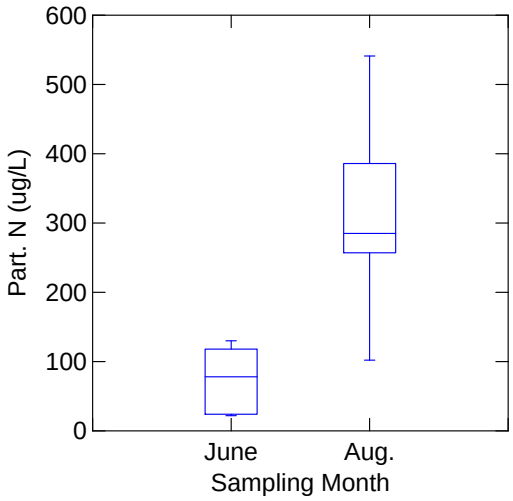


Figure 3.44. Boxplot of particulate N in Maumee Bay on June 20, 2006 and Aug. 22, 2006. Crib was not sampled in June.

TN appeared to be higher at shallow stations in both June and August (Figure 3.45) but there were no statistically significant differences (Table 3.5). The three deeper stations in August showed little variation in TN (Figure 3.45). Shallow stations had a mean TN that was higher in June (2571 µg/L, N=3) than in August (1852 µg/L, N=4) but this trend was not statistically significant (*P*=0.128). DON was calculated by subtracting DIN and particulate N from TN. Because NH_3 values were unavailable for June, June DON estimates were unable to be reliably computed. DON for August had a mean of 1076 µg/L which represented 62% of the mean TN from August. One outlier, from station Clear (Appendix B), had a quite high DON. C to N seston ratios were higher in June than August (Figure 3.46) but this was not a statistically significant difference (*P*=0.110). C to N ratios were evenly distributed among shallow and deep stations (Table 3.5). In June, Clear constituted an outlier with the highest C:N ratio observed in Maumee Bay (14.7 molar ratio, indicative of extreme N deficiency) but in August had the lowest C:N ratio of any station observed (6.5 molar ratio, indicative of no N deficiency). Excluding station Clear, in June two stations were

showing signs of moderate N deficiency while the three others had C:N ratios suggesting no deficiency. In August, the C:N ratios indicated that no N deficiency was being experienced. TN to TP ratios exhibited a significant decrease between June and August of approximately half (P<0.01) (Figure 3.47). Clear was again an outlier in August (Figure 3.47) with a high TN:TP in comparison to other stations. TN:TP were uniform between deep and shallow stations (Table 3.5).

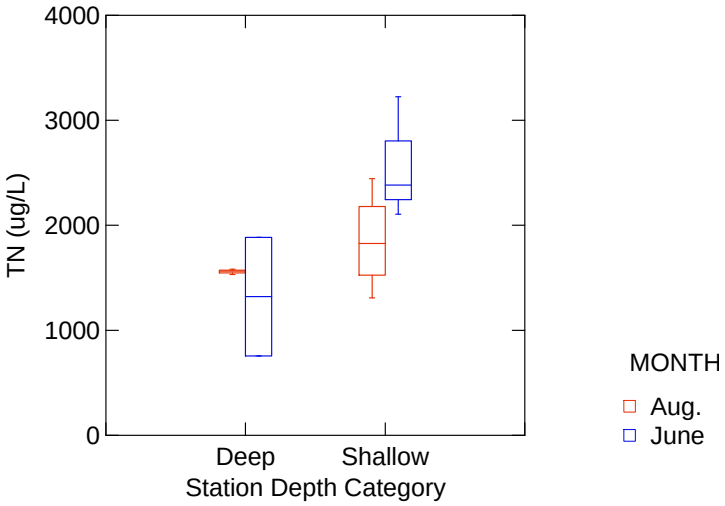


Figure 3.45. Boxplot comparing Total N on June 20, 2006 and Aug. 22, 2006 at deep and shallow stations in Maumee Bay. Note: Crib and MB18 were not sampled in June.

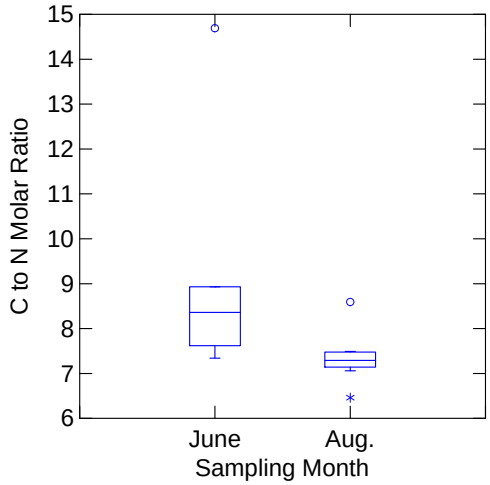


Figure 3.46. C to N molar ratio from Maumee Bay on June 20, 2006 (6 stations: Crib not sampled) and Aug. 22, 2006 (7 stations).

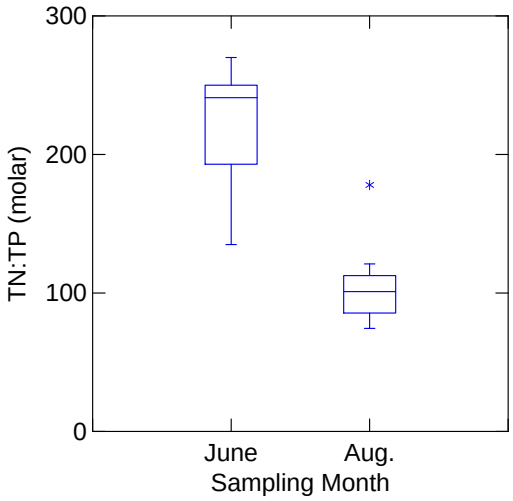


Figure 3.47. Boxplot of TN to TP ratio for Maumee Bay on June 20, 2006 and Aug. 22, 2006. June MB18 and June Crib data were unattainable.

3.3.3.4 Phytoplankton Count

The results of a detailed count to the species level on station 8M from August (station depth: 5.8m) performed by phytoplankton taxonomist Hedy Kling can be found in Table 3.6. A count on a sample from June was not performed. The majority of phytoplankton biomass in August was comprised by cyanobacteria (76%). Of the cyanophytes, almost half were *Aphanizomenon flos aquae* forma (48%), a distinctive morphotype of *Aphanizomenon flos aquae*. All *Microcystis* species comprised 22% of the cyanophyte biomass. All *Aphanocapsa* species represented a further 19% of the cyanophyte biomass. Data on heterocysts are not available.

Table 3.6. Phytoplankton count performed by Hedy Kling on a sample from station 8M from Aug. 21, 2006. Please note: *Aphanocapsa holsatica* specifically refers to *Aphanocapsa holsatica* (Lemm) Cronb. & Kom. And *Chroococcus minutus* specifically refers to *Chroococcus minutus* (Kutz) Naeg.

Maumee Bay - Aug. 21, 2006 - Station 8M				
Phytoplantkon Group	**Biomass (mg/m^3)**	**% of Total Biomass**	**Cells/L**	**% of Tot. Cells/L**
Cyanophyta	1354.5	75.8	630801254	96.5
Chlorophyta	42.2	2.4	21616360	3.3
Euglenophyta	0	0	0	0
Chrysophyceae	2.4	0.1	179028	0
Haptophyta	0	0	0	0
Diatomeae	61.5	3.4	37000	0
Cryptophyceae	316.1	17.7	1320872	0.2
Peridineae	10.6	0.6	29838	0
Xanthophyta	0	0	0	0
==========	========	==========	=======	========
TOTAL	1787.3		653984352	
Others	**Biomass (mg/m^3)**		**Cells/L**	
Protozoa	50		17000	
Mixotrophs	25		387894	
Zooplankton	523.5		1000	
Taxon	**Biomass (mg/m^3)**	**% Total Cyano. Biomass**	**Cells/L**	
Aphanizomenon flos aquae forma	654.4	48.3	135000	
Microcystis novacekii	269	19.9	5200000	
Aphanocapsa sp	218.7	16.1	417732000	
micro bluegreens	66.1	4.9	126274416	
Aphanizomenon klebanii	62.8	4.6	32000	
Aphanocapsa holsatica	41.9	3.1	80000000	
Microcystis flos aquae	22.5	1.7	1000000	
aphanizomenon akinete	7.3	0.5	10000	
Planktolyngbya limnetica	6.4	0.5	29838	
Microcystis ichthyoblabe	4.5	0.3	208000	
Chroococcus minutus	0.5	0.04	4000	
Chroococcus dispersus	0.3	0.02	8000	
Pseudanabaena mucioli	0.1	0.007	18000	
Aphanocapsa minutissima	0.1	0.007	150000	
All *Aphanizomenon*	717.2	52.9		
All *Microcystis*	296	21.9		
All *Aphanocapsa*	260.7	19.2		

3.3.3.5 Fluoroprobe Phytoplankton Estimates

The three Fluoroprobe profiles performed in June, all on deep stations, show very little chlorophyll with a mean of 1.4 µg/L (Appendix B). The percent cyanobacteria is also very low at 4% (Appendix B). As can be seen in the Fluoroprobe profiles (Figure 3.34) the distribution of chlorophyll follows the pattern of stratification with more phytoplankton in the epilimnion. At the stations profiled, cryptophytes were the largest phytoplankton group identified by the Fluoroprobe.

In August, the proportion of cyanobacteria within the total phytoplankton community, as measured by the Fluoroprobe, was 68% (Appendix B) which is near the 76% level from the phytoplankton count. Station 7M had the lowest concentration of both cyanobacterial pigments and total chlorophyll of all stations in August (Appendix B). The estimate of percentage of total chlorophyll attributed to cyanobacteria by the Fluoroprobe can be seen in Figure 3.48. The increase in percent cyanobacteria from a mean of 4.3% in June to a mean of 73.3% in August is substantial. Figure 3.49 shows a comparison of chlorophyll values obtained through extraction and the Fluoroprobe. In June, the extraction method and Fluoroprobe chlorophyll estimates were similar (extraction mean: 0.34 µg/L, Fluoroprobe mean: 1.41 µg/L). However, in August the Fluoroprobe (mean: 6.52 µg/L) estimated much less chlorophyll than did the extraction method (mean: 17.39 µg/L).

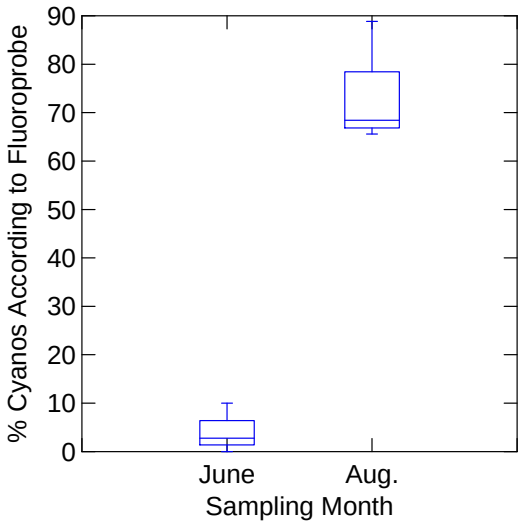


Figure 3.48. Percent cyanobacteria as detected by the Fluoroprobe on June 20, 2006 and August 22, 2006 in Maumee Bay.

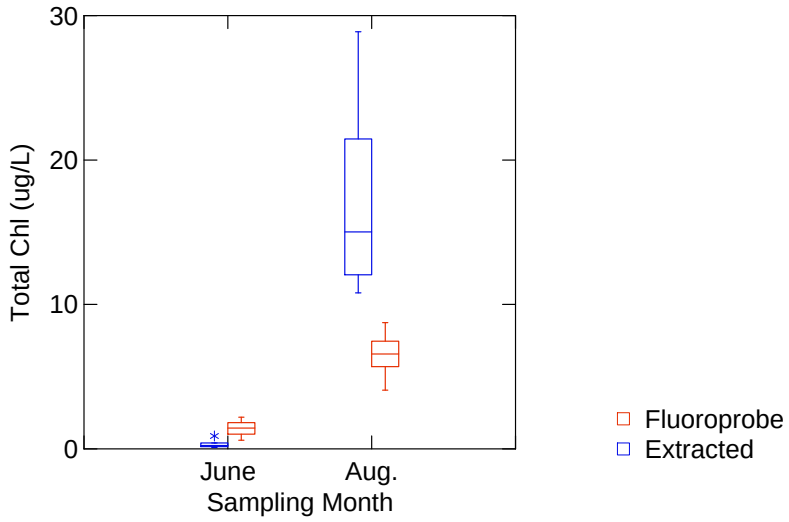


Figure 3.49. Comparison of Maumee Bay chlorophyll estimates from laboratory acetone extraction of samples from 1m or the surface (Appendix B) and *in situ* Fluoroprobe chlorophyll estimates averaged over the mixed layer.

3.3.3.6 Microcystin

In June, both dissolved and total microcystin were below the PPIA detection limit of 0.05 µg/L microcystin (Figures 3.50 and 3.51) and so levels in August were significantly higher (dissolved: $P<0.01$; total: $P<0.001$). In August, total microcystin levels were all above the W.H.O.'s 1 µg/L maximum allowable exposure level with values ranging from 2.0 to 7.6 µg/L (Figure 3.51). There was no statistical difference between deep and shallow sites in August ($P=0.39$). Crib, the water intake site, had the lowest total microcystin. Percent dissolved microcystin in August had a wide range (Figure 3.52) and a mean of 13.7%. Only one station in August, MB19, had a dissolved microcystin level above 1 µg/L.

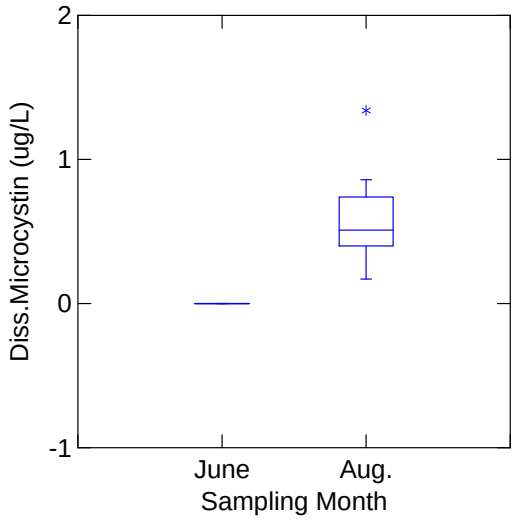


Figure 3.50. Boxplot of dissolved microcystin in Maumee Bay on June 20, 2006 and Aug. 22, 2006. Data is in equivalents of microcystin-LR.

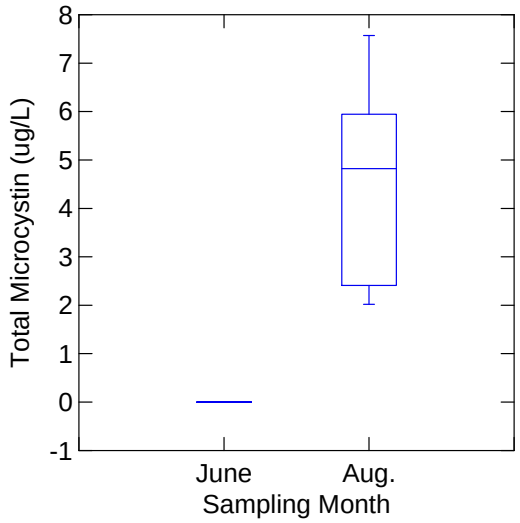


Figure 3.51. Boxplot of total microcystin in Maumee Bay on June 20, 2006 and Aug. 22, 2006. Data is in equivalents of microcystin-LR and includes both intracellular and extracellular toxin.

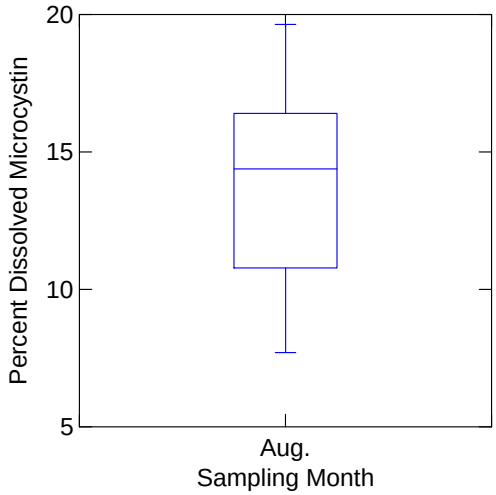


Figure 3.52. Boxplot showing distribution of percent dissolved microcystin values for Aug. 22, 2006 in Maumee Bay. June data is not presented as microcystin was below detection.

3.3.3.7 Photosynthetic Efficiency

ETRmax values had a wide range in June (Figure 3.53) but there was no trend with depth (P=0.427). In August, the two deep stations sampled showed very little variation in ETRmax and were lower

than the shallow stations, although this relationship was not significant (P=0.105) (Figure 3.54). Neither ETRmax nor Fv/Fm (Figure 3.55) was significantly different between June and August (Table 3.5).

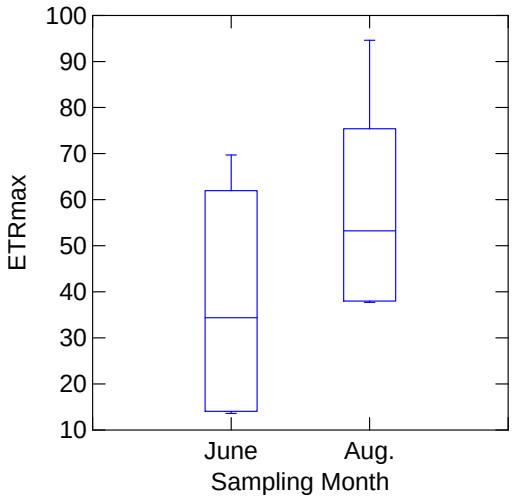


Figure 3.53. Boxplot of the PAM's ETRmax values from Maumee Bay. June 8M, June Crib, and Aug. Crib were not sampled.

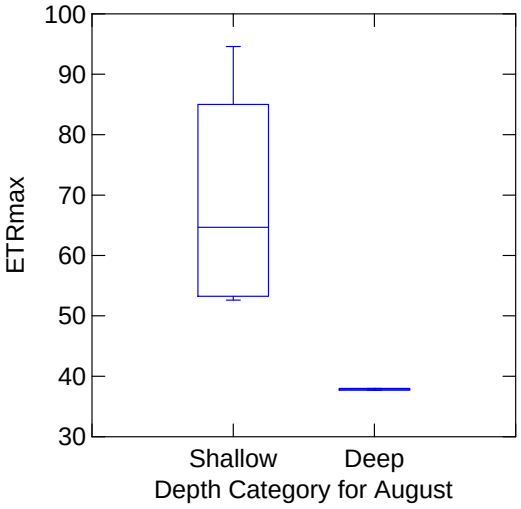


Figure 3.54. Boxplot of ETRmax, a PAM parameter, at two depth categories in Maumee Bay in August, 2006.

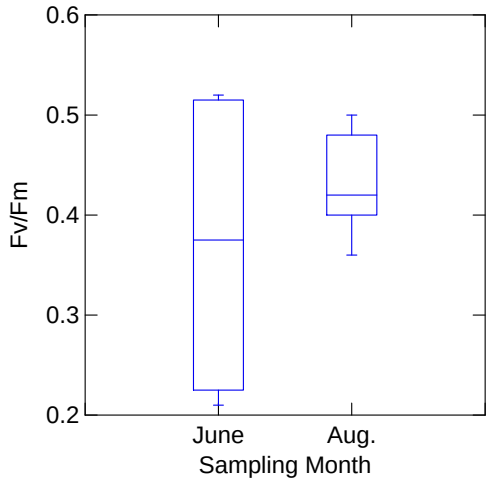


Figure 3.55. Boxplot of Fv/Fm values from the PAM. N=4 for June and N=6 for August.

3.4 Discussion

3.4.1 Bay of Quinte Discussion

3.4.1.1 Water Transparency, Chlorophyll, Phosphorus, and Water Column Stability

The significant decrease in Secchi depth in September was a symptom of higher phytoplankton biomass. This was evidenced by higher extracted chlorophyll *a* values (Figure 3.3), higher Fluoroprobe total chlorophyll estimates, and higher phytoplankton biomass as estimated through counts on station NA (Tables 3.1 and 3.2). It was expected that lower water transparency would promote microcystin production, so this hypothesis was refuted in the Bay of Quinte. Although particulate P significantly increased between July and September, TP remained similar (Figures 3.2 and 3.3). With such a marked increase in phytoplankton biomass but the same TP supply, it is not surprising that phytoplankton cells were less P rich in September, as was seen in the significantly higher C to P ratio (Figure 3.16). Since Guildford et al. (1994) lists a C to P ratio of over 258 as indicative of extreme nutrient deficiency, the September C to P ratio suggests that the phytoplankton

were severely P limited. The July C to P ratio then suggests that phytoplankton at that time were moderately P deficient. When P limitation was lower in the Bay of Quinte, microcystin concentrations were higher. Therefore, the hypothesis that cells with lower nutrient deficiency will be associated with higher microcystin is, therefore, supported. Because TP did not vary significantly between July and September, higher TP was not associated with higher microcystin concentrations.

Both SRP and TDP were significantly drawn down in September in comparison to July. This is likely attributable to the higher phytoplankton biomass in September. As was hypothesized, higher SRP was associated with higher microcystin levels. SRP and TDP showed a strong trend of being significantly lower at shallow stations in July. This corresponded to significantly higher levels of chlorophyll at those shallow stations and, therefore, higher P demands by phytoplankton. It is unclear exactly why there was higher phytoplankton biomass at shallow stations since no other variables showed a major difference with depth in July. Perhaps N runoff was being taken up by the phytoplankton at the shallow stations so that it promoted the growth of additional biomass and was not present in the water to be detected. Or, perhaps higher mean irradiance occurred at shallow stations which may have given phytoplankton there an advantage over deeper stations. The Fluoroprobe temperature profiles for stations in July (Figure 3.25) show mixing to the bottom or nearly the bottom (in the case of station GPt) so higher mean irradiance at shallow stations would have been possible. There was little evidence for stratification in either July or September, therefore increased water column stability was not associated with higher microcystin concentrations.

3.4.1.2 Nitrogen Levels

Ammonia levels were always higher than nitrate levels for all stations, with the exception of the DS (shoreline) station in July and the MBO station in September. One would expect ammonia to be lower than nitrate for two main reasons. Firstly, because the Bay of Quinte is shallow and usually mixed to the bottom, conditions should be oxic under which the majority of inorganic N is found in

its most oxidized form, nitrate (Kalff 2003). Secondly, it is more energetically efficient for phytoplankton to take up reduced ammonium than it is to take up nitrate, so phytoplankton uptake usually drives down ammonia levels (Kalff 2003).

It is possible that the ammonia values were high because of human and livestock sewage inputs, since these are known to be rich in ammonia (Kalff 2003). Furthermore, cows were observed drinking directly from the bay on certain sampling trips (Stephanie Guildford, personal communication). No major differences were observed in DIN between or within sampling periods with the exception of significantly higher NO_2 at shallow stations in September. This could have resulted from manure runoff from land.

Lower C to N ratios in September (Figure 3.12) suggest that phytoplankton were more N rich then than they were in July. One deep and one shallow station in July had C:N ratios high enough that moderate N deficiency was indicated (Guildford et al. 1994). The significantly higher TN and particulate N values in September could explain how the phytoplankton contained more N at that time period. In September, deep stations had significantly lower C:N ratios than did shallow stations. The reasons for this pattern are unclear. Differences among TN:TP ratios between July and September were not seen.

Based on the measurements of particulate N and dissolved inorganic N, the majority of the nitrogen making up the TN appears to be DON (Appendix A). This is not surprising as DON is known to commonly exceed dissolved inorganic N (DIN) (Berman 2001). High productivity in the Bay of Quinte could contribute to this DON pool since phytoplankton have been shown to release DON (Bronk et al. 1994). It should be noted that the DON pool may also be used as a source of N by phytoplankton (McCarthy 1972).

Remarkably, the particulate N mean for July 4, 2006 of 161.4 µg/L (with an outlier removed) was equivalent to a July 17, 1974 measurement from the Bay of Quinte of 161 µg/L (Liao 1977).

This shows a high level of continuity in the nitrogen environment over 33 years. This could be expected given that the Bay of Quinte Remedial Action Plan targeted P reduction and not N levels (Johnson and Hurley 1986).

3.4.1.3 Phytoplankton Community

Historically, diatoms and cyanophytes were the two most important phytoplankton groups in the Bay of Quinte, with diatoms typically being the most dominant (Nicholls and Heintsch 1986). Data from 1945, before phosphorus abatement, show this trend, as well as data from 1981, after phosphorus abatement (Nicholls and Heintsch 1986). The data presented here contrasts with that pattern as cyanobacteria were the predominant group and diatoms were hardly represented (Tables 3.2 and 3.3). Nonetheless, evidence of diatom biomass changes was observed. Soluble reactive Si was significantly lower in September than it was in July, which corresponds to an increase in diatom biomass (based on the phytoplankton counts) from 288 mg/m^3 in July to 704 mg/m^3 in September (Tables 3.2 and 3.3).

The switch to cyanobacterial dominance has widely been attributed to selective feeding by dreissenids, which had become abundant in the Bay of Quinte by 1995 (Nicholls et al. 2002). *Microcystis* colonies are often too large to be filtered out by dreissenids (Vanderploeg et al. 2001) and so dreissenids could certainly have contributed to the dominance of the various *Microcystis* species observed in July by grazing down their competitors.

To understand phytoplankton community dynamics one can look to the characteristics and tolerances of individual species. It is notable that *Anabaena spiroides*, which dominated in September, is a known N-fixer. The number of heterocysts as a percentage of total potential producers was 1.73 in July but actually decreased 5-fold to 0.335 in September. Therefore, it appears that less nitrogen fixation was occurring in September and that the possession of heterocysts does not explain how *Anabaena spiroides* came to dominate over *Microcystis*.

Another member of the *Anabaena* genus, *Anabaena flos-aquae*, has been shown to have a maximum P uptake rate that is over five times higher than that of *Microcystis aeruginosa* (Holm and Armstrong 1981, Nalewajko and Lean 1978, Reynolds 1988, Reynolds 2006). It is plausible that *Anabaena spiroides* also has a higher maximum P uptake rate which may have given it a competitive advantage in September when it appears that P was extremely limited.

3.4.1.4 2006 Microcystin

Total microcystin, dissolved microcystin, percent dissolved microcystin, and particulate microcystin per unit chlorophyll *a* were all significantly higher in July than September. With the overall cyanobacterial biomass and percent cyanobacteria being greater in September, one might expect higher microcystin at that time, but this was not the case. The multiple *Microcystis* species and *Anabaena spiroides*, which dominated in July and September, respectively, are all potential toxin producers. Therefore, the hypothesis that dominance by potentially toxic species will be positively associated with microcystin concentrations cannot be supported. It is possible, however, that the strain(s) of *Anabaena spiroides* in the Bay of Quinte has a lower toxin producing capacity than the strains of *Microcystis* present there, as it is known that toxin production per unit cyanobacteria varies with species (Reynolds 2006). The high percentage of dissolved toxin in July suggests that the *Microcystis* cells were lysing at the time of sampling. This may have been due to photoinhibition (Reynolds 2006) as the *Microcystis* had formed a visible scum on the surface. *Microcystis* has the potential to exhibit twice the positive buoyancy of *Anabaena*, therefore *Microcystis* has a greater potential for surface scum formation (Reynolds 2006). Once exposed to high light intensities for an extended period of time, many types of cyanobacteria do not survive (Sabour et al. 2005)

3.4.1.5 Comparison of 2005 and 2006 Microcystin

Microcystin levels were much higher in 2006 than they were in 2005. Detected microcystin was relatively low in 2005 (Table 3.4), but its presence shows that potential microcystin producers were still thriving in the Bay of Quinte.

3.4.2 Maumee Bay Discussion

3.4.2.1 Chlorophyll and Water Transparency

The extremely low chlorophyll observed in June may have been due to seasonal growth just beginning or due to a clearwater phase brought about by heavy grazing by zooplankton. The increase in phytoplankton biomass, as indicated by higher chlorophyll concentrations, in August was immediately obvious when sampling due to the surface scum that had accumulated. The decreased Secchi depth and increased particulate P in August can be attributed to this increase in biomass. The very low water transparency observed at station MB19 in both June and August was likely due to sediment loading from the Maumee River as MB19 is the station closest to the river mouth. Sediment-rich water was observed at MB19 while sampling. The decreased light caused by the Maumee River sediment plume has been proposed as a factor that promotes *Microcystis* over other phytoplankton types (Bridgeman 2005), presumably by limiting light and giving buoyancy-regulating *Microcystis* an advantage. At all stations, decreased water transparency was associated with higher microcystin concentrations.

3.4.2.2 Stratification, Dissolved Nutrients and Particulate N and P

The stratification present in June may have prevented water column mixing and likely contributed to the significant differences in TDP, SRP, and NO_3 concentrations that were seen between sites in June. Typically deeper stations had lower nutrient levels in June, as measured from the epilimnion. This may have been because runoff was supplying shallower stations with additional nutrients while

nutrient renewal was prevented at deeper stations. It is unlikely that greater phytoplankton biomass was drawing down nutrients more quickly in deeper stations because the chlorophyll *a* levels were similar for all depths. In August, when stratification was weak or absent, stations exhibited greater uniformity in nutrient concentrations, with the exception of NO_2. Since microcystin levels were higher in August, greater water column stability was not directly associated with higher toxin concentrations in Maumee Bay.

Between June and August, the significant decreases in the dissolved nutrients (TDP, SRP, NO_3, and NO_2) can likely be attributed to nutrient uptake by the greatly augmented phytoplankton biomass. This nutrient drawdown was observed in spite of the water column mixing that may have accompanied the weakly stratified conditions in August. Therefore, higher SRP was not associated with elevated microcystin concentrations. The significantly higher particulate N and strong trend of higher particulate P in August illustrate the movement of nutrients from the dissolved fraction to the particulate fraction as a result of having been incorporated into phytoplankton tissue.

3.4.2.3 TP and TN:TP Ratios

The significantly higher TP in August was likely the result of the mixing of the water column that could have occurred when stratification broke. The average TP found in June 2006 in Maumee Bay (25.6 µg/L) and the average August 2006 TP (36.7 µg/L) are comparable to western Lake Erie TP values in the literature. Holland and collaborators (1995) observed concentrations similar to June's in 1990-1993 in western Lake Erie and concentrations similar to August's in 1984-1987. Higher TP was associated with greater microcystin levels as was hypothesized.

The significant decrease in the TN to TP ratio between June and August was accompanied by a 69% mean increase in percentage cyanobacteria present. The TN:TP decrease also coincided with an increase in microcystin levels, as was hypothesized. The TN:TP ratios were still relatively high in August with a mean of 107. For instance, Smith (1982) considered TN:TP ratios over 35 to indicate

that chlorophyll *a* concentrations were no longer influenced by TN. The TN:TP ratios were always well over the level of 20 listed in Guildford and Hecky (2000) as being suggestive of the potential for N deficiency and always in the range indicative of the potential for P deficiency (> 50).

3.4.2.4 Nutrient Status Indicators and Photosynthetic Parameters

Even with the differences in dissolved and particulate nutrients observed over the season in Maumee Bay, there were no significant differences between June and August in the nutrient status indicators (C:P and C:N). The extreme fluctuation in station Clear's C to N ratio from being indicative of extreme N deficiency to no deficiency at all may have been the result of interference by sediment in the elemental readings. The C to N ratios indicate that likely no N deficiency was occurring in Maumee Bay. The C to P ratios indicate the presence of moderate P deficiency, which had worsened by August. Because these nutrient deficiency indicators did not change significantly over the summer, the hypothesis that indicators of greater nutrient deficiency will be negatively associated with microcystin levels is not supported in Maumee Bay.

Fv/Fm and ETRmax also did not vary significantly between June and August, but they were, on average, higher in August. Based on the Fv/Fm values published in Behrenfeld et al. (1996), the Fv/Fm values observed in Maumee Bay (June mean: 0.37, August mean: 0.43) would not be considered low but they are below the 0.5 to 0.6 Fv/Fm range recorded upon fertilization with a limiting nutrient.

3.4.2.5 Phytoplankton Community

Most of the cyanobacteria present in August were *Aphanizomenon*, a potential N fixer but not a potential microcystin producer (Falconer 2005). In contrast, many other recent cyanobacterial blooms in western Lake Erie have been mainly composed of *Microcystis* (Budd et al. 2001, Conroy et al. 2005, Vincent et al. 2004). Of the phytoplankton species identified in the August 8M sample, only

the *Microcystis* species present are known to be capable of producing microcystin (Falconer 2005). If 75.8% of the phytoplankton biomass at 8M were cyanobacteria and only 21.9% of those were *Microcystis*, relatively little *Microcystis* biomass was producing 5.9 µg/L total microcystin. This suggests that the *Microcystis* strains present in Maumee Bay have a high capacity for microcystin production. The hypothesis that an increased abundance of toxic cyanobacteria will be associated with greater microcystin concentrations is supported on the basis of the Fluoroprobe cyanobacteria estimates.

3.4.2.6 Microcystin

Microcystin levels well over the World Health Organization's 1.0 µg/L exposure level are not a new phenomenon in western Lake Erie. In 2003, Rinta-Kanto et al. (2005) measured 15.4 µg/L microcystin-LR equivalents with PPIA near the mouth of the Maumee River. The MB19 station near the Maumee River in this study had the highest total microcystin concentration in August 2006 with 7.6 µg/L. In 2004 Rinta-Kanto et al. (2005) observed microcystin levels up to 1.0 µg/L at other western Lake Erie sites. In both 2003 and 2004, *Microcystis* spp. containing the *mcyD* (microcystin-producing) gene were detected in Western Lake Erie (Rinta-Kanto et al. 2005). The August 2006 microcystin concentrations displayed consistency between stations in that all were above 2 µg/L. This was not found in the work by Rinta-Kanto et al. (2005), but they surveyed a larger area than this study. The persistent presence of microcystin over the years supports the need for consistent monitoring of microcystin levels in western Lake Erie, particularly because it is a source of drinking water.

3.5 Conclusion

It is possible that correspondence between microcystin levels and various environmental variables is coincidental since these data comprise only two snapshots of the 2006 season in both the Bay of

Quinte and Maumee Bay. Nonetheless, distinctive patterns have emerged from the significant differences found in the Bay of Quinte and Maumee Bay datasets. In the Bay of Quinte, the cyanobacteria in July produced more microcystin and experienced lower nutrient (P) stress and had more TDP and SRP available to them than in September. In July, the dominant cyanobacteria were *Microcystis* spp. whereas in September Anabaena spiroides dominated. Significant differences were found that were contrary to expectations: there was more microcystin present when there was greater water transparency, there was some apparent N deficiency, and there was a lower potentially toxic cyanobacterial biomass. In Maumee Bay, more microcystin was produced in August when there was a higher cyanobacterial biomass, decreased water transparency, increased TP, and decreased TN:TP ratios in comparison to June. Maumee Bay and the Bay of Quinte exhibit very distinctive patterns in the environmental variables that appear to influence their microcystin concentrations.

Chapter 4
Grand River Reservoirs

4.1 Study Sites

Belwood Lake, Constogo Lake, and Guelph Lake are reservoirs in the Grand River basin in southern Ontario. Belwood Lake resulted from the damming of the Grand River near Fergus, Ontario in 1942. Conestogo Lake resulted from the damming of the Conestogo River in 1958. Guelph Lake resulted from the damming of the Speed River near Guelph, Ontario in 1975 (Grand River Conservation Authority 1980). Because discharge from the reservoirs is carefully regulated, their depths vary dramatically throughout the year (Grand River Conservation Authority 1980). Agricultural and urban uses in the Grand River catchment basin have contributed to the eutrophic state of these reservoirs. Their warm, stratified, calm waters provide ideal conditions for excess algal growth (Grand River Conservation Authority 1980). All three reservoirs had reported hypolimnetic oxygen depletion problems in a 1980 assessment (Grand River Conservation Authority 1980). Recently, a massive cyanobacterial bloom occurred on Belwood Lake in the late summer of 2004, warranting a study into environmental variables that may be predictors of blooms as well as potential microcystin production. The field work for this study was performed by Miss Lesley-Ann Chiavaroli and assistants in conjunction with her Biol 499 project with Dr. Guildford. Miss Chiavaroli performed all PAM work and analyzed physical parameters and Fluoroprobe profiles. Miss Chiavaroli also compiled the majority of the data presented here for her Biol 499 report (2006) which was used with permission. Dr. Yuri Kozlov performed all chemical analyses with the exception of phosphorus and microcystin which were measured by myself. Dr. Stephanie Guildford prepared the fluoroprobe profile graphs. We are grateful to Miss Chiavaroli and Dr. Kozlov for their substantial contributions

to this chapter and their work will be acknowledged through authorship on any publication of this study.

4.1.1 Microcystin Background

A detailed background of microcystin can be found in Chapter 1. In other studies, high microcystin concentrations have been associated with environmental variables such as TP, SRP, TN, the N to P ratio, chlorophyll *a*, light, and dissolved O_2, although results have been varied (Billam et al. 2006, Kardinaal and Visser 2005). Microcystin dynamics can be somewhat unique in different water bodies. It is theorized that toxigenic strains generally produce the most microcystin under their optimal growth conditions, which typically include elevated nutrient concentrations (Kardinaal and Visser 2005, Sivonen and Jones 1999). The exact environmental variables found to best explain microcystin concentrations may be strain-specific, however (Orr and Jones 1998). The seasonal succession of cyanobacterial species and strains is likely very important to microcystin concentrations and can vary between study sites (Billam et al. 2006, Codd et al. 2005).

4.1.2 Hypotheses

Microcystin concentrations and various biological, chemical, and physical parameters were investigated in Belwood Lake, Conestogo Lake, and Guelph Lake to better understand cyanobacterial dynamics and to identify any microcystin production. This work was carried out under the following hypotheses:

1) If nutrient status affects microcystin concentrations and favourable growth conditions result in more microcystin production, then indicators of greater nutrient deficiency will be negatively associated with microcystin levels.

2) If the abundance of different cyanobacterial groups contributes to microcystin concentrations, then dominance by particular potentially toxic species will be associated with higher microcystin concentrations.

3) Greater water column stability, a low N to P ratio, higher SRP and TP, and decreased water transparency all promote the production of microcystin and that they will be positively associated with microcystin concentrations.

4.2 Methods

4.2.1 Sampling Procedure

Both Belwood and Conestogo Lakes were sampled biweekly between July 6 and September 22, 2005. Belwood was additionally sampled on Oct. 13, 2005 following the appearance of an unexpected cyanobacterial bloom. Guelph Lake was sampled biweekly between July 6 and Sept. 5, 2005.

On each sampling occasion, secchi depth was read and pH was measured using a portable pH meter. A Fluoroprobe was deployed for an *in situ* measurement of total chlorophyll and the characteristic pigments of chlorophytes, cyanophytes, diatoms, and cryptophytes. A CTD profiler was also deployed to measure photosynthetically available radiation (PAR) throughout the water column. Using a 5L Niskin bottle, 20L of water was collected from a depth of 2m and again from a depth of 6-7m to be representative of the epilimnion and hypolimnion, respectively. Upon returning to the lab, whole water was prescreened through 200 µm mesh in order to remove large grazers. Water was then filtered, through either a 0.7 µm glass microfibre filter (GF/F) or 0.2 µm polycarbonate filter as required for analysis. All water and filters were frozen until analysis with the exception of the PAM filters. These were read immediately using a Walz Diving-PAM.

4.2.2 Light Calculations

The light attenuation coefficient (K_d) was determined by taking the slope of the line of the regression between ln(PAR) and depth. The depth of the euphotic zone was then determined through the equation: z_{eu}= ln(100)/ k_d. Mean irradiance within the mixed layer as a percentage of surface PAR was calculated through the equation:

$$\text{Mean I (\%)} = \frac{\text{(Surface PAR- Mixing Depth PAR)*100}}{\ln(\text{Surface PAR/ Mixing Depth PAR})}$$

Equations can be found in Kalff (2003).

4.2.3 Nutrient and Chlorophyll Analyses

All analyses were performed using standard operating procedures compiled by Dr. Yuri Kozlov which were based on Stainton et al. (1977) and Standard Methods for the Examination of Water and Wastewater (American Public Health Association 1992), and other references mentioned below. TDP, TP, and Part P were measured by potassium persulfate digestion followed by the ascorbic acid method. TDP samples had been run through a 0.2-µm polycarbonate filter while Part P was measured on a 0.7-µm GF/F filter. SRP was also measured using the ascorbic acid method on GF/F filtrate. Particulate C and N was determined on pre-combusted GF/F filters that were packed into metal capsules and read in an Exeter CEC-440 Elemental Analyzer by David Depew after Grasshoff et al. (1983). Total N samples were digested by alkaline oxidation, passed through cadmium reduction columns, and read on a spectrophotometer following colour generation. NH_3 samples were first run through a 0.2-µm polycarbonate filter then measured with the orthophtaldialdehyde method outlined in Holmes et al. (1999). Filtered samples were measured for nitrate and nitrite on an Ion Chromatograph Dionex ICS 2500. For the measurement of soluble reactive silica, unfiltered samples were acidified, colour was generated from the addition of molybdate and stannous chloride, and samples were read on a spectrophotometer. Particulate silica was collected on a polycarbonate filter,

digested with sodium hydroxide, neutralized, and read as soluble reactive silica. Chlorophyll was protected from light, extracted cold from GF/F filters with acetone, and read in a Turner fluorometer.

4.2.4 Microcystin Analysis

Whole water was analyzed for total microcystin and GF/F filtrate was analyzed for dissolved microcystin. The protein phosphatase inhibition assay (PPIA) outlined in Bouaicha et al. (2002) was followed for toxin analysis except for the enzyme concentration used. For samples with 0.05-0.1 µg/L microcystin, 22 mUnits of enzyme were used and for samples with 0.1-0.25 µg/L microcystin, 48mUnits of enzyme were used. Samples with greater than 0.25 µg/L microcystin were diluted until they fit on the standard line. Assays were read in a SPECTRAmax GEMINI XS Dual Scanning Microplate Spectrofluorometer. In order to be confident in the microcystin data obtained from the newly established PPIA, samples were retested until multiple assay runs yielded consistent results.

4.2.5 Data Analysis

Systat Version 9 (SPSS, 1998) was used to generate most graphs and to perform statistical analyses. Microsoft Excel 2002 was used to generate some graphs. One-way ANOVA was used with Bonferroni post-hoc tests to look for significant differences among major variables between stations and dates within a water body. All data were tested for normality prior to statistical analysis and were log-transformed if they were not normally distributed. Table 4.1 lists which variables were log-transformed. In the figures, sampling trips are numbered 1 through 7. The dates that correspond to these trips are listed in Table 4.2.

Table 4.1. GRCA variables that were normal or required log-transformations prior to statistical analysis.

Variable	**Belwood**	**Conestogo**	**Guelph**
pH	normal	normal	normal
Temperature	normal	normal	normal
Secchi	normal	log	normal
Kd	normal	normal	log
Euphotic Depth	normal	normal	log
Mixing Depth	normal	normal	normal
Mean Irradiance	normal	normal	log
Fv/Fm	Log	normal	normal
Ext. Chl	normal	normal	log
SRP	Log	normal	normal
TDP	Log	log	normal
Part. P	normal	normal	normal
TP	normal	normal	log
NH3	normal	normal	log
NO2	Log	normal	normal
NO3	Log	normal	normal
TN	Log	normal	log
DON	Log	log	normal
TN:TP	Log	log	log
Part N	normal	normal	normal
C:N	normal	normal	normal
C:P	Log	normal	normal
SRSi	Log	normal	normal
Microcystin	Log	normal	log

Table 4.2. Numbered sampling trips as they appear in the GRCA figures and their corresponding dates.

GRCA Figure Legend	
Sampling Trip	*Date*
1	06-Jul-05
2	21-Jul-05
3	11-Aug-05
4	23-Aug-05
5	05-Sep-05
6	22-Sep-05
7	13-Oct-05

4.3 Results

4.3.1 Physical Characteristics

Because the sampling stations were intended to be the deepest points of each reservoir, the sampling station depth can be used as a proxy for lake depth at the time of sampling. Reservoir depth did not vary much as the water levels are highly regulated. An examination of Figure 4.1 shows that Belwood Lake was the deepest reservoir and Guelph Lake was the shallowest. The mixing depth of Guelph Lake stayed the most constant of all the three reservoirs (Figure 4.2). All showed a dramatic increase in mixing depth on the August 23, 2005 following a strong storm that eroded the thermocline. Evidence of this can also be found in the fluoroprobe profiles for August 23 (Figure 4.3).

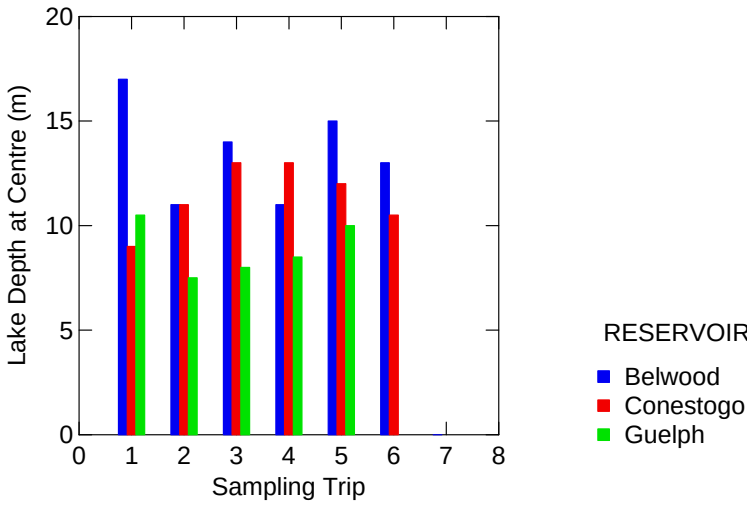


Figure 4.1. Depth of stations on each sampling trip. One station was sampled from each reservoir and it was chosen at the seemingly deepest point of the reservoir.

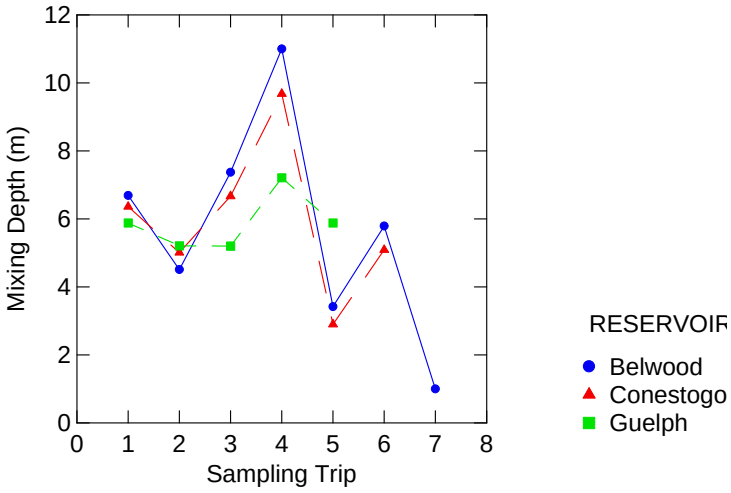
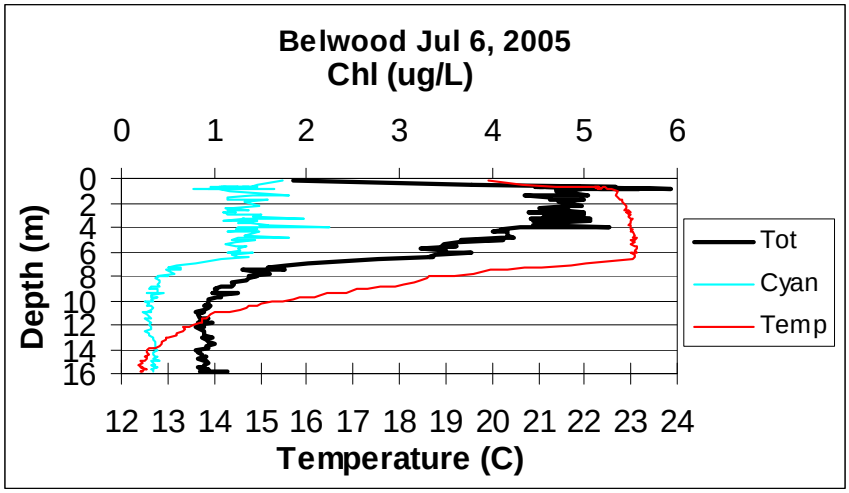
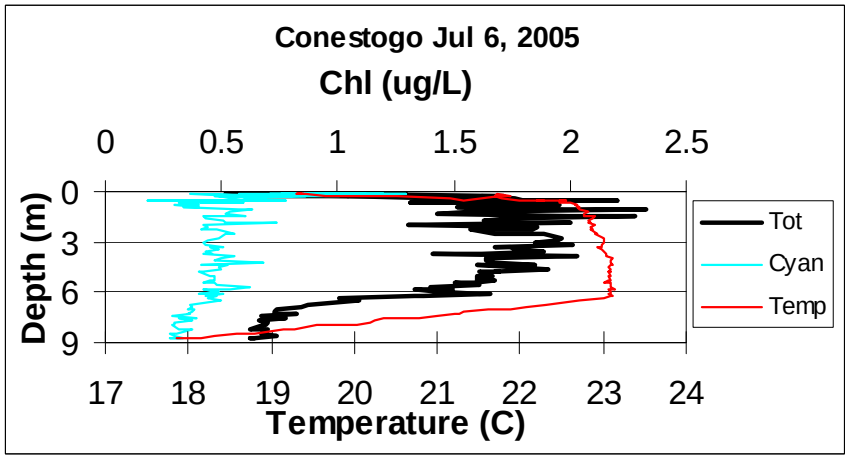
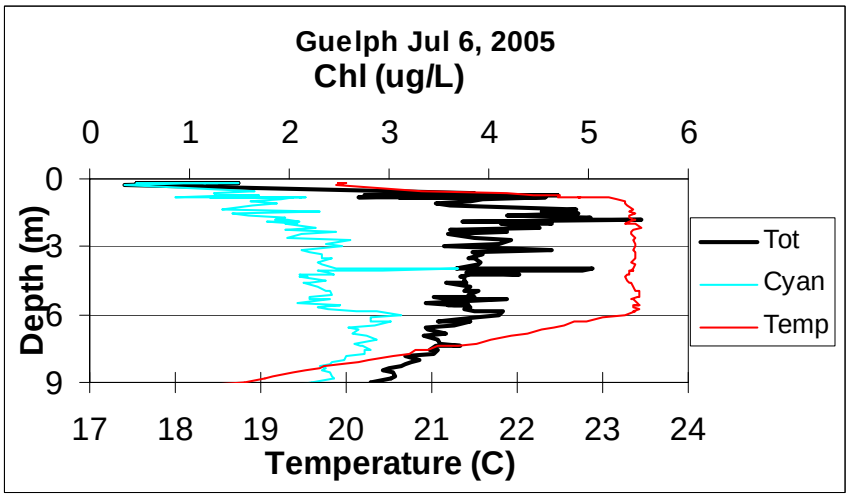


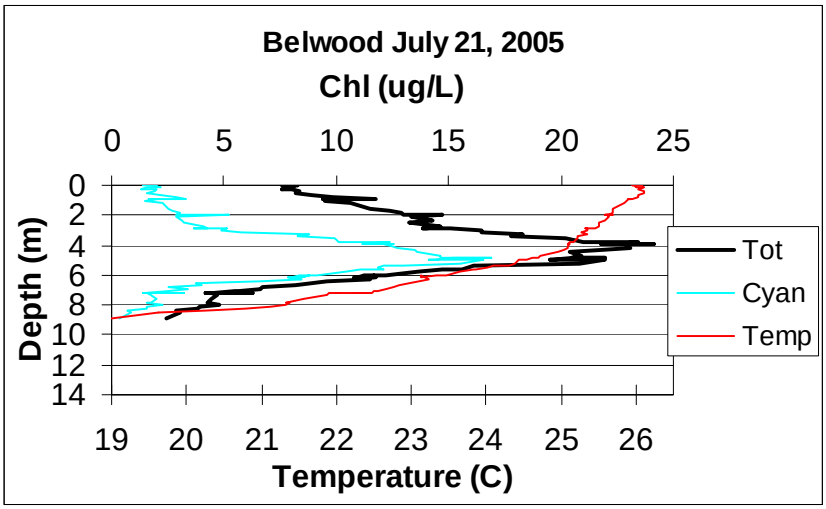
Figure 4.2. Mixing depths in Belwood, Conestogo, and Guelph lakes in 2005 as determined by fluoroprobe temperature profiles. Sampling dates are listed in Table 4.2.

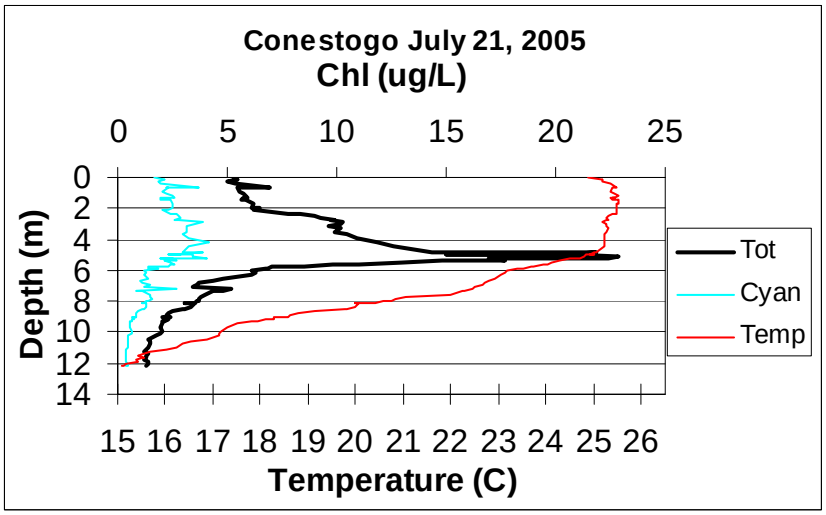
Figure 4.3. GRCA Fluoroprobe profiles *below*.

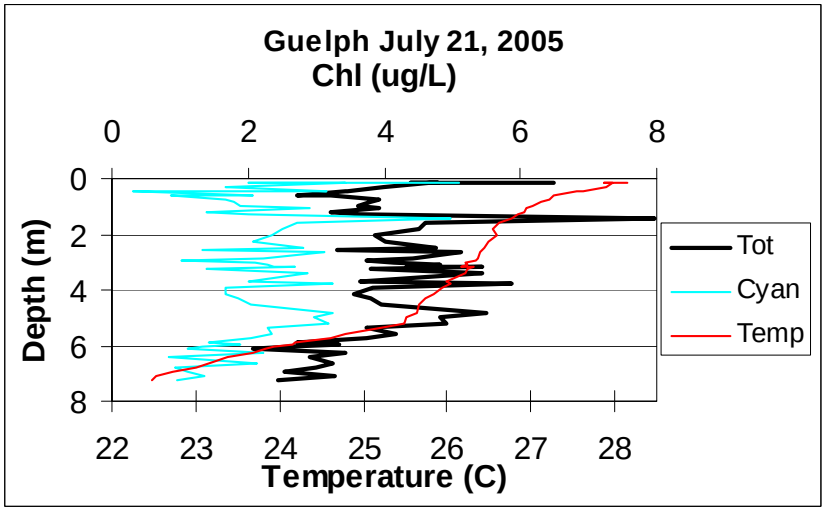


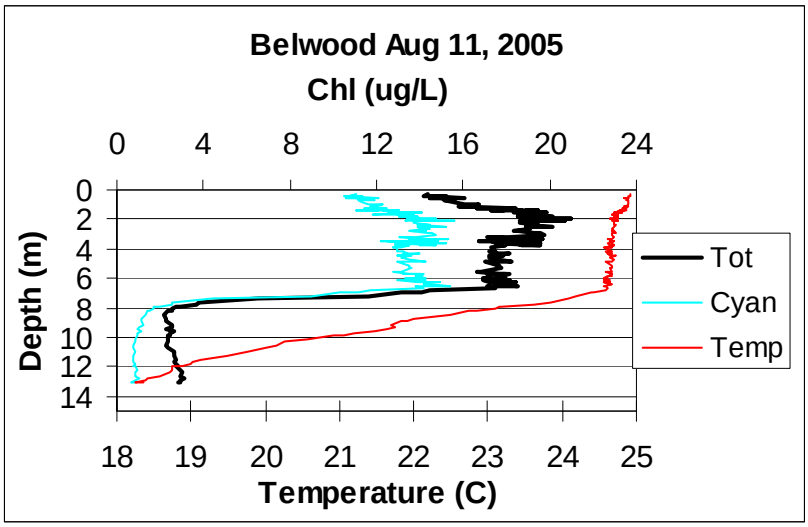


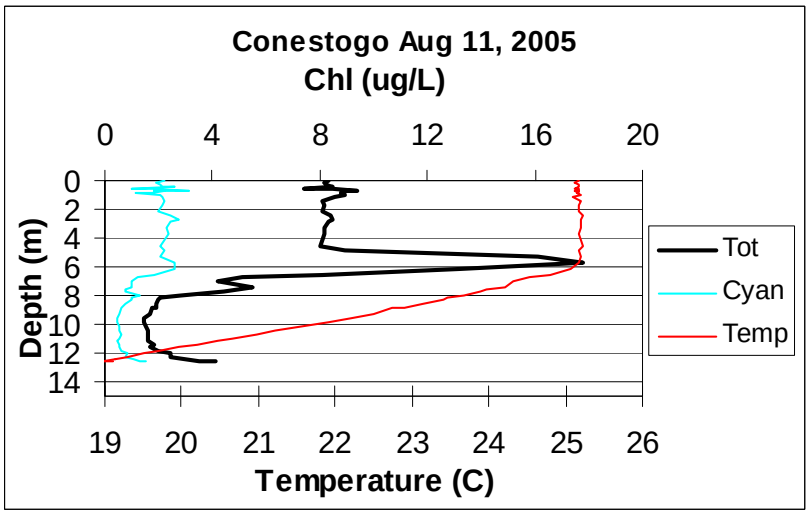


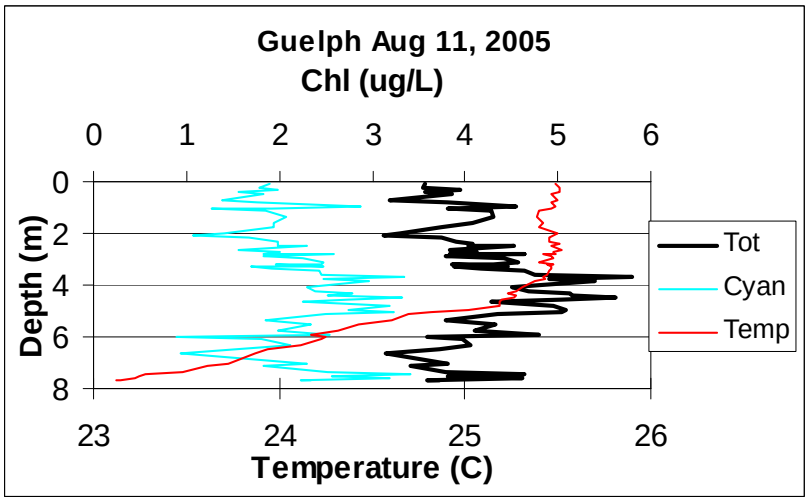

Belwood July 21, 2005
Chl (ug/L)
0 5 10 15 20 25
Depth (m)
0 2 4 6 8 10 12 14
19 20 21 22 23 24 25 26
Temperature (C)
Tot
Cyan
Temp

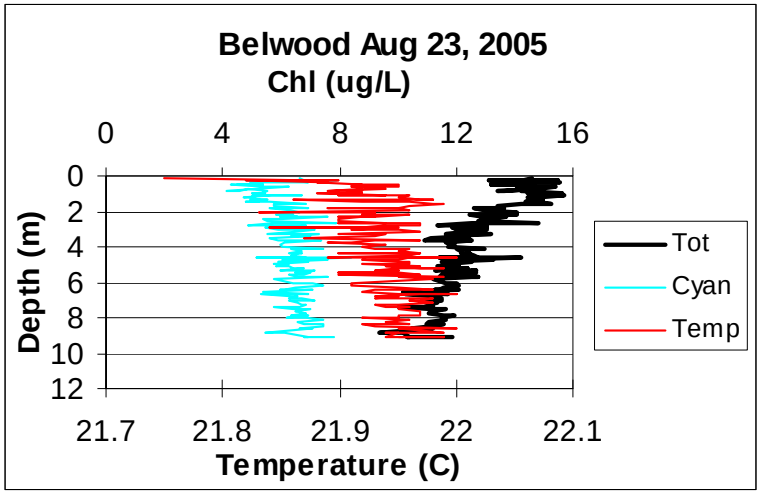

Conestogo July 21, 2005
Chl (ug/L)
0 5 10 15 20 25
Depth (m)
0 2 4 6 8 10 12 14
15 16 17 18 19 20 21 22 23 24 25 26
Temperature (C)
Tot
Cyan
Temp

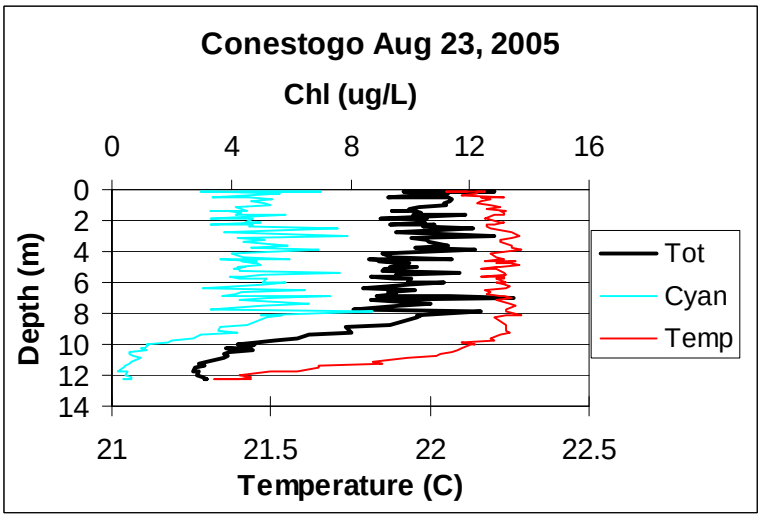

Guelph July 21, 2005
Chl (ug/L)
0 2 4 6 8
Depth (m)
0 2 4 6 8
22 23 24 25 26 27 28
Temperature (C)
Tot
Cyan
Temp

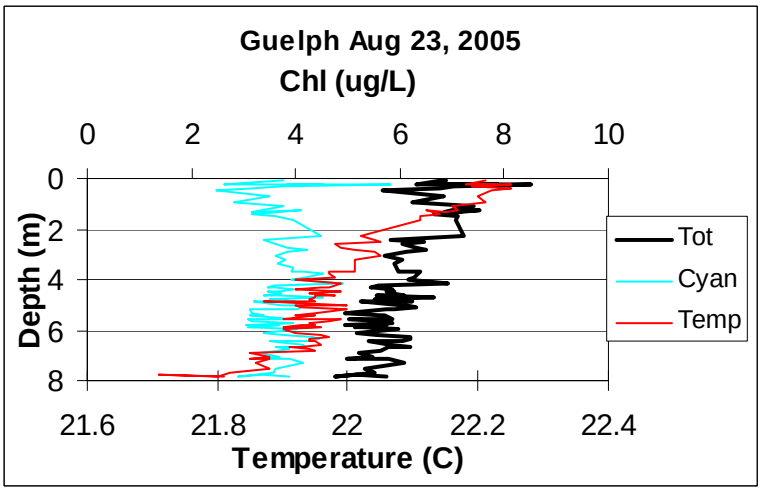

Belwood Aug 11, 2005
Chl (ug/L)
0 4 8 12 16 20 24
Depth (m)
0 2 4 6 8 10 12 14
18 19 20 21 22 23 24 25
Temperature (C)
Tot
Cyan
Temp

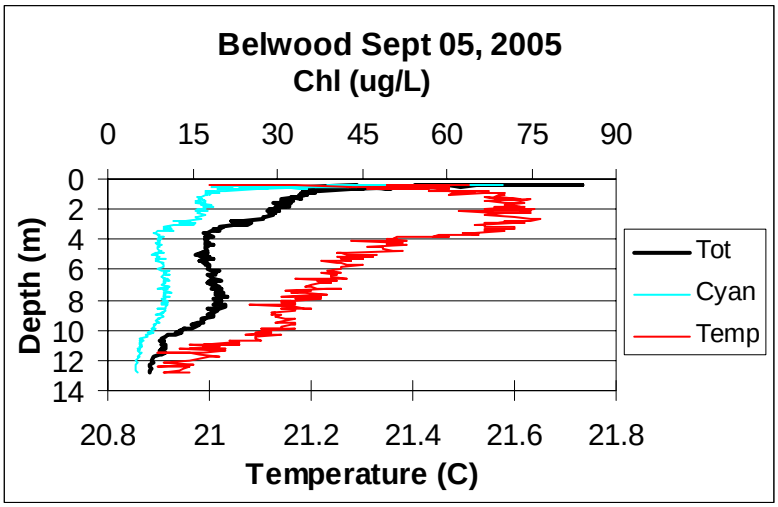

Conestogo Aug 11, 2005
Chl (ug/L)
0 4 8 12 16 20
Depth (m)
0 2 4 6 8 10 12 14
19 20 21 22 23 24 25 26
Temperature (C)
Tot
Cyan
Temp

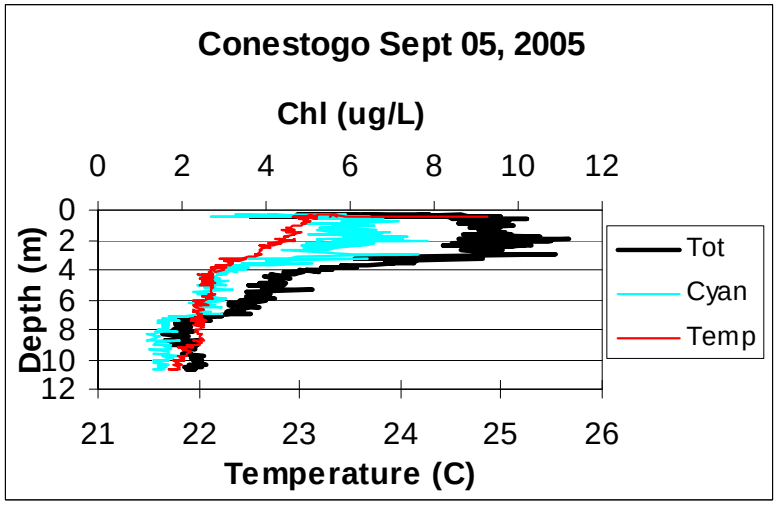

Guelph Aug 11, 2005
Chl (ug/L)
0 1 2 3 4 5 6
Depth (m)
0 2 4 6 8
23 24 25 26
Temperature (C)
Tot
Cyan
Temp

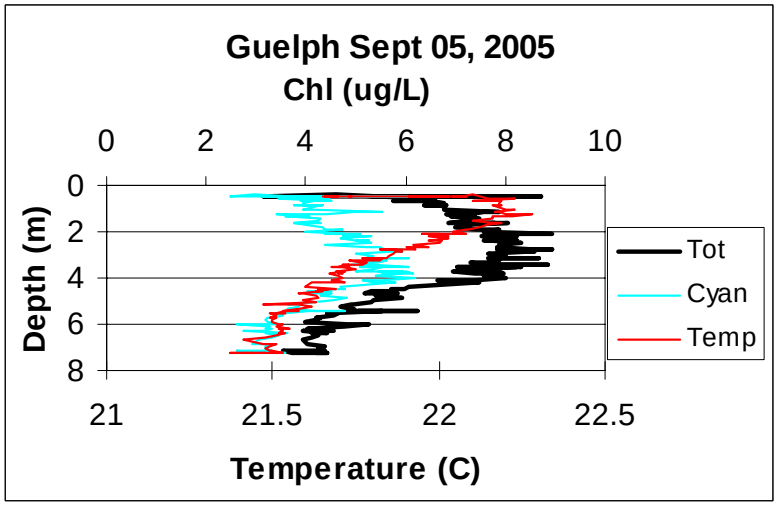

Belwood Aug 23, 2005
Chl (ug/L)
0
4
8
12
16
Depth (m)
0
2
4
6
8
10
12
21.7
21.8
21.9
22
22.1
Temperature (C)
Tot
Cyan
Temp

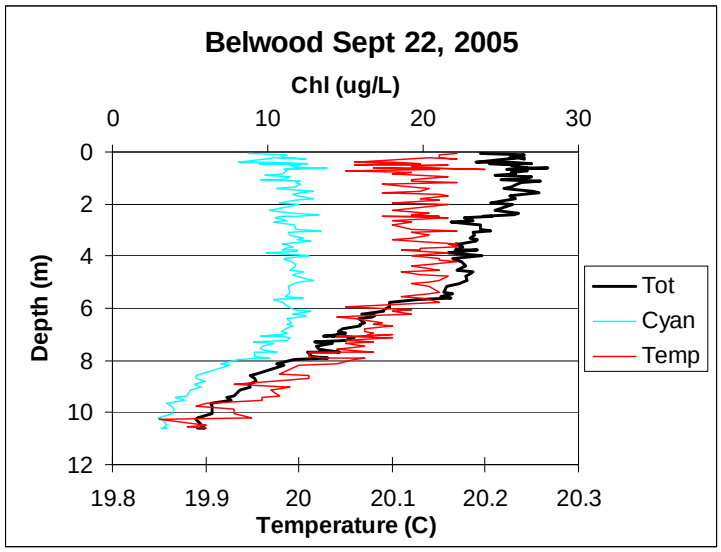

Conestogo Aug 23, 2005
Chl (ug/L)
0
4
8
12
16
Depth (m)
0
2
4
6
8
10
12
14
21
21.5
22
22.5
Temperature (C)
Tot
Cyan
Temp

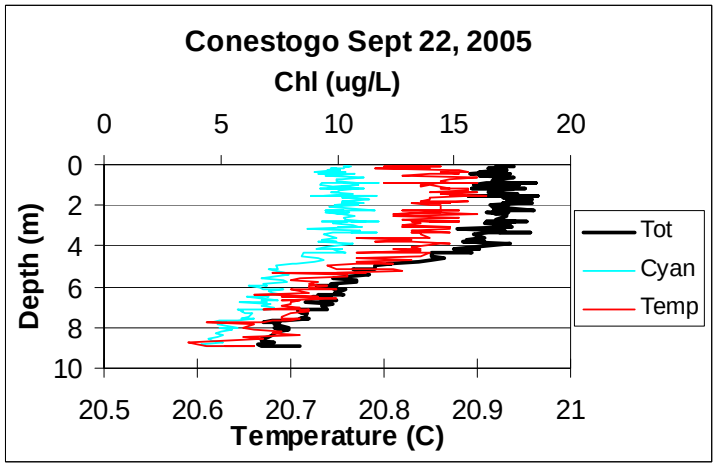

Guelph Aug 23, 2005
Chl (ug/L)
0
2
4
6
8
10
Depth (m)
0
2
4
6
8
21.6
21.8
22
22.2
22.4
Temperature (C)
Tot
Cyan
Temp

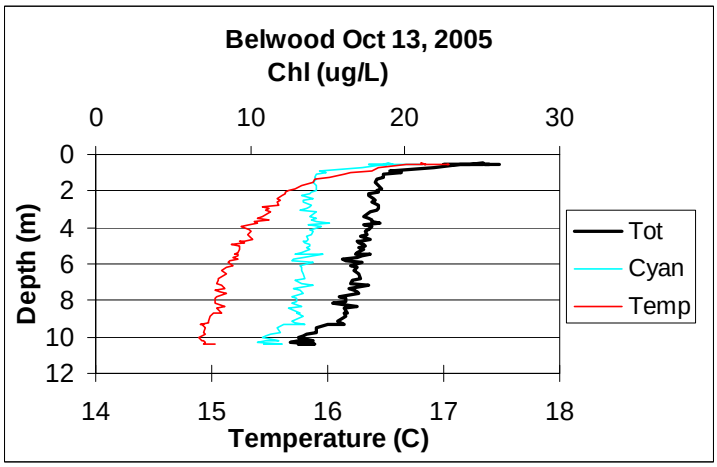

Belwood Sept 05, 2005
Chl (ug/L)
0 15 30 45 60 75 90
Depth (m)
0 2 4 6 8 10 12 14
20.8 21 21.2 21.4 21.6 21.8
Temperature (C)
Tot
Cyan
Temp


Conestogo Sept 05, 2005
Chl (ug/L)
0 2 4 6 8 10 12
Depth (m)
0 2 4 6 8 10 12
21 22 23 24 25 26
Temperature (C)
Tot
Cyan
Temp


Guelph Sept 05, 2005
Chl (ug/L)
0 2 4 6 8 10
Depth (m)
0 2 4 6 8
21 21.5 22 22.5
Temperature (C)
Tot
Cyan
Temp


Belwood Sept 22, 2005
Chl (ug/L)
0
10
20
30
0
2
4
6
8
10
12
Depth (m)
19.8
19.9
20
20.1
20.2
20.3
Temperature (C)
Tot
Cyan
Temp


Conestogo Sept 22, 2005
Chl (ug/L)
0
5
10
15
20
0
2
4
6
8
10
Depth (m)
20.5
20.6
20.7
20.8
20.9
21
Temperature (C)
Tot
Cyan
Temp


Belwood Oct 13, 2005
Chl (ug/L)
0
10
20
30
0
2
4
6
8
10
12
Depth (m)
14
15
16
17
18
Temperature (C)
Tot
Cyan
Temp

Secchi depth showed an overall decline throughout the seasons in all reservoirs (Figure 4.4) with Belwood Lake typically having the lowest water transparency (down to 0.75m on Oct. 13, 2005). Early in the summer, Conestogo Lake exhibited the highest Secchi depth (6.2m) however Guelph Lake had the lowest attenuation coefficient (0.43/m) (Figure 4.5) and the highest euphotic depth (10.7m) (Figure 4.6) observed. In July and early August, phytoplankton in Conestogo Lake experienced the greatest light environment with a mean irradiance of nearly 40% of the surface PAR (Figure 4.7). Belwood Lake consistently had the lowest mean irradiance (Figure 4.7).

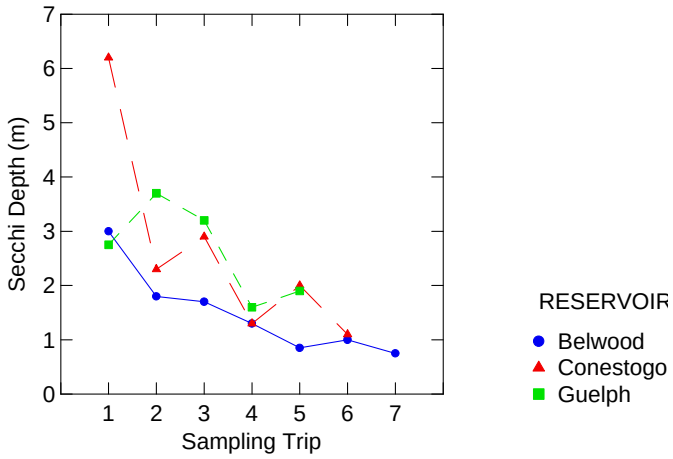


Figure 4.4. Secchi depth from Belwood, Conestogo, and Guelph lakes. Sampling dates: Table 4.2.

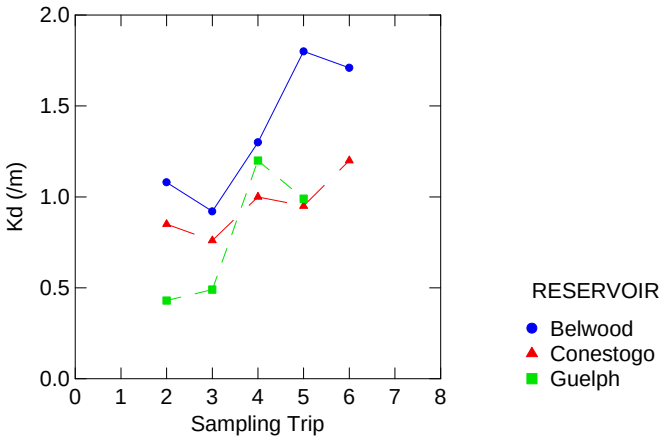


Figure 4.5. Light attenuation coefficient from Belwood, Conestogo, and Guelph lakes in 2005 as determined from CTD profiler readings. Sampling trip dates are listed in Table 4.2.

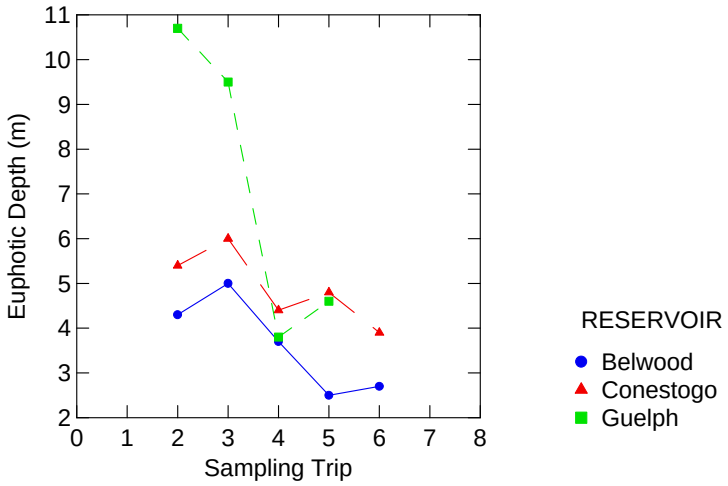


Figure 4.6. Euphotic depth in Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

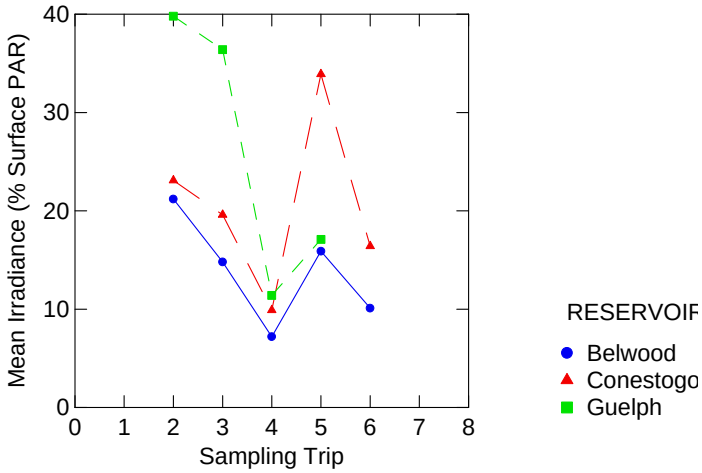


Figure 4.7. Mean irradiance from Belwood, Conestogo, and Guelph lakes in 2005. Dates of sampling trips are listed in Table 4.2.

4.3.2 Chlorophyll

Extracted chlorophyll *a* steadily increased in the epilimnia of all three reservoirs throughout the summer (Figure 4.8) although the chlorophyll decline was captured in Belwood Lake on October 13, 2005. Although all reservoirs had approximately the same chlorophyll levels in early summer, for the

rest of the summer Belwood Lake had the greatest epilimnetic chlorophyll concentration followed by Conestogo Lake and finally Guelph Lake (Figure 4.8). Hypolimnetic chlorophyll levels were much more similar between the three reservoirs (Figure 4.9) but also showed a net increase throughout the growing season.

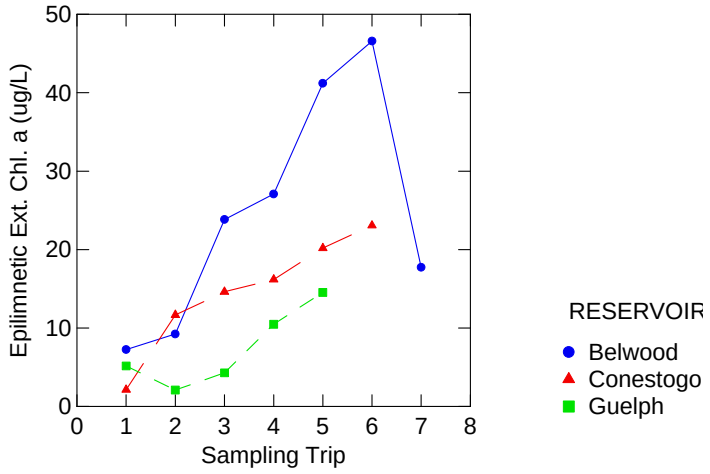


Figure 4.8. Epilimnetic extracted chlorophyll a levels from Belwood, Conestogo, and Guelph lakes from 2005. All samples were taken at a depth of 2m with the exception of that from Belwood Lake on trip #7. Sampling trip dates are listed in Table 4.2.

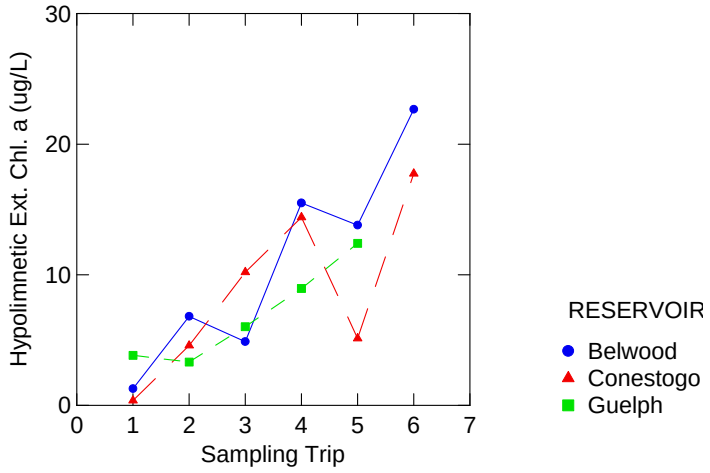


Figure 4.9. Hypolimnetic extracted chlorophyll a from Belwood, Conestogo, and Guelph lakes in 2005. Depths sampled were typically 10m for Belwood Lake, 7m for Conestogo Lake, and 6m for Guelph Lake. Sampling trip dates are listed in Table 4.2.

4.3.3 Phosphorus

The SRP levels show variation between sampling dates but were always relatively low (<5 µg/L) (Figure 4.10). All three reservoirs had undetectable SRP on Aug. 11 but this was followed by the peak SRP of each reservoir. Conestogo and Guelph lakes showed their peak measured SRP levels on August 23, 2005 and Belwood showed its peak on September 5, 2005. TDP displayed a similar pattern in that Conestogo and Guelph lakes had their highest measured levels on August 23 but Belwood had its highest level on September 5 (Figure 4.11). Particulate P steadily increased in all reservoirs until August 11 after which time some fluctuation occurred in all reservoirs (Figure 4.12). Both epilimnetic and hypolimnetic TP were very similar between reservoirs on the first sampling date, July 6, 2005 (Figures 4.13 and 4.14). In the hypolimnion, all reservoirs showed a net increase in TP over the summer but always stayed within the same range, with one exception. On September 5, 2005, Belwood Lake's hypolimnetic TP was considerably higher at 36 µg/L than the TP of the other two reservoirs (Figure 4.14). Conestogo and Guelph lakes both had a peak TP of 27 µg/L which was reached on September 22, 2005 and September 5, 2005, respectively. Epilimnetic TP in Conestogo and Guelph lakes were similar to each other on all sampling dates and never surpassed 25 µg/L (Figure 4.13). Belwood Lake showed a dramatic increase in TP which commenced August 23, 2005, and continued to increase until sampling ceased (Figure 4.13). By October 13, 2005 Belwood Lake had reached an epilimnetic TP of 54 µg/L (Figure 4.13).

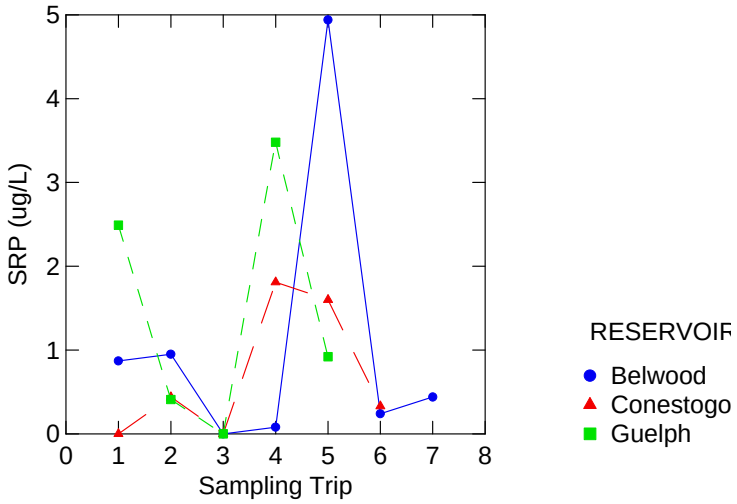


Figure 4.10. Epilimnetic soluble reactive P levels from Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

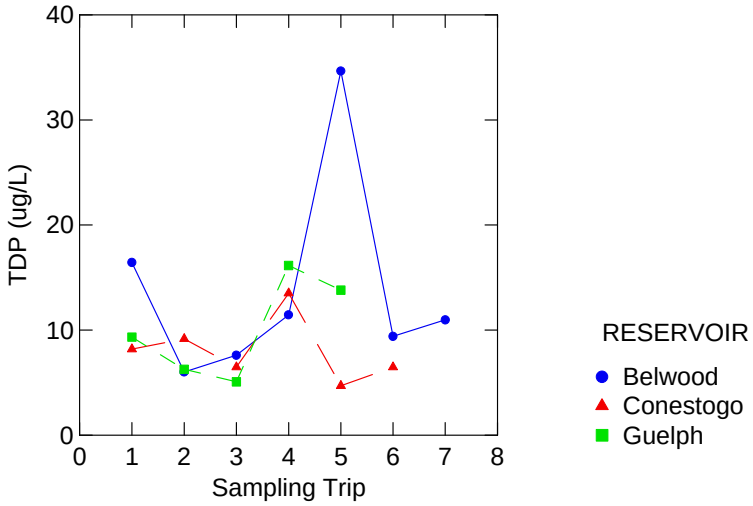


Figure 4.11. Epilimnetic total dissolved P levels from Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

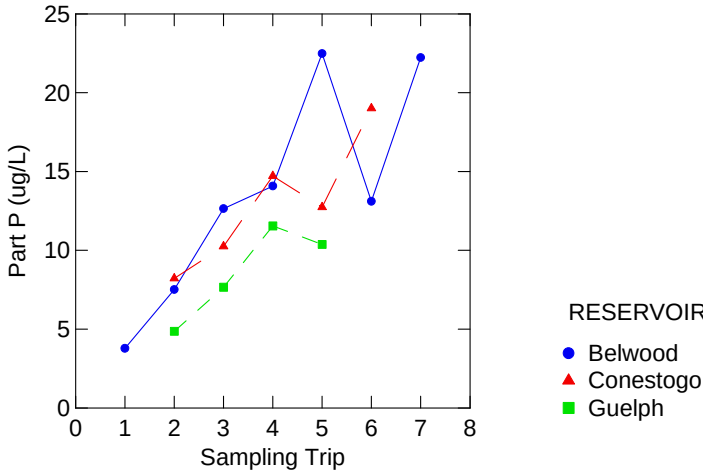


Figure 4.12. Epilimnetic particulate P levels in Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

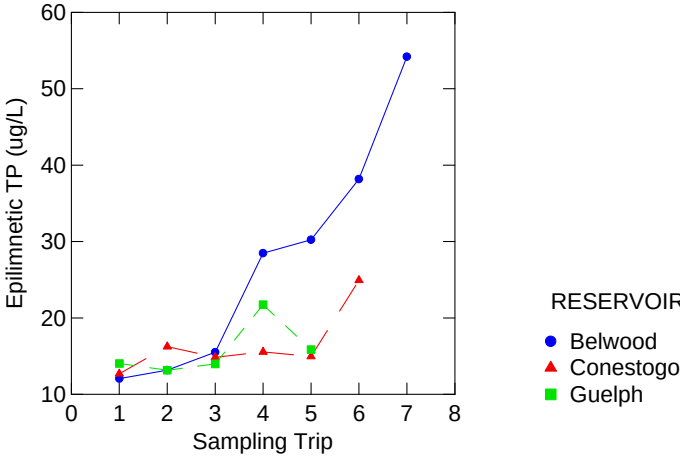


Figure 4.13. Epilimnetic total P levels in Belwood, Conestogo, and Guelph lakes in 2005. Samples were always taken from 2m with the exception of the Belwood sample on trip #7. Sampling trip dates are listed in Table 4.2.

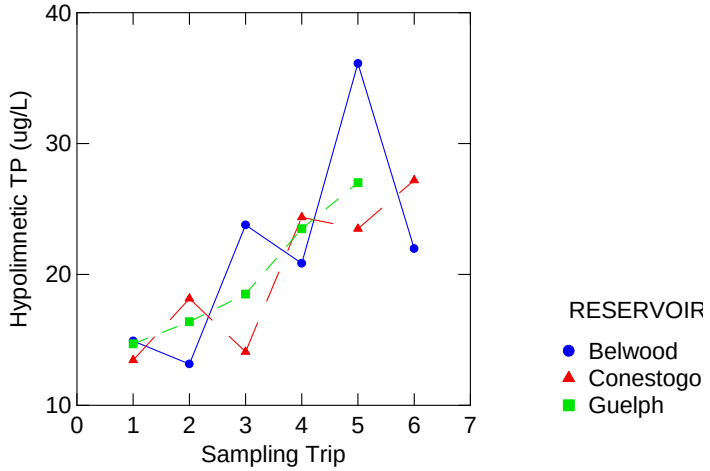


Figure 4.14. Hypolimnetic total P from Belwood, Conestogo, and Guelph lakes in 2005. Depths sampled were typically 10m for Belwood Lake, 7m for Conestogo Lake, and 6m for Guelph Lake. Sampling trip dates are listed in Table 4.2.

4.3.4 Nitrogen

NH_3 dynamics in each reservoir exhibited approximately the same pattern of increases and decreases throughout the summer (Figure 4.15). This pattern of NH_3 levels was similar to the pattern of mixing depth. Conestogo Lake contained much more dissolved inorganic nitrogen (DIN) than either Belwood or Guelph lakes. All forms of DIN were approximately twice as high in Conestogo Lake at the beginning of sampling on July 6, 2005 (Figures 4.15, 4.16, and 4.17). NO_3 levels in Conestogo lake stayed well above those in the other reservoirs throughout the summer (Figure 4.16), but showed the same drawdown as the other two reservoirs. In contrast, NO_2 levels in Constogo Lake exhibited a net increase throughout the summer while Belwood and Guelph levels declined (Figure 4.17). The concentration of NO_3 dropped below the level of NH_3 in Belwood and Guelph lakes on August 23, 2005 and in Belwood Lake on September 5, 2005. On all other occasions, NH_3 levels were lower than NO_3 levels as was expected. In comparison to NO_3 levels, NO_2 was not a substantial portion of the DIN (Figures 4.16 and 4.17).

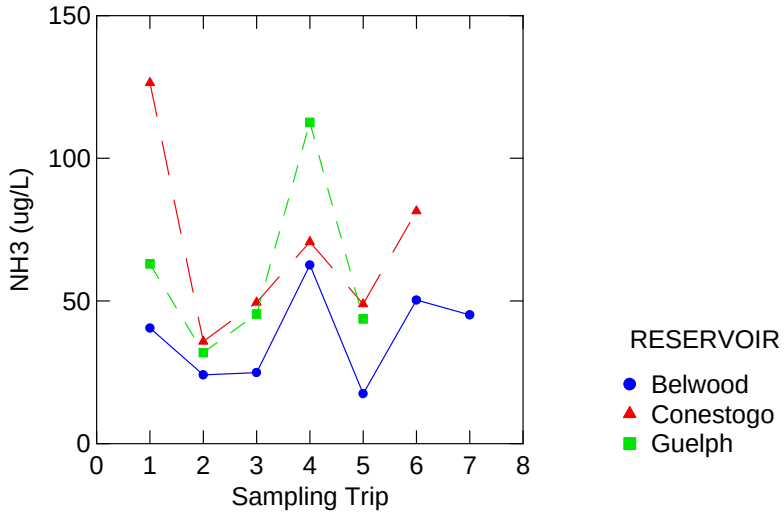


Figure 4.15. Epilimnetic ammonia levels in Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

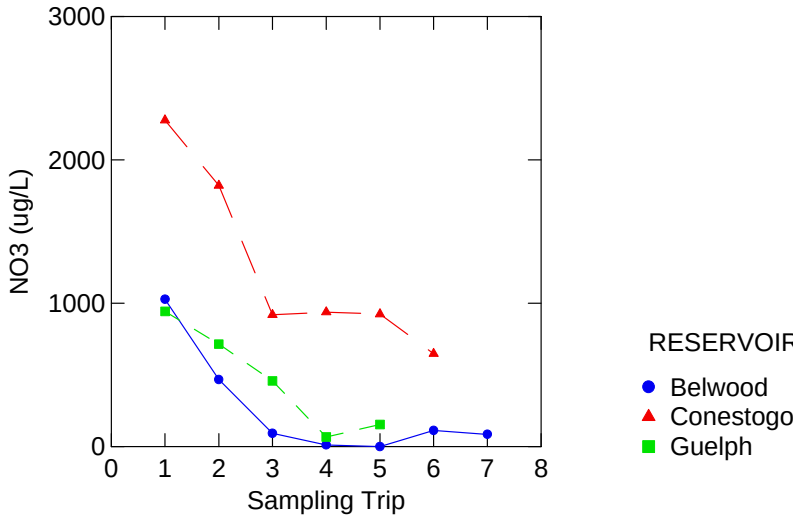


Figure 4.16. Epilimnetic nitrate levels in Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

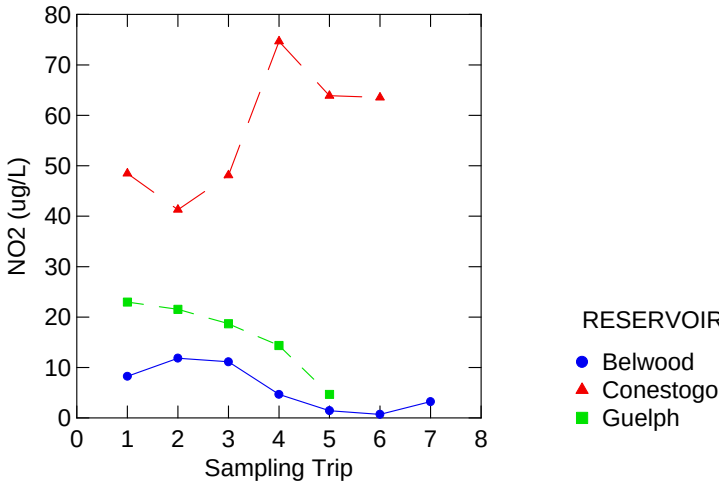


Figure 4.17. Epilimnetic nitrite levels in Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

Particulate N increased in all three reservoirs in similar increments and proportions to epilimnetic chlorophyll *a* (Figures 4.18 and 4.8). As with chlorophyll, particulate N was highest in Belwood Lake, then Conestogo Lake, and finally Guelph Lake. Epilimnetic TN peaked in each reservoir early in the season (Conestogo: July 6, 2005, 5744 µg/L; Belwood: July 21, 2005, 5340 µg/L; Guelph: July 21, 2005, 4120 µg/L). Following these peaks, TN declined and remained between 440 and 1970 µg/L in all reservoirs at all times (Figure 4.19). From August 11 on, TN was higher in the hypolimnion than it was in the epilimnion (Figures 4.20 and 4.19).

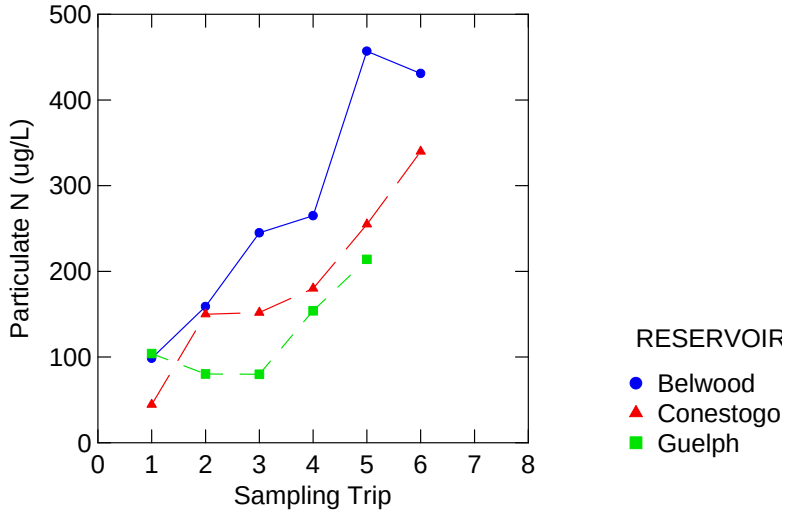


Figure 4.18. Epilimnetic particulate N in Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

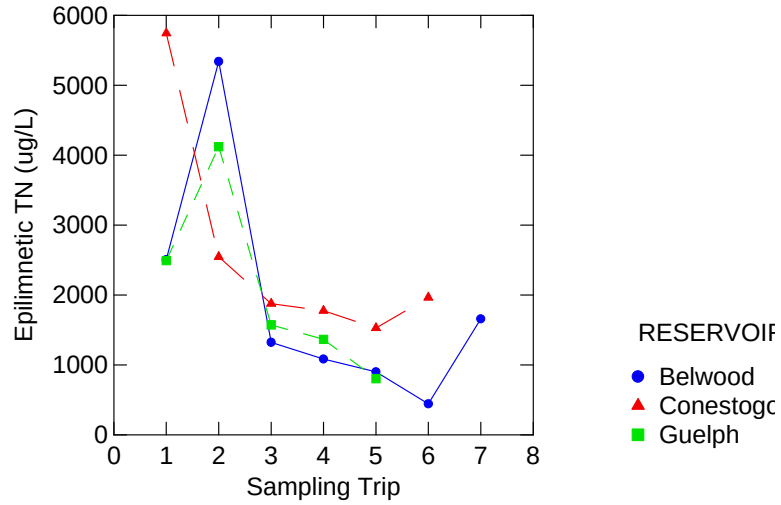


Figure 4.19. Epilimnetic total N levels in Belwood, Conestogo, and Guelph lakes in 2005. Samples were always taken from 2m with the exception of the Belwood sample on trip #7. Sampling trip dates are listed in Table 4.2.

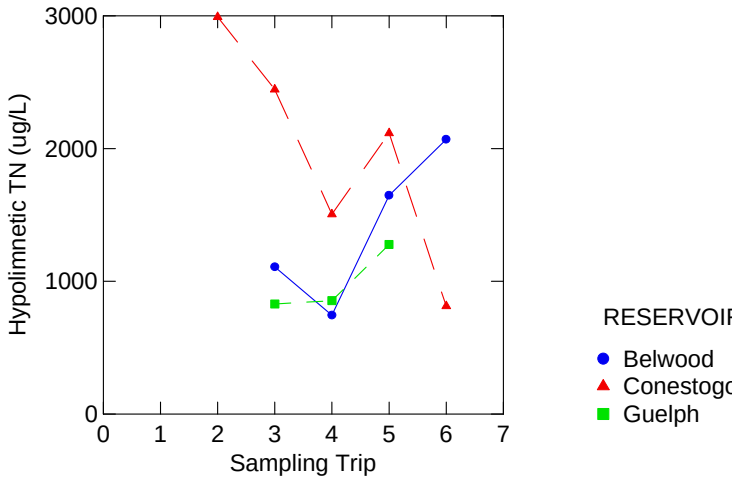


Figure 4.20. Hypolimnetic total N levels in Belwood, Conestogo, and Guelph lakes in 2005. Depths sampled were typically 10m for Belwood Lake, 7m for Conestogo Lake, and 6m for Guelph Lake. Sampling trip dates are listed in Table 4.2.

4.3.5 Nutrient Ratios

Epilimnetic TN:TP ratios follow nearly the same pattern as epilimnetic TN over the sampling period (Figures 4.21 and 4.19). TN:TP in the epilimnion was extremely high in July (350 - 1000 molar ratio) in all reservoirs. In August and September, TN:TP dropped in the epilimnion and Conestogo Lake consistently had the highest TN:TP followed by Guelph Lake and finally Belwood Lake. In the hypolimnion, Belwood and Guelph lakes showed a remarkably similar pattern of TN:TP that had a modest range of 79-105 molar ratio (Figure 4.22). The pattern of TN:TP in the hypolimnion in Conestogo Lake was distinct from the other two reservoirs and ranged widely from 136 to 388 molar ratio (Figure 4.22).

Particulate C to N ratios were relatively low in the three reservoirs throughout the sampling period (Figure 4.23). Belwood Lake C to N ratio were always in the range indicative of no N deficiency (Guildford et al. 1994). In July and early August, three samples from Conestogo Lake and two from Guelph Lake were just above the threshold of moderate N deficiency and all other samples from Conestogo and Guelph reservoirs showed no signs of deficiency. The particulate C to P ratios

were relatively high and all indicated some level of P deficiency (Figure 4.24). Belwood Lake showed signs of extreme P deficiency throughout the entire sampling period. Conestogo Lake seston had C to P ratios suggesting extreme deficiency on three trips and moderate deficiency on two trips. Guelph Lake showed extreme P deficiency on July 21 and September 5 but moderate P deficiency in August.

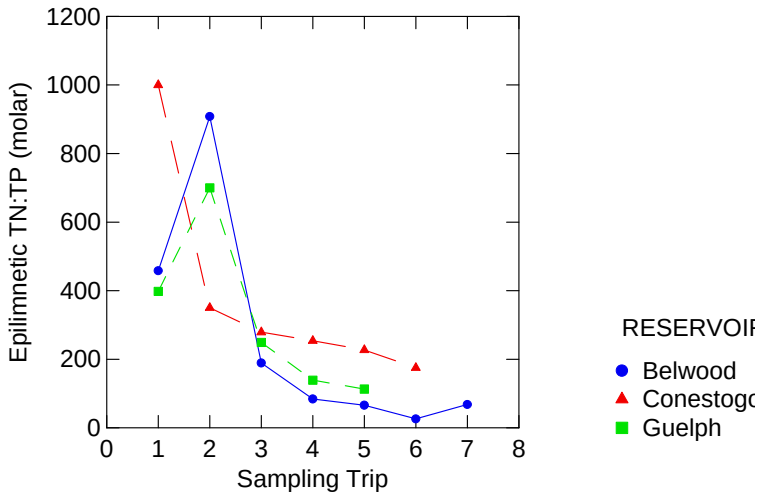


Figure 4.21. Epilimnetic TN to TP molar ratios from Belwood, Conestogo, and Guelph lakes in 2005. Samples were taken from 2m with the exception of the Belwood sample on trip #7. Sampling trip dates are listed in Table 4.2.

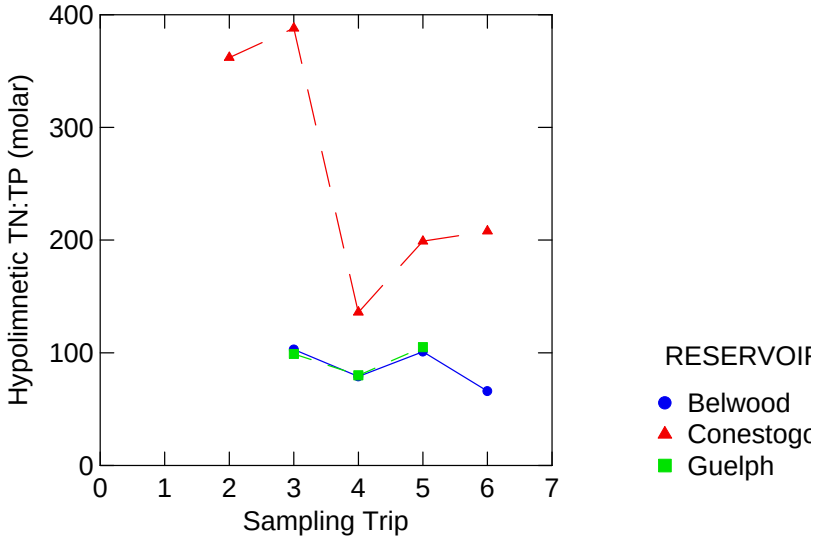


Figure 4.22. Hypolimnetic TN to TP molar ratios from Belwood, Conestogo, and Guelph lakes in 2005. Depths sampled were typically 10m for Belwood Lake, 7m for Conestogo Lake, and 6m for Guelph Lake. Sampling trip dates are listed in Table 4.2.

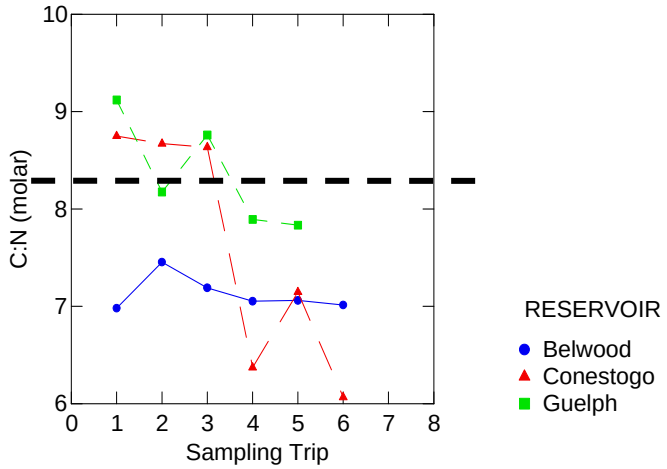


Figure 4.23. Epilimnetic particulate C to N molar ratios from Belwood, Conestogo and Guelph lakes from 2005. The line indicates a ratio of 8.3 above which moderate N deficiency is suggested. Sampling dates can be found in Table 4.2.

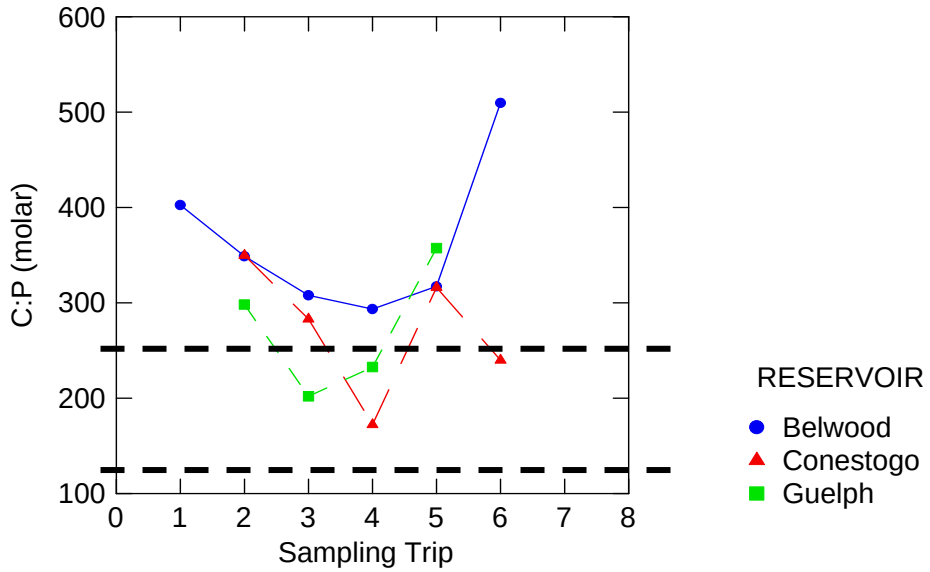


Figure 4.24. Epilimnetic particulate C to P molar ratios from Belwood, Conestogo and Guelph lakes in 2005. The lines indicate the range of ratios between 129 and 258 that suggests moderate P deficiency. Ratios above 258 suggest extreme P deficiency. Sampling dates can be found in Table 4.2.

4.3.6 Fluoroprobe and PAM

Fluoroprobe profiles can be found in Figure 4.3. The concentrations of cyanobacterial pigments as detected by the fluoroprobe at 2m are shown in Figure 4.25. According to the Fluoroprobe, cyanobacterial abundance was very similar between reservoirs in July. Guelph Lake showed very little increase in cyanobacterial abundance over the summer. After July, cyanobacterial abundance in Belwood Lake increased dramatically and increased as well, although to a lesser degree, in Conestogo Lake (Figure 4.25). Figure 4.26 illustrates the percentage of total chlorophyll detected by the Fluoroprobe that was attributed to Cyanobacteria. Guelph Lake had the most constant percent cyanobacteria that hovered around 50%. Constestogo Lake showed some fluctuations in July but then a steady increase throughout August and September, reaching a peak of 63% on September 22, 2005.

Belwood Lake exhibited the widest fluctuations and also the highest percent cyanobacteria, which peaked at 76% on October 13, 2005 during the cyanobacterial bloom.

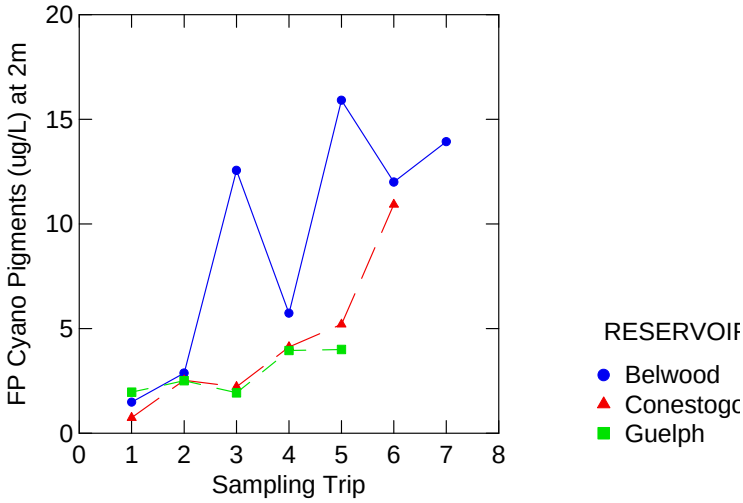


Figure 4.25. 2005 concentrations of cyanobacteria-specific pigments in Belwood, Conestogo, and Guelph lakes as determined by the Fluoroprobe.

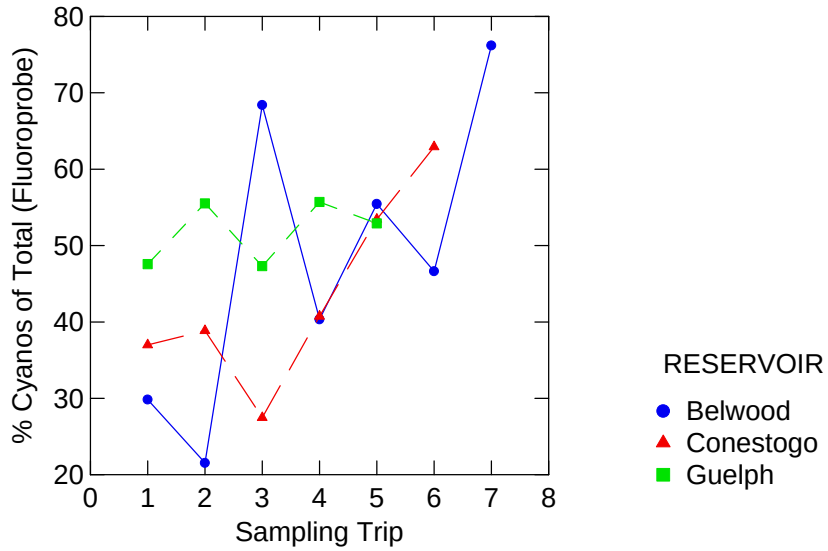


Figure 4.26. Percent cyanobacteria as determined by the fluoroprobe in Belwood, Conestogo, and Guelph lakes in 2005.

From the Fluoroprobe profiles it can be seen that all reservoirs were strongly stratified on July 6, 2005 and all had epilimnia of approximately 6m in depth (Figure 4.3). Only Belwood shows a hypolimnion of uniform depth, however. Guelph and Conestogo lakes exhibit steadily decreasing temperatures from the bottom of the epilimnion until the profile ends. On July 21, 2005 Conestogo remained strongly stratified but Belwood and Guelph lakes show a temperature gradient in the epilimnion. By August 11, 2005, strong stratification had been restored in all three reservoirs. The August 23, 2005 profiles show the effects of strong winds from the recent storm. Belwood Lake mixed deeply as did Guelph Lake although Conestogo still possessed a thermocline and shallow hypolimnion. Stratification was partially restored in all reservoirs by September 5, 2005 and continued through September 22. When Belwood Lake was profiled on October 13, 2005 the water column showed some temperature structure and the surface bloom which was comprised mainly of cyanobacteria is evident from 0 to 1m.

The Fv/Fm values (Figure 4.27) are quite variable temporally and between reservoirs. One pattern that can be seen is that all three reservoirs had relatively high variable fluorescence on August 23, 2005. This coincides with the major storm and the peak concentrations of TDP and SRP.

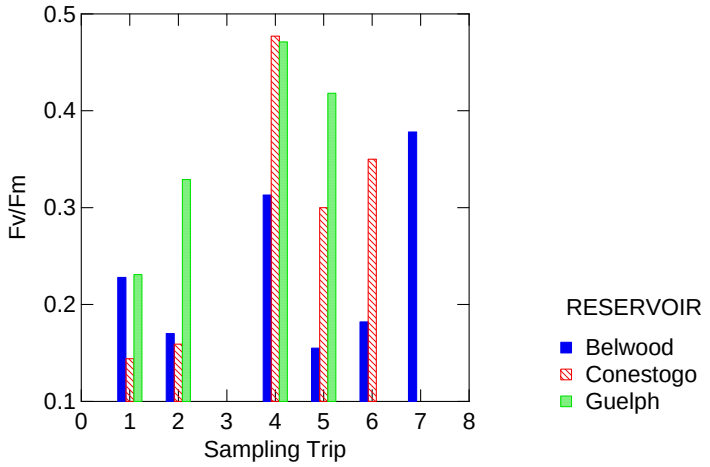


Figure 4.27. Variable fluorescence (Fv/Fm) as determined by the Diving-PAM in Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

4.3.7 Microcystin

Microcystin dynamics, which are illustrated in Figure 4.28, were distinct in each reservoir. The data presented are total microcystin concentrations (particulate and dissolved) in microcystin-LR equivalents. Only Belwood Lake surpassed the World Health Organization's safety threshold level of 1 µg/L (on September 22, 2005). Conestogo Lake had consistently low levels of microcystin which never exceeded 0.2 µg/L. Guelph Lake exhibited a nearly linear increase in microcystin from July 21 to September 5, 2005 but measured levels still did not exceed 0.6 µg/L. Because sampling past September 5 did not occur in Guelph Lake possible further increases in microcystin concentrations were not observed. Microcystin levels in Belwood Lake increased in an exponential fashion from July 6 to September 22, 2005, after which the microcystin concentration dramatically dropped off.

Linear regression was performed with total microcystin as the dependent variable to identify statistical relationships. The statistically significant and nearly significant results are listed in Table 4.3. Microcystin had significant positive regressions (P<0.05) with chlorophyll *a* (Figure 4.29), the attenuation coefficient, C:P ratios, the cyanobacteria estimate at 2m by the Fluoroprobe, and TP. Microcystin had significant negative regressions (P<0.05) with TN, the TN:TP ratio, and NO_3. Microcystin had nearly statistically significant negative regressions with DON (P=0.051) and Secchi depth (P=0.55).

Table 4.3. Summary of linear regressions performed with total microcystin as the dependent variable. Significant regressions are in bold. Regressions that neared statistical significance are also listed. 'Epi. Avg.'= average of entire mixed layer.

Independent Variable	*R2*	*P-value*
Chlorophyll *a*	**0.53**	**<0.01**
Kd	**0.50**	**<0.01**
TN	**0.34**	**<0.05**
TN:TP	**0.30**	**<0.05**
C:P	**0.28**	**<0.05**
NO3	**0.28**	**<0.05**
FP Cyanos - 2m	**0.23**	**<0.05**

TP	**0.22**	**<0.05**
DON	0.23	0.051
Secchi	0.21	0.055

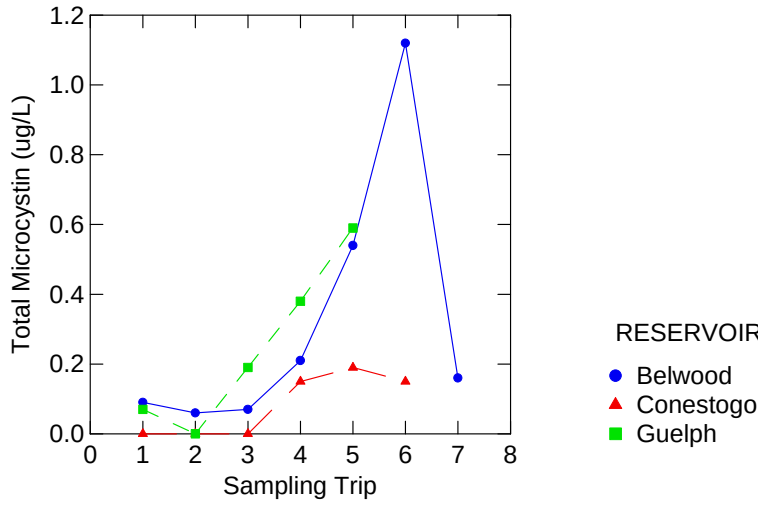


Figure 4.28. Total microcystin levels (epilimnetic) in Belwood, Conestogo, and Guelph lakes in 2005. Sampling trip dates are listed in Table 4.2.

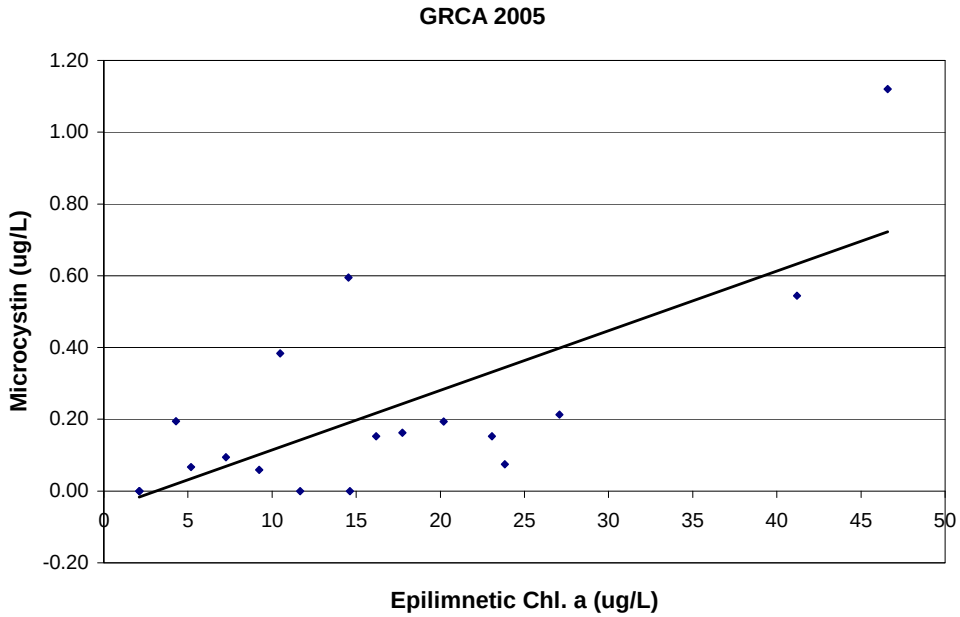


Figure 4.29. Linear regression of epilimnetic microcystin and epilimnetic chlorophyll in all three GRCA reservoirs on all dates in 2005, R^2=0.53, P<0.01.

4.4 Discussion

Because Belwood, Conestogo, and Guelph reservoirs are in close proximity, they experience very similar climatic influences. Also, because they are all managed by the GRCA, they presumably experience similar discharge regimes. Many parameters presented here follow a similar pattern in all three reservoirs. In particular, the same pattern in mixing depth seen in all reservoirs shows the effect of commonly experienced weather patterns. Comparing these reservoirs is, therefore, valid and useful, particularly to better understand how and why they differ. Belwood Lake stands out from the other two reservoirs in that it had the highest microcystin and cyanobacteria levels. Observing which variables separate Belwood Lake from the other reservoirs could help explain the occurrence of microcystin and blooms.

4.4.1 Light and Water Column Stability

The steady decline in Secchi depth observed follows the steady increase in chlorophyll *a*, therefore water transparency decreases can mainly be attributed to increased phytoplankton biomass and not suspended sediment. Belwood Lake had the lowest light environment for phytoplankton as indicated by mean irradiance. Buoyancy-regulating cyanobacteria in Belwood Lake may then have had an advantage over other phytoplankton if light was limiting as they could migrate closer to the surface (Ganf and Oliver 1982). In fact, microcystin had a highly significant positive relationship with the light attenuation coefficient (R^2=0.5, P<0.01). Therefore, microcystin-producing cyanobacteria thrived under low light conditions. This phenomenon has been observed in other studies such as Sabour and coworkers (2005) who found that the occurrence of an *Anabaena* bloom coincided with strong light attenuation.

For the majority of the summer, the water columns of these reservoirs were stratified, which is a condition known to promote cyanobacteria. It is unlikely that water column stability was important in controlling cyanobacterial abundance and microcystin levels, however, because all

reservoirs were stratified while cyanobacterial biomass and microcystin concentrations varied greatly between reservoirs.

4.4.2 Phosphorus

Conestogo and Guelph exhibited their peak SRP and TDP immediately following the major summer storm which broke stratification and allowed for epilimnetic nutrient replenishment. Belwood Lake, however, had a two-week delay before its peak SRP and TDP. Because Belwood had a higher phytoplankton biomass than the other two reservoirs, perhaps phytoplankton rapidly took up the surge of P when overturn began. This could have caused the observed delay increase in dissolved P.

The fact that TP was so similar between reservoirs in early July but differed greatly late in the summer is intriguing. Belwood Lake's TP increased dramatically throughout the season. More fertilizer runoff could have been reaching Belwood than the other reservoirs. Or, perhaps decomposition of the higher phytoplankton biomass in Belwood caused greater hypolimnetic oxygen depletion resulting in P release from the sediments (Kalff 2003). Whatever the cause, TP, cyanobacterial biomass, and microcystin were all higher in Belwood. The significant positive relationship between total microcystin and TP supports part of my third hypothesis. The hypothesized positive relationship between SRP and microcystin was not supported.

4.4.3 Nitrogen and TN:TP

Conestogo Lake was much richer in DIN than were the other two reservoirs. The Grand River catchment basin is home to a large number of livestock (Dorner et al. 2004) which produce N-rich wastes. Since the Conestogo catchment basin has the highest percentage of agricultural land use of any of the reservoirs (Table 4.4), high levels of agricultural runoff may have contributed to this elevated DIN.

Microcystin showed a significant negative relationship with TN:TP as was hypothesized. In general, the TN to TP ratios in these reservoirs were not sensitive to changes in TP since the levels of TN were so high. The decreased TN:TP reflects a substantial drop in TN.

Table 4.4. Breakdown of Belwood, Conestogo, and Guelph watersheds by land type. 2005 data was used with permission of Luis Leon and originally compiled by Lesley-Ann Chiavaroli.

Watershed Characteristic	Belwood	Conestogo	Guelph
Agricultural (%)	80.8	89.9	69.7
Forested (%)	9.2	7	20.7
Water (%)	5.4	2.1	1.7
Wetlands (%)	3.2	0.1	0.4
Urban (%)	1.2	0.8	0.7

4.4.4 Chlorophyll and Fluoroprobe Results

Epilimnetic chlorophyll *a* had the strongest positive relationship with microcystin that was observed (R^2=0.53, P<0.01). This association is a useful finding which, if confirmed in other years, could be used to identify lakes in need of microcystin testing.

The percent cyanobacteria, as determined by the Fluoroprobe, in Guelph and Conestogo lakes declined between July 21 and August 11 while the percent cyanobacteria in Belwood Lake increased from 22% to 68% during the same period. This occurred when NH_3 and NO_3 were low in all reservoirs, but were lowest in Belwood Lake. Perhaps N fixation or migration to N sources by cyanobacteria gave them an advantage over other types of phytoplankton in Belwood Lake. This is plausible since microcystin had significant negative relationships with both TN and NO_3.

Although percent cyanobacteria was not related to microcystin levels, cyanobacterial biomass at 2m, as detected by Fluoroprobe, had a significant positive regression with microcystin. This supports my second hypothesis that higher microcystin levels will be associated with potentially

toxic species. Because no microscopic identification was performed on the GRCA samples, no more is known about the predominant cyanobacterial species present.

4.4.5 Nutrient Status Indicators

Based on the C:N molar ratios, it appears that there was little to no N deficiency in Guelph and Conestogo lakes and no N deficiency in Belwood Lake. In contrast, the C:P ratios suggest that there was moderate and extreme P limitation in Guelph and Conestogo lakes and always extreme P limitation in Belwood Lake. The C:P ratio had a significant positive relationship with microcystin concentrations. This means that more microcystin was found when phytoplankton was relatively poor in P. This is contrary to my first hypothesis that cells in favourable growth conditions will be associated with higher microcystin levels. If lower C:P is correlated with higher growth rate in the GRCA reservoirs, then this observation suggests that cyanobacteria with slower growth rates produced more microcystin. This would be contrary to the assertions of some researchers that growth rate and microcystin production rate are positively related (Oh et al. 2000). However, the microcystin was relatively low in the reservoirs.

4.5 Conclusion

In these three reservoirs, a gradient of environmental variables was observed between sampling dates and between reservoirs. This allowed for the comparison of reservoirs and the conditions that affected microcystin conditions in all of them. Regressions between microcystin and environmental parameters across all three reservoirs revealed that chlorophyll *a* (epilimnetic) concentrations explained the greatest amount of variation in microcystin. Differences in light attenuation, TN, TN:TP ratios, C:P ratios, NO_3, cyanobacterial biomass, and TP also exhibited significant relationships with microcystin concentrations. Even though it appears that little to no N limitation

was occurring, N levels were still associated with microcystin levels. Extreme P limitation was observed in Belwood Lake, the reservoir with the highest microcystin concentrations.

The major summer storm that occurred in 2005 appears to have strongly affected the concentrations of nutrients, namely dissolved P and NH_3, that were available for phytoplankton uptake. As this likely affected microcystin concentrations, the potential importance of external physical drivers must be considered when studying microcystin in a system.

These data suggest that the occurrence of a cyanobacterial surface bloom does not necessarily indicate worrisome levels of microcystin. However, cyanobacteria with the potential to produce microcystin do occur in all three of these reservoirs and cyanobacteria were seen to make up a large portion of the total phytoplankton biomass throughout the summer.

Chapter 5
Conclusions

5.1 Summary of Hypothesis Testing for Individual Water Bodies

1) If nutrient status affects microcystin concentrations and favourable growth conditions result in more microcystin production, then indicators of greater nutrient deficiency will be negatively associated with microcystin levels.

Using the C:P and C:N seston ratios to indicate nutrient status, all study sites showed evidence that likely no N limitation was taking place. The C:P ratios suggest that in the GRCA reservoirs there was moderate and extreme P limitation, in the Bay of Quinte there was moderate P limitation in July and extreme P limitation in September, and in Maumee Bay there was moderate P limitation.

Hypothesis #1 was supported by the C to P ratio data from the Bay of Quinte. In July when C:P indicated moderate P deficiency a mean of 2.3 μg/L microcystin was detected, but in September when ratios suggested extreme P deficiency the microcystin level was a mere 0.58 μg/L. No relationship with PAM parameters was seen. A trend suggested that lower C:N ratios were seen when microcystin concentrations were lower. If this trend is valid, it is in the opposite direction as was hypothesized.

With the Maumee Bay data no significant differences between sampling dates in C:N ratios, C:P ratios, or PAM parameters were found.

Data from the Grand River reservoirs show the opposite pattern of what was expected. Higher microcystin concentrations were associated with higher C:P ratios. This occurred while P limitation was likely present in all three reservoirs.

2) If the abundance of different cyanobacterial groups contributes to microcystin concentrations, then dominance by particular potentially toxic species will be associated with higher microcystin concentrations.

In the Bay of Quinte potentially toxic cyanobacterial biomass in general was not associated with higher microcystin concentrations. It may be that *Microcystis* in the Bay of Quinte is particularly toxic and that its biomass is associated with higher microcystin levels but further testing would be required to address this.

In Maumee Bay increased potentially toxic cyanobacterial abundance (*Microcystis*) was associated with higher microcystin levels. One must be cautious, however, to not overemphasize this result since there were only two time points sampled and one had extremely low phytoplankton biomass.

In the GRCA reservoirs, microcystin concentrations were positively correlated with cyanobacterial biomass, but it is not known how much of that cyanobacteria was potential toxin producers.

3) Greater water column stability, a low N to P ratio, higher SRP and TP, the presence of dreissenids, and decreased water transparency all promote the production of microcystin and that they will be positively associated with microcystin concentrations.

Table 5.1 lists several of the above measured parameters and whether or not they were positively associated with microcystin concentrations. Maumee Bay and the GRCA reservoirs shared the same pattern of variables which were potential promoters of elevated microcystin concentrations: low TN:TP, high TP, and low water transparency. The Bay of Quinte showed the opposite pattern and only high SRP was associated with increased microcystin levels. Although none of the study sites showed a positive relationship between water column stability and microcystin concentrations, all of these sites are sheltered and relatively calm.

Table 5.1. Summary of variables hypothesized to be associated with higher microcystin concentrations and the results hypothesis testing.

	Association with Higher Microcystin?		
Variable	**Bay of Quinte**	**Maumee Bay**	**GRCA Reservoirs**
Greater Water Column Stability	No	No	No
Decreased TN:TP	No	Yes	Yes
Increased SRP	Yes	No	No
Increased TP	No	Yes	Yes
Decreased Transparency	No	Yes	Yes

The presence of dreissenids was not analyzed, however some observations can be made. The Bay of Quinte and Maumee Bay both have problems with high *Microcystis* abundance that is likely promoted by dreissenids (Nicholls and Hopkins 1993, Nicholls et al. 2002, Vanderploeg et al. 2001). These bays also had the highest microcystin levels observed in this study. The Grand River reservoirs are not known to contain dreissenids and they contained much less microcystin than the Bay of Quinte and Maumee Bay.

5.2 Bloom Formation and Implications for Toxicity

In each of the Bay of Quinte, Maumee Bay, and Belwood Lake a surface cyanobacterial bloom was observed. These blooms represented a gradient of toxicity, with Belwood Lake's October 2005 bloom being barely toxic (0.16 ug/L total microcystin, Appendix C), the Bay of Quinte's July 2006 bloom being moderately toxic (mean total microcystin: 2.25 ug/L, Appendix A) and Maumee Bay's August 2006 bloom being the most toxic (mean total microcystin: 4.65 ug/L, Appendix B). The occurrence of a bloom cannot be used as a proxy for microcystin measurement, although concern over toxicity is warranted given the high concentration of potentially toxic species. The Bay of Quinte's bloom was dominated by *Microcystis* (97%) but Maumee Bay's bloom was dominated by a non-microcystin producer, *Aphanizomenon,* and had only 22% *Microcystis*, so even detailed counts cannot necessarily indicate toxicity.

5.3 Overall Trends with Microcystin

By combining all of the data from the Bay of Quinte, Maumee Bay, and the GRCA reservoirs, a wider range of variables is represented and any general trends between microcystin concentrations and environmental parameters can be seen. The relationship between total microcystin and C:P ratio is illustrated in Figure 5.1. Linear regression was performed and this relationship is not statistically significant (R^2=0.041, *P*=0.245). However, many of the high microcystin values occur at lower C:P ratios. Based on the observation that microcystin production is higher under ideal nutrient conditions, this is logical (Orr and Jones 1998). The plot of total microcystin against total extracted chlorophyll *a* can be seen in Figure 5.2 and this relationship was also non-significant (R^2=0.06, *P*=0.113). No high microcystin concentrations were found at chlorophyll *a* levels below 10 µg/L. The relationship between total microcystin and cyanobacterial chlorophyll, as determined by the Fluoroprobe, was examined as well (Figure 5.3). Although no correlation was found (R^2=0.007, *P*=0.615), all high microcystin concentrations occurred when the Fluoroprobe cyanobacterial chlorophyll was within the range of 2.7 to 6.0 µg/L.

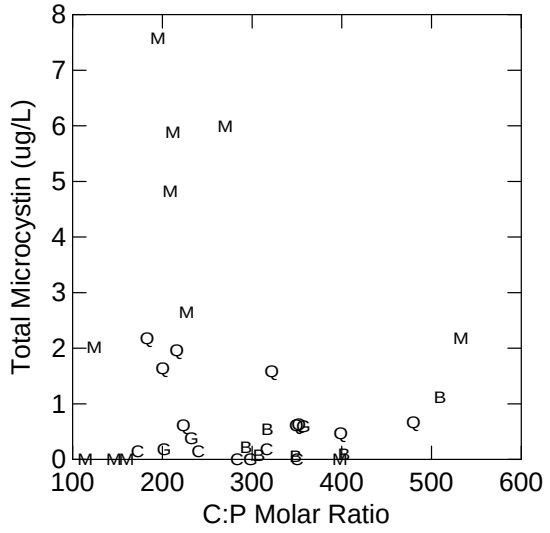


Figure 5.1. Plot of total microcystin vs. C:P molar ratio for all water bodies sampled in this study. M=Maumee Bay, Q=Bay of Quinte, B=Belwood Lake, C=Conestogo Lake, and G=Guelph Lake.

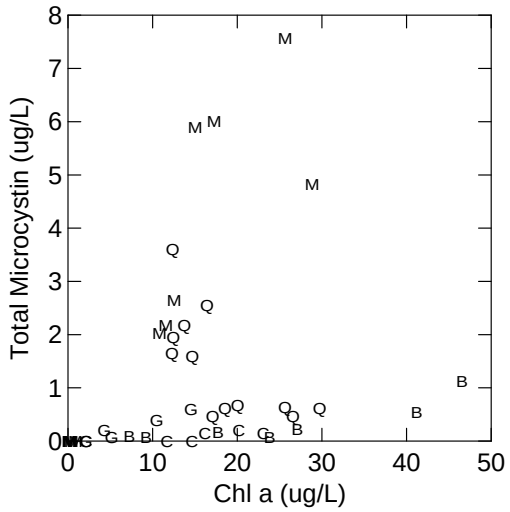


Figure 5.2. Total microcystin plotted against extracted chlorophyll *a* for all Maumee Bay, Bay of Quinte, and GRCA data. Symbols are as in Figure 5.1.

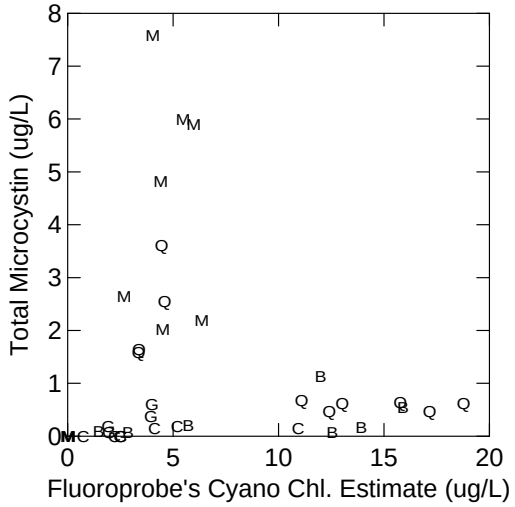


Figure 5.3. Total microcystin plotted against the Fluoroprobe's estimate of chlorophyll attributable to cyanobacteria for all water bodies in this study. Symbols are as in Figure 5.1.

A nearly significant negative relationship between total microcystin and TN:TP molar ratios is illustrated in Figure 5.4 (R^2=0.118, *P*=0.063). This graph is presented on a log x-axis to show the spread of data at lower TN:TP ratios where all of the higher microcystin concentrations occurred.

This relationship may not have been very strong because many of the TN:TP ratios encountered were relatively high whereas the reported optimal N:P ratio for *Microcystis* spp. is 4.1 (Smith 1982, Rhee and Gotham 1980). Another nearly significant relationship was observed between total microcystin and Fv/Fm for all pooled data (Figure 5.5). This positive relationship had an R^2 of 0.104 and a *P*-value of 0.059. The high microcystin values are clustered around the middle of the x-axis, just above 0.4. High microcystin values did not occur at low Fv/Fm values that are indicative of poor physiological health. This observation also supports the hypothesis that microcystin production is greater when phytoplankton are in better physiological condition.

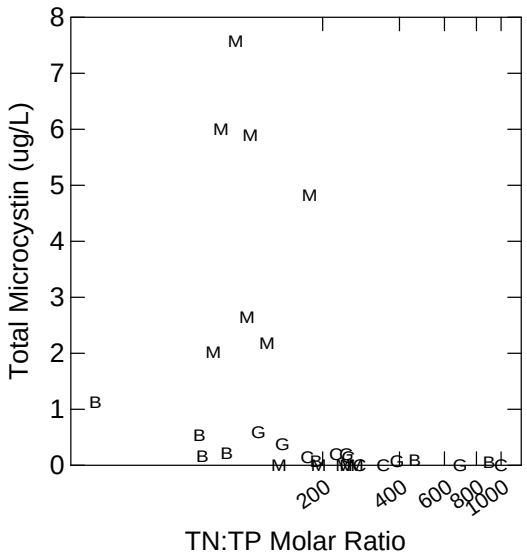


Figure 5.4. Total microcystin plotted against TN:TP molar ratios in all study sites. The x-axis in a log scale. Symbols are as in Figure 5.1.

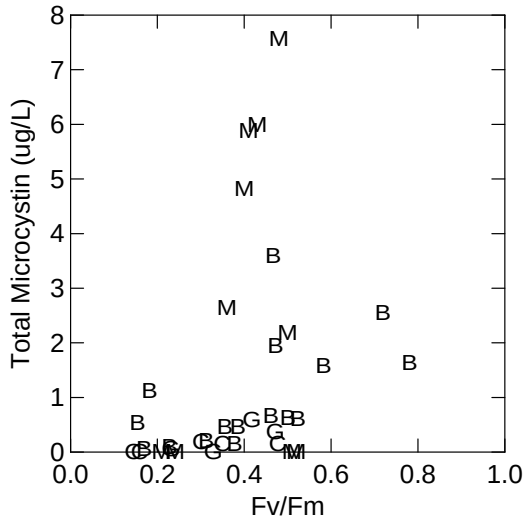


Figure 5.5. Total microcystin plotted against Fv/Fm variable fluorescence ratios for all study sites. Symbols are as in Figure 5.1.

The relationship between total microcystin and TP (Figure 5.6) was the most statistically significant found. Because of the non-linear nature of this relationship, the logarithms of the variables are presented. The log plus one transformation was used for total microcystin as some data points were 0.00 and could not be transformed by log alone. Linear regression on the transformed data revealed a highly significant relationship with an R^2 value of 0.290 and a *P*-value below 0.001. This relationship between TP and microcystin is similar to the results of Giani et al. (2005) who found that TP and TN were the best predictors of microcystin concentrations along a trophic gradient of southern Quebec lakes. Total microcystin was also significantly related to water temperature (Figure 5.7), although not as highly as were total microcystin and TP. This positive correlation had an R^2 of 0.131 and a *P*-value less than 0.05. High microcystin concentrations were typically found around 25^{o}C with some high levels occurring at temperatures just over 25^{o}C. This slightly exceeds the general temperature range of 18^{o}C to 25^{o}C in which toxic content was found to be highest in most studies reviewed by Sivonen and Jones (1999).

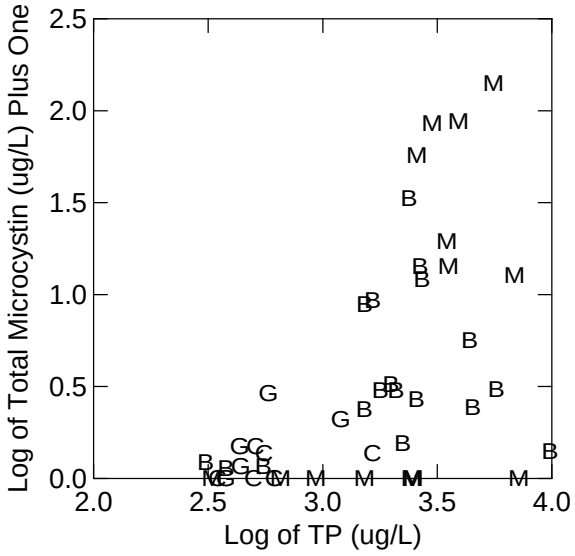


Figure 5.6. The log plus one of total microcystin plotted against the log of TP. The log plus one was used for the y-axis as some data points were 0.00. All water bodies in this study are represented. Symbols are as in Figure 5.1.

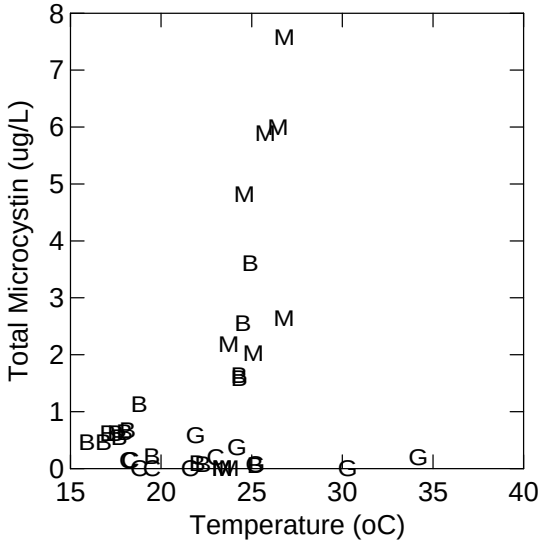


Figure 5.7. Total Microcystin plotted against Temperature for all study sites. Symbols are as in Figure 5.1.

5.4 Final Thoughts

Over the water bodies studied, TP was the best predictor of total microcystin concentrations, explaining 29% of the variation in toxin levels. Total microcystin's significant relationship with temperature and its nearly significant relationships with Fv/Fm and TN:TP ratios suggest that multiple variables were influencing microcystin levels at the same time. A multivariate analysis of these data is the next logical step. This approach would wisely be applied to future studies as well due to the complexity of factors that likely affect microcystin production. Because of the risks associated with microcystin exposure to people, aquatic ecosystems, and terrestrial animals, consistent microcystin testing is recommended, at least until this toxin's production is better understood.

Literature Cited

American Public Health Association. 1992. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington.

An J., W. W. Carmichael. 1994. Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. Toxicon **32**:1495-1507.

Arnott D. L., M. J. A. Vanni. 1996. Nitrogen and phosphorus recycling by the zebra mussel (*Dreissena polymorpha*) in the western basin of Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences **53**:646-659.

Baganz D., G. Staaks, S. Pflugmacher, and C. E. W. Steinberg. 2004. Comparative study of microcystin-LR-induced behavioral changes of two fish species, *Danio rerio* and *Leucaspius delineatus*. Environmental Toxicology **19**:564-570.

Bailey R. C., L. Grapentine, T. J. Stewart, T. Schaner, M. E. Chase, J. S. Mitchell, and R. A. Coulas. 1999. Dreissenidae in Lake Ontario: Impact assessment at the whole lake and Bay of Quinte spatial scales. Journal of Great Lakes Research **25**:482-491.

Behrenfeld M. J., A. J. Bale, Z. S. Kolber, J. Aiken, and P. G. Falkowski. 1996. Confirmation of iron limitation of phytoplankton photosynthesis in the Equatorial Pacific Ocean. Nature **383**:508-511.

Berman T. 2001. The role of DON and the effect of N:P ratios on occurrence of cyanobacterial blooms: Implications from the outgrowth of *Aphanizomenon* in Lake Kinneret. Limnology and Oceanography **46**:443-447.

Billam M., L. L. Tang, Q. S. Cai, S. Mukhi, H. X. Guan, P. W. Wang, Z. M. Wang, C. W. Theodorakis, R. T. Kendall, and J. S. Wang. 2006. Seasonal variations in the concentration of microcystin-LR in two lakes in western Texas, USA. Environmental Toxicology and Chemistry **25**:349-355.

Blomqvist P., A. Pettersson, and P. Hyenstrand. 1994. Ammonium-nitrogen: A key regulatory factor causing dominance of non-nitrogen-fixing cyanobacteria in aquatic systems. Archiv fur Hydrobiologie.Stuttgart **132**:141-164.

Bouaicha N., I. Maatouk, G. Vincent, and Y. Levi. 2002. A colorimetric and fluorometric microplate assay for the detection of microcystin-LR in drinking water without preconcentration. Food and Chemical Toxicology **40**:1677-1683.

Bridgeman T. B. 2005. The *Microcystis* blooms of western Lake Erie (2003-2004). Annual Conference on Great Lakes Research [Ann.Conf.Great Lakes Res.].Vol.48 .

Bronk D. A., P. M. Glibert, and B. B. Ward. 1994. Nitrogen uptake, dissolved organic nitrogen release, and anew production. Science **265**:1843-1846.

Budd J. W., A. M. Beeton, R. P. Stumpf, D. A. Culver, and W. C. Kerfoot. 2001. Satellite observations of *Microcystis* blooms in western Lake Erie. Verhandlungen Internationale Vereinigung fur Theorestische und Angewandte Limnologie **27**:3787-3793.

Bur M., J. Deller, D. Einhouse, B. Hass, T. Johnson, J. Markham, C. Murray, L. Rudstam, M. Thomas, B. Trometer, J. Tyson, and L. Witzel. 2002. Report of the Lake Erie Forage Task Group: March 2002. State of New York.

Bykova O., A. Laursen, V. Bostan, J. Bautista, and L. McCarthy. 2006. Do zebra mussels (*Dreissena polymorpha*) alter lake water chemistry in a way that favours *Microcystis* growth? Science of the Total Environment **371**:362-372.

Carmichael W. W. 1992. Cyanobacteria Secondary Metabolites - the Cyanotoxins. Journal of Applied Bacteriology **72**:445-459.

Carmichael W. W., J. An. 1999. Using an Enzyme Linked Immunosorbent Assay (ELISA) and a Protein Phosphatase Inhibition Assay (PPIA) for the Detection of Microcystins and Nodularins. Natural Toxins **7**:377-385.

Chorus I., J. Bartram. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring, and management. E & FN Spon, London.

Codd G. A., J. Lindsay, F. M. Young, L. F. Morrison, and J. S. Metcalf. 2005. Harmful Cyanobacteria: From mass mortalities to management measures. Pages 1-24 *In* J. Huisman, H. C. P. Matthijs, and P. M. Visser, editors. Harmful Cyanobacteria, Springer, Dordrecht, The Netherlands.

Conroy J. D., W. J. Edwards, R. A. Pontius, D. D. Kane, H. Zhang, J. F. Shea, J. N. Richey, and D. A. Culver. 2005. Soluble nitrogen and phosphorus excretion of exotic freshwater mussels (*Dreissena spp*.): potential impacts for nutrient remineralisation in western Lake Erie. Freshwater Biology **50**:1146-1162.

Dorner S. M., P. M. Huck, and R. M. Slawson. 2004. Estimating Potential Environmental Loadings of *Cryptosporidium spp*. and *Campylobacter spp*. from Livestock in the Grand River Watershed, Ontario, Canada. Environmental Science & Technology **38**:3370-3380.

Downing J. A., S. B. Watson, and E. McCauley. 2001. Predicting cyanobacteria dominance in lakes. Canadian Journal of Fisheries and Aquatic Sciences **58**:1905-1908.

Engelke C. J., L. A. Lawton, and M. Jaspars. 2003. Elevated microcystin and nodularin levels in cyanobacteria growing in spent medium of *Planktothrix agardhii*. Archiv fuer Hydrobiologie **158**:541-550.

Falconer I. R. 2005. Cyanobacterial toxins of drinking water supplies: cylindrospermopsins and microcystins. CRC Press, Boca Raton.

Falconer I. R. 1993. Mechanism of toxicity of cyclic peptide toxins from blue-green algae. Pages 177-186 *In* I. R. Falconer, editor. Algal Toxins in Seafood and Drinking Water, Academic Press, Cambridge.

Falkowski P. G., J. A. Raven. 1997. Aquatic photosynthesis, Blackwell Science, Malden, Mass.

Ferber L. R., S. N. Levine, A. Lini, and G. P. Livingston. 2004. Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? Freshwater Biology **49**:690-708.

Figueredo C. C., A. Giani, and D. F. Bird. 2007. Does allelopathy contribute to *Cylindrospermopsis raciborskii* (cyanobacteria) bloom occurrence and geographic expansion? Journal of Phycology

43:256-265.

Fontal O. I., M. R. Vieytes, J. M. V. B. de Sousa, M. C. Louzao, and L. M. Botana. 1999. A fluorescent microplate assay for microcystin-LR. Analytical Biochemistry **269**:289-296.

Fraleigh P. C., P. L. Klerks, and R. C. Stevenson. 1991. Temporal changes in zebra mussel (*Dreissena polymorpha* Pall.) veliger densities and veliger settling rates in western Lake Erie near Maumee Bay. Journal of Shellfish Research **10**:248.

Ganf G. G., R. L. Oliver. 1982. Vertical separation of light and available nutrients as a factor causing replacement of green algae by blue-green algae in the plankton of a stratified lake. Journal of Ecology **70**:829-844.

Genty B., J.M. Briantais, and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta **990**:87-92.

Giani A., D. F. Bird, Y. T. Prairie, and J. F. Lawrence. 2005. Empirical study of cyanobacterial toxicity along a trophic gradient of lakes. Canadian Journal of Fisheries and Aquatic Sciences **62**:2100-2109.

Goldberg J., H. B. Huang, Y. G. Kwon, P. Greengard, A. C. Nairn, and J. Kuriyan. 1995. 3-Dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. Nature **376**:745-753.

Graham L. E., L. W. Wilcox. 2000. Algae. Prentice-Hall, Inc., Upper Saddle River, NJ.
Grand River Conservation Authority. 1984. Guelph Lake Conservation Area: Times Past to Times Present. Grand River Conservation Authority, Cambridge.

Grand River Conservation Authority. 1980. Reservoirs: Water Quality. GRCA **1**:1-91.

Grasshoff K., M. Erhardt, and K. Kremling. 1983. Methods of Seawater Analysis 2**.**

Guildford S. J., L. L. Hendzel, H. J. Kling, E. J. Fee, G. G. C. Robinson, R. E. Hecky, and S. E. M. Kasian. 1994. Effects of lake size on phytoplankton nutrient status. Canadian Journal of Fisheries and Aquatic Sciences **51**:2769-2783.

Guildford S. J. and R. E. Hecky. 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnology and Oceanography **45**:1213-1223.

Harada K., K. Tsuji. 1998. Persistence and decomposition of hepatotoxic microcystins produced by cyanobacteria in natural environment. Journal of Toxicology: Toxin Reviews **17**:385-403.

Hartman WL. 1978. Effects of Exploitation, Environmental Changes, and New Species on the Fish Habitats and Resources of Lake Erie. Report nr 22.

Heresztyn T., B. C. Nicholson. 2001. Determination of cyanobacterial hepatotoxins directly in water using a protein phosphatase inhibition assay. Water Research **35**:3049-3056.

Holland R. E., T. H. Johengen, and A. M. Beeton. 1995. Trends in nutrient concentrations in Hatchery Bay, western Lake Erie, before and after *Dreissena polymorpha*. Canadian Journal of Fisheries and Aquatic Sciences **52**:1202-1209.

Holm N. P., D. E. Armstrong. 1981. Role of nutrient limitation and competition in controlling the populations of *Asterionella formosa* and *Microcystis aeruginosa* in semicontinuous culture. Limnology and Oceanography **26**:622-634.

Holmes R. M., A. Aminot, R. Kerouel, B. A. Hooker, and B. J. Peterson. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences **56**:1801-1808.

Honkanen R. E., J. Zwiller, R. E. Moore, S. L. Daily, B. S. Khatra, M. Dukelow, and A. L. Boynton. 1990. Characterization of microcystin-LR, a potent inhibitor of type 1 and type 2A protein phosphatases. Journal of Biological Chemistry **265**:19401-19404.

Huisman J., J. Sharples, J. M. Stroom, P. M. Visser, W. E. A. Kardinaal, J. M. H. Verspagen, and B. Sommeijer. 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology **85**:2960-2970.

Hyenstrand P., J. S. Metcalf, K. A. Beattie, and G. A. Codd. 2001. Losses of the cyanobacterial toxin microcystin-LR from aqueous solution by adsorption during laboratory manipulations. Toxicon **39**:589-594.

Ibelings B. W., L. R. Mur, and A. E. Walsby. 1991. Diurnal changes in buoyancy and vertical distribution in populations of *Microcystis* in two shallow lakes. Journal of Plankton Research **13**:419-436.

Johnson M. G., D. A. Hurley. 1986. Overview of Project Quinte - 1972-82. Pages 1-6 *In* C. K. Minns, D. A. Hurley, and K. H. Nicholls, editors. Project Quinte: Point-Source Phosphorus Control and Ecosystem Response in the Bay of Quinte, Lake Ontario, Canadian Special Publication of Fisheries and Aquatic Sciences 86, Ottawa.

Jones G. J., P. T. Orr. 1994. Release and degradation of microcystin following algicide treatment of a *Microcystis aeruginosa* bloom in a recreational lake, as determined by HPLC and protein phosphatase inhibition assay. Water Research **28**:871-876.

Kaebernick M., B. A. Neilan. 2001. Ecological and molecular investigations of cyanotoxin production. FEMS Microbiology Ecology **35**:1-9.

Kaebernick M., B. A. Neilan, T. Boerner, and E. Dittmann. 2000. Light and the Transcriptional Response of the Microcystin Biosynthesis Gene Cluster. Applied and Environmental Microbiology **66**:3387-3392.

Kalff J. 2003. Limnology. Prentice-Hall, Upper Saddle River.

Kardinaal W. E. A., P. M. Visser. 2005. Dynamics of Cyanobacterial Toxins: Sources of variability in microcystin concentrations. Pages 41-64 *In* J. Huisman, C. P. Matthijs, and P. M. Visser, editors. Harmful Cyanobacteria, Springer, Dordrecht, The Netherlands.

Kearns K. D., M. D. Hunter. 2001. Toxin-producing *Anabaena flos-aquae* Induces Settling of *Chlamydomonas reinhardtii*, a competing motile alga. Microbial Ecology **42**:80-86.

Kemeny D. M., S. J. Challacombe. 1988. ELISA and Other Solid Phase Immunoassays. John Wiley and Sons, Chichester, Great Britain.

Kotak B.G., A.K.-Y. Lam, E.E. Prepas, and S.E. Hrudey. 2000. Role of chemical and physical variables in regulating microcystin-LR concentration in phytoplankton of eutrophic lakes. Canadian Journal of Fisheries and Aquatic Sciences **57**:1584-1593.

Lam A. K.-Y., P. M. Fedorak, and E. E. Prepas. 1995. Biotransformation of the cyanobacterial hepatotoxin microcystin-LR, as determined by HPLC and protein phosphatase bioassay. Environmental Science and Technology **29**:242-246.

Lambert T. W., M. P. Boland, C. F. B. Holmes, and S. E. Hrudey. 1994. Quantitation of the microcystin hepatotoxins in water at environmentally relevant concentrations with the protein phosphatase bioassay. Environmental Science & Technology **28**:753-755.

Levine S. N., D. W. Schindler. 1999. Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. Canadian Journal of Fisheries and Aquatic Sciences **56**:451-466.

Liao C. F. 1977. The effect of nutrient enrichment of nitrogen fixation activity in the Bay of Quinte, Lake Ontario. Hydrobiologia **56**:273-279.

Lukac M., R. Aegerter. 1993. Influence of trace metals on growth and toxin production of *Microcystis aeruginosa*. Toxicon **31**:293-305.

Lyck S. 2004. Simultaneous changes in cell quotas of microcystin, chlorophyll *a*, protein and carbohydrate during different growth phases of a batch culture experiment with *Microcystis aeruginosa*. Journal of Plankton Research **26**:727-736.

MacIsaac H. J. 1996. Potential abiotic and biotic impacts of zebra mussels on the inland waters of North America. American Zoologist **36**:287-299.

MacKintosh C., K. A. Beattie, S. Klumpp, P. Cohen, and G. A. Codd. 1990. Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Letters **264**:187-192.

Mazur H., M. Plinski. 2001. Stability of cyanotoxins, microcystin-LR, microcystin-RR and nodularin in seawater and BG-11 medium of different salinity. Oceanologia **43**:329-339.

McCarthy J. J. 1972. The uptake of urea by natural populations of marine phytoplankton. Limnology and Oceanography **17**:738-748.

McElhiney J., L. A. Lawton, and C. Leifert. 2001. Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure. Toxicon **39**:1411-1420.

Meisner K., E. Dittmann, and T. Borner. 1996. Toxic and non-toxic strains of the cyanobacterium *Microcystis aeruginosa* contain sequences homologous to peptide synthetase genes. FEMS Microbiology Letters **135**:295-303.

Minns C. K. 1995. Approaches to assessing and managing cumulative ecosystem change, with the Bay of Quinte as a case study: an essay. Journal of Aquatic Ecosystem Health **4**:1-24.

Minns C. K., D. A. Hurley, and K. H. Nicholls. 1986. Project Quinte: Point-Source Phosphorus Control and Ecosystem Response in the Bay of Quinte, Lake Ontario. Canadian Special Publication of Fisheries and Aquatic Sciences 86, Ottawa.

Mountfort, D. P. Holland, J. Sprosen. 2005. Method for detecting classes of microcystins by combination of protein phosphatase inhibition assay and ELISA: comparison with LC-MS. Toxicon **45**:199-206.

Mountfort D. O., G. Kennedy, I. Garthwaite, M. Quilliam, P. Truman, and D. J. Hannah. 1999. Evaluation of the fluorometric protein phosphatase inhibition assay in the determination of okadaic acid in mussels. Toxicon **37**:909-922.

Nalewajko C., D. R. S. Lean. 1978. Phosphorus kinetics-algal growth relationships in batch cultures. Mitteilungen der internationale Vereinigung fur theoretische und angewandte Limnologie **21**:184-192.

Nicholls K. H., L. Heintsch, and E. Carney. 2002. Univariate step-trend and multivariate assessments of the apparent effects of P loading reductions and zebra mussels on the phytoplankton of the Bay of Quinte, Lake Ontario. Journal of Great Lakes Research **28**:15-31.

Nicholls K. H., G. J. Hopkins. 1993. Recent changes in Lake Erie (north shore) phytoplankton: Cumulative impacts of phosphorus loading reductions and the zebra mussel introduction.

Journal of Great Lakes Research **19**:637-647.

Nicholls K. H., L. Heintsch. 1986. A Comparison of the Net Phytoplankton in the Lower Bay of Quinte Near Indian Point, 1945 and 1981. Pages 159-176 *In* C. K. Minns, D. A. Hurley, and K. H. Nicholls, editors. Project Quinte: Point-Source Phosphorus Control and Ecosystem Response in the Bay of Quinte, Lake Ontario, Canadian Special Publication of Fisheries and Aquatic Sciences 86, Ottawa.

Nicholls K. H., L. Heintsch, and E. C. Carney. 2004. A multivariate approach for evaluating progress towards phytoplankton community restoration targets: Examples from eutrophication and acidification case histories. Aquatic Ecosystem Health & Management **7**:15-30.

Niessan W. M. A., J. van der Greef. 1992. Liquid Chromatography- Mass Spectrometry. Marcel Dekker, Inc., New York.

Nizan S., C. Dimentman, and M. Shilo. 1986. Acute toxic effects of the cyanobacterium *Microcystis aeruginosa* on *Daphnia magna*. Limnology and Oceanography **31**:497-502.

Oh H. M., S. J. Lee, M. H. Jang, and B. D. Yoon. 2000. Microcystin production of *Microcystis aeruginosa* in P-limited chemostat. Applied Environmental Microbiology. **66**:176-179.

Orr P. T., G. J. Jones. 1998. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. Limnology and Oceanography **43**:1604-1614.

Ozawa K., H. Fujioka, M. Muranaka, A. Yokoyama, Y. Katagami, T. Homma, K. Ishikawa, S. Tsujimura, M. Kumagai, M. F. Watanabe, and H. Park. 2005. Spatial distribution and temporal variation of *Microcystis* species composition and microcystin concentration in Lake Biwa. Environmental Toxicology **20**:270-276.

Pflugmacher S. 2002. Possible allelopathic effects of cyanotoxins, with reference to microcystin-LR, in aquatic ecosystems. Environmental Toxicology **17**:407-413.

Porta D., M. A. J. Fitzpatrick, and G. D. Haffner. 2005. Annual variability of phytoplankton primary production in the western basin of Lake Erie (2002-2003). Journal of Great Lakes Research

31:63-71.

Porter K. G. 1976. Enhancement of algal growth and productivity by grazing zooplankton. Science **192**:1332-1334.

Raikow D. F., O. Sarnelle, A. E. Wilson, and S. K. Hamilton. 2004. Dominance of the noxious cyanobacterium *Microcystis aeruginosa* in low-nutrient lakes is associated with exotic zebra mussels. Limnology and Oceanography **49**:482-487.

Rapala J., K. Erkomaa, J. Kukkonen, K.Sivonen, K. Lahti. 2002. Detection of microcystins with protein phosphatase inhibition assay, high-performance liquid chromatography-UV detection and enzyme-linked immunosorbent assay - Comparison of methods. Anal. Chim. Acta **466**:213-231.

Reynolds C. S. 2006. Ecology of Phytoplankton. Cambridge University Press, Cambridge.

Reynolds C. S. 1988. Functional morphology and the adaptive strategies of freshwater phytoplankton. Pages 338-433 *In* C. D. Sandgren, editor. Growth and Reproductive Strategies of Freshwater Phytoplankton, Cambridge University Press, Cambridge.

Rhee G. -Y., I. J. Gotham. 1980. Optimum N:P ratios and coexistence of planktonic algae. Journal of Phycology **16**:486-489.

Rinta-Kanto J. M., A. J. A. Ouellette, G. L. Boyer, M. R. Twiss, T. B. Bridgeman, and S. W. Wilhelm. 2005. Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. Environmental Science and Technology **39**:4198-4205.

Rippka, R., J. Deruelles, J. Waterbury, M. Herdman, and R. Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology **111**:1-61.

Robarts R. D., T. Zohary. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. New Zealand Journal of Marine and Freshwater Research **21**:391-399.

Rott E., H. C. Duthie, and E. Pipp. 1998. Monitoring organic pollution and eutrophication in the Grand River, Ontario, by means of diatoms. Canadian Journal of Fisheries and Aquatic Sciences

55:1443-1453.

Sabour B., M. Loudiki, B. Oudra, V. Vasconcelos, S. Oubraim, and B. Fawzi. 2005. Dynamics and toxicity of *Anabaena aphanizomenoides* (cyanobacteria) waterblooms in the shallow brackish Oued Mellah lake (Morocco). Aquatic Ecosystem Health & Management **8**:95-104.

Sarnelle O., A. E. Wilson, S. K. Hamilton, L. B. Knoll, and D. F. Raikow. 2005. Complex interactions between the zebra mussel, *Dreissena polymorpha*, and the harmful phytoplankter, *Microcystis aeruginosa*. Limnology and Oceanography **50**:896-904.

Schindler D. W. 1977. Evolution of phosphorus limitation in lakes. Science **195**:260-262.

Schulze E. -D., M. M. Caldwell. 1994. Ecophysiology of photosynthesis. Springer-Verlag, Berlin.

Sedmak B., T. Elersek. 2005. Microcystins induce morphological and physiological changes in selected representative phytoplanktons. Microbial Ecology **50**:298-305.

Sedmak B., G. Kosi. 2002. Harmful cyanobacterial blooms in Slovenia- bloom types and microcystin producers. Acta Biologica Slovenica **45**:17-30.

Sim A. T. R., L. Mudge. 1993. Protein phosphatase activity in cyanobacteria: Consequences for microcystin toxicity analysis. Toxicon **31**:1179-1186.

Sivonen K. 1990. Effects of light, temperature, nitrate, orthophosphate and bacteria on growth of and hepatoxin production by *Oscillatoria agardhii* strains. Applied Environmental Microbiology **56**:2658-2666.

Sivonen K., G. Jones. 1999. Cyanobacterial Toxins. Pages 41-112 *In* I. Chorus and J. Bartram, editors. Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management, E and FN Spon, London.

Smith V. H. 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: An empirical and theoretical analysis. Limnology and Oceanography **27**:1101-1112.

Stainton M. P., M. J. Capel, and A. J. Armstrong. 1977. The Chemical Analysis of Freshwater. Freshwater Institute, Winnipeg.

Thostrup L., K. Christoffersen. 1999. Accumulation of microcystin in *Daphnia magna* feeding on toxic *Microcystis*. Archiv fuer Hydrobiologie **145**:447-467.

U.S. Army Engineer District, Buffalo. Maumee Bay State Park, Ohio: Shoreline Erosion, Beach Restoration Study: Volume 2: Appendices. US Army Corps of Engineers; 1983. Report nr 2.

Utkilen H., N. Gjolme. 1995. Iron-stimulated toxin production in *Microcystis aeruginosa*. Applied and Environmental Microbiology **61**:797-800.

Vanderploeg H. A., J. R. Liebig, W. W. Carmichael, M. A. Agy, T. H. Johengen, G. L. Fahnenstiel, and T. F. Nalepa. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences **58**:1208-1221.

Vincent R. K., X. Qin, R. M. L. McKay, J. Miner, K. Czajkowski, J. Savino, and T. Bridgeman. 2004. Phycocyanin detection from LANDSAT TM data for mapping cyanobacterial blooms in Lake Erie. Remote Sensing of Environment **89**:381-392.

Vogt C. 1999. Creating Long Documents using Microsoft Word. Published on the Web at the University of Waterloo.

Wiegand C., S. Pflugmacher. 2005. Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. Toxicology and Applied Pharmacology **203**:201-218.

Zurawell R.W., H. Chen, J.M. Burke, and E.E. Prepas. 2005. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. Journal of Toxicology and Environmental Health, Part B **8**:1-37.

Appendix A

Bay of Quinte 2006 Dataset

Parameter	July 4, 2006						
	Shallow			Deep			
Station	DS	FI	NR	GPt	MBO	NA	Avg.
Date	July 5/06	July 4/06	July 4/06	July 4/06	July 5/06	July 4/06	
GPS Latitude	44.188140	44.180230	44.180300	44.170150	44.186800	44.191300	
GPS Longitude	-77.053642	-77.053300	-77.039950	-77.049690	-77.047370	-77.025810	
Sampling Depth (m)	1	1	1	2	2	1	
Station Depth (m)	2.4	2	1.7	6	6.1	4.8	
Secchi Depth (m)	1.85	2.1	1.5	2.25	1.95	2.08	1.96
SRP (ug/L)	3.4	2.8	2.8	4.0	5.4	3.7	3.68
TDP (ug/L)	11.8	12.4	N/A	13.0	12.7	12.7	12.51
Part P (ug/L)	13.5	9.5	N/A	11.1	10.4	11.8	11.26
TP (ug/L)	30.7	24.1	N/A	25.0	31.0	29.3	28.01
TP (umol/L)	0.991	0.779	N/A	0.807	1.00	0.945	0.90
SrSi (ug/L)	3817	4178	N/A	4121	4141	3162	3883.85
PartSi (ug/L)	978	1388	1452	1012	974	1033	1139.45
NH3 (ug/L)	10.7	21.8	14.8	18.1	28.9	21.8	19.35
NO2 (ug/L)	2.40	3.39	3.06	2.08	3.06	1.75	2.62
NO3 (ug/L)	454.1	5.0	7.7	bdl	bdl	3.1	117.45
Part N (ug/L)	151	179	207	135	135	19	137.63
N Arith. (ug/L)	617.7	209.6	232.2	155.0	167.0	45.9	
TN (ug/L)	923	1144	1133	978	1006	895	1013.04
TN (umol/L)	65.9	81.7	80.9	69.8	71.8	63.9	72.31
TN:TP (molar)	66.4	105	N/A	86.5	71.7	67.6	79.43
F (ug/L)	62.7	27.5	29.1	29.0	31.1	28.0	34.56
Cl (mg/L)	12.5	7.2	5.6	7.8	7.8	5.2	7.69
SO4 (mg/L	16.0	5.0	3.8	5.2	5.1	3.5	6.41
Ext. Chl (ug/L)	13.76	14.69	16.41	12.31	12.46	12.38	13.67
Part C (ug/L)	954	1185	1562	866	868	156	931.89
Part N (ug/L)	151	179	207	135	135	19	137.63
CN (molar)	7.39	7.71	8.82	7.50	7.50	9.42	8.05
CP (molar)	183	322	N/A	200	216		230.29
Mean Diss. Mcyst (ug/L)	0.61	0.68*	1.16**	0.77*	0.44*	0.92	0.76
CV for Diss. Mcyst	8.67	7.26	10.71	7.38	1.36	5.36	6.79
Mean Total Mcyst (ug/L)	2.17	1.58*	2.55	1.64***	1.96**	3.60*	2.25
CV for Total Mcyst	9.91	9.23	2.46	20.60	17.43	5.99	10.94
% Dissolved Mcyst	27.88	42.98	45.56	46.79	22.31	25.57	35.18
Part. Mcyst (arith.) ug/L	1.57	0.90	1.39	0.87	1.52	2.68	1.49
Part Mcyst/Chl	0.11	0.06	0.08	0.07	0.12	0.22	0.11
Dark Adapted fv/fm	n/a	0.58	0.72	0.78	0.47	0.47	0.60
Etrm	n/a	56.6	25.8	196.5	55.1	46.3	76.07
Temp. (oC)	n/a	24.31	24.51	24.31	n/a	24.92	24.51
FP Green (ug/L)	n/a	0.00	0.07	0.00	n/a	0.00	0.02
FP Cyanos (ug/L)	n/a	3.37	4.61	3.38	n/a	4.46	3.95

FP Diatoms (ug/L)	n/a	0.01	0.00	0.15	n/a	0.01	0.04
FP Cryptophyta (ug/L)	n/a	1.93	0.86	1.54	n/a	1.72	1.51
FP Total Conc. (ug/L)	n/a	5.31	5.53	5.08	n/a	6.19	5.53
FP % Cyanos of Total	n/a	63.38	83.30	66.66	n/a	72.04	71.34
Depth Avg'd	n/a	0.1-1.5m	0.3-1.3m	0-1.7m	n/a	0-0.5m	
Stratified y/n	n/a	n	n	y	n/a	n	
Depth of Strat. (m)	n/a	n/a	n/a	4-5 m	n/a	n/a	

Parameter	**Sept. 22, 2006**						
	Shallow			**Deep**			
Station	DS	FI	NR	GPT	MBO	NA	Avg.
Date	Sept 22/06	Sept 22/06	Sept 22/06	Sept 22/06	Sept 22/06	Sept 22/06	
Sampling Depth (m)	2	1	0.5	2	2	2	
Station Depth (m)	2.4	1.8	1.2	6.4	5.2	5.2	
Secchi Depth (m)	1.2	1	1.25	0.9	1.2	1	1.09
SRP (ug/L)	1.8	1.8	2.7		2.7	2.8	2.36
TDP (ug/L)	6.5	6.5	5.3	5.6	7.4	7.4	6.42
Part P (ug/L)	13.6	25.0	N/A	22.6	26.1	19.0	21.26
TP (ug/L)	27.1	42.9	24.1	27.6	25.9	38.7	31.03
TP (umol/L)	0.874	1.38	0.777	0.893	0.835	1.25	1.00
SrSi (ug/L)	3759	3387	3078	3163	3620	3240	3374.54
PartSi (ug/L)	1131	1827	1175	875	981	739	1121.27
NH3 (ug/L)	9.3	9.0	7.4	21.4	6.1	188.5	40.28
NO2 (ug/L)	3.39	2.40	2.73	1.75	1.42	2.08	2.29
NO3 (ug/L)	bdl	bdl	bdl	bdl	18.5	bdl	18.48
Part N (ug/L)	394	546	423	535	377	484	459.88
N Arith. (ug/L)	406.6	557.3	433.2	558.5	402.9	674.7	
TN (ug/L)	1244	1304	1620	1985	923	1686	1460.18
TN (umol/L)	88.8	93.1	116	142	65.9	120	104.22
TN:TP (molar)	102	67.3	149	159	78.9	96.3	108.59
F (ug/L)	28.0	42.6	32.8	32.3	29.2	39.3	34.04
Cl (mg/L)	8.9	7.8	9.0	8.4	8.4	9.1	8.60
SO4 (mg/L	4.7	4.0	4.8	4.6	4.5	4.9	4.58
Ext. Chl (ug/L)	20.06	25.65	17.10	29.73	18.55	26.57	22.95
Part C (ug/L)	2531	3421	2832	3057	2261	2936	2839.70
Part N (ug/L)	394	546	423	535	377	484	459.88
CN (molar)	7.50	7.31	7.81	6.66	7.00	7.07	7.22
CP (molar)	480	352	N/A	349	223	399	360.73
Mean Diss. Mcyst (ug/L)	0.07	0.05**	0.06****	0.06**	0.08	0.07	0.06
CV for Diss. Mcyst	28.13	44.71	51.10	43.41	9.47	23.62	33.41
Mean Total Mcyst (ug/L)	0.67	0.63	0.46	0.62*	0.61*	0.47	0.58
CV for Total Mcyst	11.15	12.19	2.17	9.37	7.07	24.64	11.10
% Dissolved Mcyst	10.03	8.14	12.36	10.07	12.96	15.18	11.46
Part. Mcyst (arith.) ug/L	0.60	0.58	0.40	0.55	0.54	0.40	0.51
Mcyst/Chl	0.03	0.02	0.02	0.02	0.03	0.01	0.02
Dark Adapted fv/fm	0.46	0.50	0.36	0.52	n/a	0.39	0.45

Etrm	21.3		13.5	26.7	n/a	18.0	19.89
Temp. (oC)	18.15	18.00	15.92	17.07	17.50	16.84	17.25
FP Green (ug/L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FP Cyanos (ug/L)	11.10	15.79	12.41	18.78	13.03	17.18	14.71
FP Diatoms (ug/L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FP Cryptophyta (ug/L)	1.52	2.63	1.29	2.38	1.80	1.88	1.92
FP Total Conc. (ug/L)	12.62	18.42	13.70	21.16	14.83	19.06	16.63
FP % Cyanos of Total	87.94	85.69	90.63	88.77	87.86	90.13	88.50
Depth Avg'd	0-1.6m	0.3-2.1m	0-0.5m	0-2.2m	0.2-3.2m	0-2.1m	
Stratified y/n	n	overturn start	n	overturn start	n	overturn start	
Depth of Strat. (m)	n/a	n/a	n/a	n/a	n/a	n/a	

Bay of Quinte Appendix Legend	
Term Used	**Definition/ Explanation**
T	Total
SR or Sr	Soluble reactive
Part	Particulate
Extracted Chl *a*	Average of 2 measurements of extracted chlorophyll a
Diss.	Dissolved
Mcyst	Microcystin-LR equivalents
CV	Coefficient of Variation
Part Mcyst	Particulate Microcystin estimated from equation: (Total MCYST - Diss. MCYST)
% Diss. Mcyst	Percentage of Total Microcystin made up by Dissolved Microcystin
Fv/Fm	PAM: Variable fluorescence (optimal quantum yield)
ETRmax	PAM: Maximum electron transport rate (photosynthetic capacity)
FP	Fluoroprobe estimate of pigment concentrations
Depth Avg'd	Fluoroprobe values were averaged over these depths to represent the mixed portion of the water column
*	Mean from 2 PPIA runs
**	Mean from 4 PPIA runs
***	Mean from 5 PPIA runs
****	Mean from 6 PPIA runs
bdl	Below detection limit (below 3 ug/L for NO3)

Appendix B

Maumee Bay 2006 Dataset

Parameter	June 20, 2006							
	Shallow				Deep			Mean
Station	MB19	Clear	MB18	MB15	7M	8M	Crib	
Sampling Depth (m)	1	1	1	1	1	1	1	
Station Depth (m)	2.2	2.3	2.6	3	5.5	6	5.8	
GPS Latitude (N)	41.72933	41.73324	41.74223	41.70636	41.73345	41.78897	41.70031	
GPS Longitude (W)	-83.43099	-83.32628	-83.40189	-83.33385	-83.29715	-83.33506	-83.26189	
Secchi Depth (m)	0.55	2.3	2.6	3	2.5	3.2	2.7	2.4
Secchi Depth (m)	0.55	*2.3*	*2.6*	*3*	2.5	3.2	2.7	2.4
SRP (ug/L)	29.6	9.5	33.1	12.7	4.3	5.4	n/a	15.8
TDP (ug/L)	47.9	14.1	47.3	18.4	7.2	8.7	13.0	22.4
Part P (ug/L)	15.2	15.2	n/a	2.7	20.3	5.3	14.2	12.2
TP (ug/L)	29.6	19.5	47.3	24.1	12.4	16.7	29.8	25.6
TP (umol/L)	0.954	0.631	1.53	0.779	0.400	0.539	0.964	0.828
SrSi (ug/L)	2054	1319	2086	1513	1533	1221	n/a	1621
PartSi (ug/L)	1967	744	620	610	347	598	n/a	814
NH3 (ug/L)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
NO2 (ug/L)	24.0	13.2	38.4	13.2	12.6	10.6	n/a	18.7
NO3 (ug/L)	2076	1322	3096	1547	782.4	871.8	n/a	1616
Part N (ug/L)	88	68	118	24	22	130	n/a	75
DON Arith. (ug/L)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
TN (ug/L)	3224	2383	n/a	2106	757	1885	n/a	2071
TN (umol/L)	230	170	n/a	150	54.0	135	n/a	148
TN:TP (molar)	241	270	n/a	193	135	250	n/a	218
F (ug/L)	58.3	61.0	60.8	57.5	34.9	57.0	n/a	54.9
Cl (mg/L)	14.1	11.2	18.2	11.3	9.2	10.1	n/a	12.4
SO4 (mg/L	19.6	15.3	24.8	16.0	13.0	13.7	n/a	17.0
Ext. Chl (ug/L)	0.8902	0.2132	0.4144	0.1607	0.2637	0.1030	n/a	0.3409
Part C (ug/L)	674	857	772	166	164	816	n/a	575
Part N (ug/L)	88	68	118	24	22	130	n/a	75
CN (molar)	8.93	14.69	7.62	8.09	8.63	7.34	n/a	9.22
CP (molar)	114	146	n/a	160	21	398	n/a	168
Mean Diss. Mcyst (ug/L)	BDL*	BDL	BDL*	BDL*	BDL*	BDL*	BDL	
CV for Diss. Mcyst	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
Mean Total Mcyst (ug/L)	BDL	BDL	BDL	BDL	BDL	BDL	BDL*	
CV for Total Mcyst	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
% Dissolved Mcyst	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
Part. Mcyst (arith.) ug/L	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
Part Mcyst/Chl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
Dark Adapted fv/fm		0.52	0.51	0.24	0.21	n/a	n/a	0.37
ETRmax		69.7	54.2	14.5	13.6	n/a	n/a	38.0
Temp. (oC)	n/a	n/a	n/a	n/a	23.39	23.37	23.76	23.50

FP Green (ug/L)	n/a	n/a	n/a	n/a	0.21	0.08	0.44	0.24
FP Cyanos (ug/L)	n/a	n/a	n/a	n/a	0.04	0.06	0.00	0.03
FP Diatoms (ug/L)	n/a	n/a	n/a	n/a	0.24	0.14	0.41	0.26
FP Cryptophyta (ug/L)	n/a	n/a	n/a	n/a	0.95	0.32	1.34	0.87
FP Total Conc. (ug/L)	n/a	n/a	n/a	n/a	1.44	0.60	2.19	1.41
FP % Cyanos of Total	n/a	n/a	n/a	n/a	2.86	9.72	0.00	4.20
Depth Avg'd	n/a	n/a	n/a	n/a	0.51-2.31m	0.43-2.35m	0.37-1.5m	
Stratified y/n	n/a	n/a	n/a	n/a	y	y	y	
Depth of Strat. (m)	n/a	n/a	n/a	n/a	2.3	2.4	2	

Parameter	**Aug. 22, 2006**							
	Shallow				**Deep**			**Mean**
Station	MB15	MB18	MB19	Clear	7M	8M	Crib	
Sampling Depth (m)	0	0	0	1	2	2	2	
Station Depth (m)	1.3	1.9	2.2	3.8	5.7	5.8	5.8	
GPS Latitude (N)	41.70636	41.74223	41.72933	41.73324	41.73345	41.78897	41.70031	
GPS Longitude (W)	-83.33385	-83.40189	-83.43099	-83.32628	-83.29715	-83.33506	-83.26189	
Secchi Depth (m)	0.6	1.7	1.45	1.6	1.75	1	1.4	1.4
Secchi Depth (m)	0.6	1.7	1.45	1.6	1.75	1	1.4	1.4
SRP (ug/L)	48.9	3.3	19.1	7.8	10.4	14.9	n/a	17.4
TDP (ug/L)	10.9	5.0	19.0	7.7	13.9	15.1	n/a	11.9
Part P (ug/L)	7.2	22.2	33.9	37.0	20.0	20.9	15.6	22.4
TP (ug/L)	34.8	36.3	42.3	30.3	34.5	32.4	46.4	36.7
TP (umol/L)	1.12	1.17	1.36	0.979	1.11	1.05	1.50	1.19
SrSi (ug/L)	2453	1048	1327	665	694	847	n/a	1172
PartSi (ug/L)	1779	729	1177	556	606	618	n/a	911
NH3 (ug/L)	24.6	12.1	23.6	10.3	131.1	29.2	n/a	38.5
NO2 (ug/L)	7.97	8.96	10.6	12.6	5.35	6.99	n/a	8.74
NO3 (ug/L)	481.6	134.8	491.8	10.7	188.5	358.9	n/a	277.7
Part N (ug/L)	240	362	410	541	274	285	102	316.32
DON Arith. (ug/L)	1159	792	806	1869	982	851	n/a	1076
TN (ug/L)	1913	1310	1741	2444	1581	1531	1564	1726
TN (umol/L)	137	93.5	124	174	113	109	112	123
TN:TP (molar)	121	79.8	91.1	178	101	104	74.5	107
F (ug/L)	65.8	30.0	66.4	25.9	38.3	61.4	n/a	48.0
Cl (mg/L)	15.4	6.6	18.0	7.5	4.7	9.3	n/a	10.2
SO4 (mg/L	18.9	9.6	21.7	5.1	6.8	12.9	n/a	12.5
Ext. Chl (ug/L)	11.55	17.27	25.65	28.88	12.55	15.02	10.81	17.39
Part C (ug/L)	1485	2318	2560	2994	1758	1724	755	1942
Part N (ug/L)	240	362	410	541	274	285	102	316
CN (molar)	7.22	7.46	7.29	6.46	7.49	7.06	8.59	7.37
CP (molar)	533	270	195	209	227	212	124	253
Mean Diss. Mcyst (ug/L)	0.17**	0.86**	1.34***	0.62**	0.40	0.51**	0.40**	0.61
CV for Diss. Mcyst	0.06	0.08	0.06	0.06	0.15	0.10	0.11	0.09
Mean Total Mcyst (ug/L)	2.18	6.00	8.97	4.82	2.64	5.89	2.02**	4.65
CV for Total Mcyst	0.11	0.09	0.05	0.05	0.08	0.08	0.02	0.07

% Dissolved Mcyst	7.70	14.38	14.97	12.88	15.08	8.67	19.64	13.33
Part. Mcyst (arith.) ug/L	2.01	5.14	7.63	4.20	2.24	5.38	1.62	4.03
Part Mcyst/Chl	0.17	0.30	0.30	0.15	0.18	0.36	0.15	0.23
Dark Adapted fv/fm	0.50	0.43	0.48	0.40	0.36	0.41	n/a	0.43
Etrm	53.9	52.6	94.6	75.4	38.0	37.7	n/a	58.7
Temp. (oC)	23.73	26.45	26.80	24.60	26.79	25.76	25.08	25.60
FP Green (ug/L)	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.01
FP Cyanos (ug/L)	6.36	5.47	4.04	4.43	2.69	5.98	4.51	4.79
FP Diatoms (ug/L)	0.01	0.01	0.25	0.00	0.00	0.00	0.05	0.05
FP Cryptophyta (ug/L)	0.79	2.28	1.82	2.13	1.36	2.75	0.66	1.68
FP Total Conc. (ug/L)	7.16	7.76	6.16	6.57	4.06	8.74	5.22	6.52
FP % Cyanos of Total	88.92	70.49	65.64	67.46	66.36	68.48	86.46	73.40
Depth Avg'd		0.59-1.82m	0.61-2.23m	0.1-2.4m	0.13-3.0m	0.6-3.0m		
Stratified y/n	n	n	n	n	y	y	y?	
Depth of Strat. (m)	n/a	n/a	n/a	n/a	1	1.2	3	

Maumee Bay Appendix Legend	
Term Used	**Explanation**
All as per Bay of Quinte Appendix except for...	
*	Mean from 1 PPIA run
**	Mean from 3 PPIA runs
***	Mean from 4 PPIA runs

Appendix C

GRCA Dataset by Date

	Belwood	Belwood	Conestogo	Conestogo	Guelph	Guelph
Station #	GR5109	GR5110	GR5107	GR5108	GR5105	GR5106
Date	6-Jul-05	6-Jul-05	6-Jul-05	6-Jul-05	6-Jul-05	6-Jul-05
Time (24hr)	11:19:00	11:19:00	8:45:00	9:24:00	13:15:00	13:15:00
Lake Depth (m)	17	17	9	9	10.5	10.5
Sample Depth (m)	2	10	2	7	2	8
pH	8.61		8.69		8.67	
Temp. (oC)	22		18.8		25.2	
Secchi (m)	3		6.2		2.75	
Kd (m-1)						
Euphotic Depth (m)						
Mixed Depth (m)	6.69		6.36		5.88	
Mean Irradiance (% PAR)						
FP Chl (ug/L) - 2m	4.96		2		4.12	
FP Green (ug/L) - 2m	0		0		0	
FP Cyano (ug/L) - 2m	1.48		0.74		1.96	
FP Diatoms (ug/L) - 2m	1.32		0.68		1.68	
FP Crypto (ug/L) -2m	2.16		0.58		0.48	
FP CDOM (ug/L) -2m	2.11		1.09		1.98	
FB CDOM - avg. (ug/L)	2.55		1.12		1.7	
FP Chl. Avg. (ug/L)	2.70		1.43		3.77	
FB Cyano Avg. (ug/L)	0.82		0.44		2.24	
Fv/Fm (PAM)	0.228		0.144		0.231	
SRP (ug/L)	0.87		0.00		2.49	
TDP (ug/L)	16.43		8.19		9.33	
PartP (ug/L)	3.78					
TP (ug/L)	12.06	14.92	12.70	13.45	14.04	14.70
NH3 (ug/L)	40.5		126.5		63.0	
SrSi (ug/L)	1698		881		1730	
NO2 (ug/L)	8.26		48.47		22.98	
NO3 (ug/L)	1027.9		2277.3		942.8	
TN (ug/L)	2505		5744		2491	
Ext. Chl. a (ug/L)	7.26	1.28	2.13	0.37	5.17	3.83
TN (mM/L)	178.91		410.26		179.11	
TP (mM/L)	0.39	0.48	0.41	0.43	0.45	0.47
TN:TP (molar)	458		1000		398	
Norg (ug/L)	1428.06		3291.41		1462.05	
Part C (mg/L)	590		333		813	
Part N (mg/L)	98.6		44.4		104	
C:N (molar)	7.0		8.8		9.1	
C:P (molar)	403.1					

Total Microcystin (ug/L)	0.09		BDL		0.07	
# PPIA Runs	3		3		6	
CV - Microcystin	0.11		9.60		0.22	

	Belwood	**Belwood**	**Conestogo**	**Conestogo**	**Guelph**	**Guelph**
Station #	GR5143	GR5146	GR5144	GR5145	GR5142	GR5147
Date	21-Jul-05	21-Jul-05	21-Jul-05	21-Jul-05	21-Jul-05	21-Jul-05
Time (24hr)	10:19:00	10:19:00	8:25:00	8:25:00	12:35:00	12:35:00
Lake Depth (m)	11	11	11	11	7.5	7.5
Sample Depth (m)	2	8	2	7	2	6
pH	8.91		8.91		8.71	
Temp. (oC)	25.2		21.6		30.3	
Secchi (m)	1.8		2.3		3.7	
Kd (m-1)	1.08		0.85		0.43	
Euphotic Depth (m)	4.3		5.4		10.7	
Mixed Depth (m)	4.51		5.01		5.21	
Mean Irradiance (% PAR)	21.2		23.1		39.8	
FP Chl (ug/L) - 2m	13.32		6.51		4.52	
FP Green (ug/L) - 2m	3.4		0		0	
FP Cyano (ug/L) - 2m	2.87		2.53		2.51	
FP Diatoms (ug/L) - 2m	2.56		0		0	
FP Crypto (ug/L) -2m	4.48		3.98		2.01	
FP CDOM (ug/L) -2m	1.37		0.9		1.03	
FB CDOM - avg. (ug/L)	1.94		1.24		1.33	
FP Chl. Avg. (ug/L)	11.75		6.64		4.06	
FB Cyano Avg. (ug/L)	5.74		1.77		2.16	
Fv/Fm (PAM)	0.17		0.159		0.329	
SRP (ug/L)	0.95		0.44		0.41	
TDP (ug/L)	6.02		9.17		6.27	
PartP (ug/L)	7.51		8.22		4.86	
TP (ug/L)	13.17	13.16	16.26	18.16	13.16	16.39
NH3 (ug/L)	24.1		35.8		31.9	
SrSi (ug/L)	1742		1034		1692	
NO2 (ug/L)	11.85		41.29		21.54	
NO3 (ug/L)	467.4		1821.2		714.8	
TN (ug/L)	5340		2546	2991	4120	
Ext. Chl. a (ug/L)	9.24	6.82	11.67	4.58	2.1	3.32
TN (mM/L)	381.45		181.83	213.61	294.3	
TP (mM/L)	0.42	0.42	0.52	0.59	0.42	0.53
TN:TP (molar)	908		350	362	700	
Norg (ug/L)	4837.04		647.46		3351.76	
Part C (mg/L)	1016		1115		562	
Part N (mg/L)	159		150		80.2	
C:N (molar)	7.5		8.7		8.2	
C:P (molar)	348.9		349.6		298.3	

Total Microcystin (ug/L)	0.06		BDL		BDL	
# PPIA Runs	3		3		3	
CV - Microcystin	0.09		5.30		0.69	

	Belwood	**Belwood**	**Conestogo**	**Conestogo**	**Guelph**	**Guelph**
Station #	GR5290	GR5295	GR5292	GR5294	GR5291	GR5293
Date	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05	11-Aug-05
Time (24hr)	11:20:00	11:20:00	8:51:00	8:51:00	13:42:00	13:42:00
Lake Depth (m)	14	14	13	13	8	8
Sample Depth (m)	2	10	2	7	2	6
pH	8.88		9.02		8.66	
Temp. (oC)	22.3		19.5*		34.2	
Secchi (m)	1.7		2.9		3.2	
Kd (m-1)	0.92		0.76		0.49	
Euphotic Depth (m)	5.0		6.0		9.5	
Mixed Depth (m)	7.37		6.67		5.20	
Mean Irradiance (% PAR)	14.8		19.6		36.4	
FP Chl (ug/L) - 2m	18.36		8.08		4.08	
FP Green (ug/L) - 2m	1.27		1.8		1.32	
FP Cyano (ug/L) - 2m	12.56		2.22		1.93	
FP Diatoms (ug/L) - 2m	0		2.4		0	
FP Crypto (ug/L) -2m	4.53		1.66		0.83	
FP CDOM (ug/L) -2m	1.7		0.69		1.01	
FB CDOM - avg. (ug/L)	2.02		1.09		1.32	
FP Chl. Avg. (ug/L)	14.71		6.08		4.35	
FB Cyano Avg. (ug/L)	10.73		1.52		2.29	
Fv/Fm (PAM)						
SRP (ug/L)	0.00		0.00		0.00	
TDP (ug/L)	7.61		6.48		5.08	
PartP (ug/L)	12.65		10.25		7.66	
TP (ug/L)	15.51	23.78	14.85	14.09	13.99	18.50
NH3 (ug/L)	24.9		49.5		45.4	
SrSi (ug/L)	1596		1376		1593	
NO2 (ug/L)	11.13		48.11		18.67	
NO3 (ug/L)	93.0		919.2		457.8	
TN (ug/L)	1323	1109	1876	2445	1572	829
Ext. Chl. a (ug/L)	23.84	4.88	14.63	10.2	4.29	6.03
TN (mM/L)	94.51	79.2	133.98	174.65	112.26	59.22
TP (mM/L)	0.5	0.77	0.48	0.45	0.45	0.6
TN:TP (molar)	189	103	279	388	249	99
Norg (ug/L)	1194.03		858.94		1049.73	
Part C (mg/L)	1510		1125		600	
Part N (mg/L)	245		152		79.9	
C:N (molar)	7.2		8.7		8.8	
C:P (molar)	307.9		283.0		202.0	

Total Microcystin (ug/L)	0.07		BDL		0.19	
# PPIA Runs	3		3		2	
CV - Microcystin	0.11		1.74		0.04	

	Belwood	**Belwood**	**Conestogo**	**Conestogo**	**Guelph**	**Guelph**
Station #	GR5299	GR5301	GR5298	GR5300	GR5297	GR5302
Date	23-Aug-05	23-Aug-05	23-Aug-05	23-Aug-05	23-Aug-05	23-Aug-05
Time (24hr)	11:21:00	11:21:00	8:37:00	8:37:00	13:22:00	13:22:00
Lake Depth (m)	11	11	13	13	8.5	8.5
Sample Depth (m)	2	9	2	7	2	6
pH	8.52		8.52		8.36	
Temp. (oC)	19.5		18.3		24.2	
Secchi (m)	1.3		1.3		1.6	
Kd (m-1)	1.30		1.00		1.20	
Euphotic Depth (m)	3.7		4.4		3.8	
Mixed Depth (m)	11.00		9.68		7.21	
Mean Irradiance (% PAR)	7.2		9.9		11.4	
FP Chl (ug/L) - 2m	14.2		10.11		7.09	
FP Green (ug/L) - 2m	1.84		0.98		0.76	
FP Cyano (ug/L) - 2m	5.73		4.12		3.95	
FP Diatoms (ug/L) - 2m	0.26		0		0	
FP Crypto (ug/L) -2m	6.37		5		2.38	
FP CDOM (ug/L) -2m	1.84		1.55		1.45	
FB CDOM - avg. (ug/L)	1.91		1.61		1.49	
FP Chl. Avg. (ug/L)	12.58		8.73		6.06	
FB Cyano Avg. (ug/L)	6.24		3.95		3.70	
Fv/Fm (PAM)	0.313		0.477		0.471	
SRP (ug/L)	0.08		1.81		3.48	
TDP (ug/L)	11.45		13.51		16.15	
PartP (ug/L)	14.08		14.72		11.55	
TP (ug/L)	28.48	20.85	15.56	24.37	21.73	23.49
NH3 (ug/L)	62.6		70.7		112.6	
SrSi (ug/L)	1894		1321		2319	
NO2 (ug/L)	4.67		74.67		14.36	
NO3 (ug/L)	11.5		938.0		66.7	
TN (ug/L)	1084	745	1775	1505	1365	854
Ext. Chl. a (ug/L)	27.09	15.5	16.18	14.4	10.49	8.95
TN (mM/L)	77.46	53.23	126.79	107.53	97.5	61.03
TP (mM/L)	0.92	0.67	0.5	0.79	0.7	0.76
TN:TP (molar)	84	79	254	136	139	80
Norg (ug/L)	1005.65		692.23		1171.37	
Part C (mg/L)	1602		983		1042	
Part N (mg/L)	265		180		154	
C:N (molar)	7.1		6.4		7.9	
C:P (molar)	293.4		172.2		232.7	

Total Microcystin (ug/L)	0.21		0.15		0.38	
# PPIA Runs	2		2		1	
CV - Microcystin	0.06		0.10		0.04	

	Belwood	**Belwood**	**Conestogo**	**Conestogo**	**Guelph**	**Guelph**
Station #	GR5346	GR5349	GR5347	GR5348	GR5345	GR5350
Date	5-Sep-05	5-Sep-05	5-Sep-05	5-Sep-05	5-Sep-05	5-Sep-05
Time (24hr)	8:35:00	8:35:00	13:15:00	13:15:00	10:40:00	10:40:00
Lake Depth (m)	15	15	12	12	10	10
Sample Depth (m)	2	10	2	7	2	6
pH	9.04		8.64		8.64	
Temp. (oC)	17.7		23		21.9	
Secchi (m)	0.85		2		1.9	
Kd (m-1)	1.80		0.95		0.99	
Euphotic Depth (m)	2.5		4.8		4.6	
Mixed Depth (m)	3.42		2.90		5.88	
Mean Irradiance (% PAR)	15.9		33.9		17.1	
FP Chl (ug/L) - 2m	28.69		9.73		7.56	
FP Green (ug/L) - 2m	0		0		1.12	
FP Cyano (ug/L) - 2m	15.91		5.2		4	
FP Diatoms (ug/L) - 2m	6.71		0		0.68	
FP Crypto (ug/L) -2m	6.07		4.52		1.76	
FP CDOM (ug/L) -2m	1.01		0		0.91	
FB CDOM - avg. (ug/L)	1.34		0.92		1.17	
FP Chl. Avg. (ug/L)	20.89		5.23		6.35	
FB Cyano Avg. (ug/L)	11.56		3.42		4.28	
Fv/Fm (PAM)	0.155		0.3		0.418	
SRP (ug/L)	4.94		1.60		0.92	
TDP (ug/L)	34.65		4.70		13.80	
PartP (ug/L)	22.49		12.74		10.37	
TP (ug/L)	30.24	36.12	14.97	23.49	15.86	27.01
NH3 (ug/L)	17.5		48.9		43.7	
SrSi (ug/L)	1982		313		2407	
NO2 (ug/L)	1.44		63.90		4.67	
NO3 (ug/L)	0.0		924.6		154.1	
TN (ug/L)	900	1648	1528	2116	804	1277
Ext. Chl. a (ug/L)	41.2	13.8	20.19	5.13	14.54	12.41
TN (mM/L)	64.3	117.7	109.16	151.13	57.42	91.22
TP (mM/L)	0.98	1.165	0.48	0.76	0.51	0.87
TN:TP (molar)	66	101	227	199	113	105
Norg (ug/L)	881.32		490.89		601.45	
Part C (mg/L)	2766		1562		1437	
Part N (mg/L)	457		255		214	
C:N (molar)	7.1		7.1		7.9	
C:P (molar)	317.1		316.1		357.3	

Total Microcystin (ug/L)	0.54		0.19		0.59	
# PPIA Runs	2		2		2	
CV - Microcystin	0.14		0.13		0.09	

	Belwood	**Belwood**	**Conestogo**	**Conestogo**		**Belwood**
Station #	GR5388	GR5390	GR5387	GR5391		GR5404
Date	22-Sep-05	22-Sep-05	22-Sep-05	22-Sep-05		13-Oct-05
Time (24hr)	11:15:00	11:15:00	8:45:00	8:45:00		10:30:00
Lake Depth (m)	13	13	10.5	10.5		0
Sample Depth (m)	2	6	2	5		0
pH	8.5		8.74			
Temp. (oC)	18.8		18.2			
Secchi (m)	1		1.1			0.75
Kd (m-1)	1.71		1.20			
Euphotic Depth (m)	2.7		3.9			
Mixed Depth (m)	5.79		5.09			1.00
Mean Irradiance (% PAR)	10.1		16.4			
FP Chl (ug/L) - 2m	25.73		17.37			18.28
FP Green (ug/L) - 2m	0		0.14			0
FP Cyano (ug/L) - 2m	12		10.93			13.93
FP Diatoms (ug/L) - 2m	3.01		0			0
FP Crypto (ug/L) -2m	10.71		6.3			4.34
FP CDOM (ug/L) -2m	1.75		1.3			1.47
FB CDOM - avg. (ug/L)	1.85		1.34			1.61
FP Chl. Avg. (ug/L)	19.38		13.76			17.63
FB Cyano Avg. (ug/L)	10.15		8.68			13.98
Fv/Fm (PAM)	0.182		0.35			0.378
SRP (ug/L)	0.24		0.33			0.44
TDP (ug/L)	9.40		6.47			10.97
PartP (ug/L)	13.11		19.01			22.23
TP (ug/L)	38.17	21.97	24.93	27.20		54.20
NH3 (ug/L)	50.3		81.5			45.1
SrSi (ug/L)	2372		750			2720
NO2 (ug/L)	0.72		63.54			3.23
NO3 (ug/L)	112.7		648.0			85.5
TN (ug/L)	443	2070	1964	813		1658
Ext. Chl. a (ug/L)	46.58	22.67	23.07	17.75		17.75
TN (mM/L)	31.61	58.09	140.27	58.09		118.43
TP (mM/L)	1.23	0.88	0.8	0.71		1.75
TN:TP (molar)	26	66	175	208		68
Norg (ug/L)	278.83		1170.7			1524.3
Part C (mg/L)	2591		1768			
Part N (mg/L)	431		340			
C:N (molar)	7.0		6.1			
C:P (molar)	509.6		239.8			

Total Microcystin (ug/L)	1.12		0.15			0.16
# PPIA Runs	2		2			3
CV - Microcystin	0.17		0.09			0.06