uOttawa

L'Université canadienne
Canada's university

FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTOCTORALES

uOttawa

L'Université canadienne
Canada's university

FACULTY OF GRADUATE AND POSDOCTORAL STUDIES

Angeline R. Tillmanns
AUTEUR DE LA THÈSE / AUTHOR OF THESIS

Ph.D. (Biology)
GRADE / DEGREE

Department of Biology
FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

The ecology of bloom forming cyanobacteria:
food web interactions and environmental correlates

TITRE DE LA THÈSE / TITLE OF THESIS

Frances Pick
DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

Jules Blais

Scott Findlay

Naomi Cappuccino

Ellie Prepas

Gary W. Slater
Le Doyen de la Faculté des études supérieures et postdoctorales / Dean of the Faculty of Graduate and Postdoctoral Studies

The ecology of bloom forming cyanobacteria: food web interactions and environmental correlates

Angeline R. Tillmanns

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
In partial fulfillment of the requirements
For the PhD in Biology

Department of Biology
Faculty of Science
University of Ottawa

Your file Votre référence
ISBN: 978-0-494-51806-9
Our file Notre référence
ISBN: 978-0-494-51806-9

Table of Contents

List of Tables

List of Figures

Abstract

Cyanobacterial blooms occur when one or a few species of cyanobacteria dominate an ecosystem. These blooms can be detrimental to human well being and ecosystems as many bloom-forming cyanobacterial species produce toxins. At a regional scale, cyanobacterial blooms are more likely in eutrophic lakes. In mesotrophic lakes, however, it is difficult to predict cyanobacterial blooms. This may be due in part to a lack of working models to describe cyanobacteria – zooplankton interactions as well as a lack of field studies at appropriate scales. This thesis investigates how, at different temporal scales, toxin-producing, bloom-forming cyanobacteria interact with zooplankton and the importance of biotic and abiotic factors in explaining both microcystin content and cyanobacterial population dynamics. To accomplish this objective, a range of methods was employed: meta-analysis of literature data, experimentation and time-series analysis of a shallow mesotrophic lake (Constance Lake, Ontario) based on high frequency sampling over 2-3 years.

The meta-analysis revealed high variability both within and across zooplankton species in their response to cyanobacteria. However, in most cases, zooplankton maintained positive growth rates when fed a diet containing cyanobacteria. A laboratory experiment with *Daphnia* showed that zooplankton can adapt to avoid toxic strains of cyanobacteria. Both these results suggest that zooplankton may play a role in controlling cyanobacterial biomass and potentially shifting cyanobacterial strains towards toxic genotypes. Field observations found that abiotic conditions, mainly temperature and pH, were more important than zooplankton when explaining the variability of microcystins. This lack of relationship between zooplankton and microcystins may have been due to the low cladoceran abundances in Constance Lake. Negative correlations, however, were detected between *Anabaena* and *Daphnia* and with cyclopoid copepods. Generally across cyanobacterial taxa, correlations with biotic variables such as the growth rates of rotifers and other cyanobacterial taxa, were more important than abiotic variables in explaining variability in cyanobacterial population growth rates. Surprisingly, these biotic relationships were predominantly positive suggesting that facilitation may play a larger role than previously thought in regulating cyanobacterial populations.

Acknowledgements

I would like to thank my supervisor, Dr. Frances Pick for her constant intellectual, logistical and emotional support throughout my doctoral degree. I am also grateful to Drs. S. Findlay and J. Blais for useful feedback at different stages of my research and to these gentleman as well as R. Kassen and D. Currie for many interesting and enlightening conversations. I would like to thank L. Kimpe, R. Aranda-Rodriguez and J. Harvey for helping me wield the tools of analytical chemistry and A. Wilson and O. Sarnelle for their assistance with my first chapter. I am grateful to S. Leblanc and S. Burton for help in the laboratory and in the field on many occasions. Thank-you to my colleagues C. Allaway, G. Bouchard, K. Guckert, C. Tolley and S. Wiens for constant encouragement, to J. Myers and J. Jasmine for opening my mind to evolutionary concepts and to A. Algar for his work organising the weekly ecology journal club which provided much fodder for forming ideas.

I would like to thank my family in Ottawa, Montreal and in Victoria for their support and enthusiasm throughout my degree.

I am indebted to my husband, P. Gans, for his never ending encouragement. Without his support this thesis would not have been nearly as enjoyable.

Introduction

Cyanobacterial blooms - a water quality issue

Cyanobacteria are photosynthetic prokaryotes that occur in many different habitat types across the globe (Whitton and Potts 2000). In aquatic ecosystems, planktonic cyanobacteria can dominate lakes or slow-moving rivers creating biomass maximums or "blooms" (Reynolds and Walsby 1975). Certain cyanobacterial species commonly found in these blooms produce compounds that are highly toxic to animals and people (Carmichael 1994). Generally, toxins remain within the cell and are only released into the water during cell lysis (Jones and Orr 1994; Lam et al. 1995; Sivonen and Jones 1999). This makes toxic blooms difficult to manage as water clearing technologies such as copper sulphate cause mass cell lysis and therefore the release of toxins into the water column (Kenefick et al. 1993). Ecologically, cyanobacterial blooms can disrupt normal ecosystem functions by reducing energy transfer through the food chain, phytoplankton diversity and nutrient recycling rates in the pelagic zone (Sunda et al. 2006).

The frequency of cyanobacterial blooms appears to be on the rise in Canada. Lake Winnipeg has been labelled, 'Canada's Sickest Lake', as a result of persistent cyanobacterial blooms (Ferguson 2004) and lakes monitored for cyanobacterial blooms by the Quebec Ministry of Environment and Parks show a steady increase in bloom incidence over the past four years[1] (Figure 0.1). This increase in bloom frequency may be an artifact of increased monitoring efforts and a heightened awareness among the public, but a paleolimnetic study of algal pigments in the Qu'Appelle Valley, Saskatchewan, found that although bloom-forming cyanobacteria were a component of the phytoplankton community prior to European settlement (*ca.* 1890), cyanobacterial biomass has increased on average about ten-fold since this time (Dixit et al. 2000). It is likely that the continuous cultural eutrophication of many water bodies in Canada has increased their susceptibility to cyanobacterial blooms (Schindler and Vallentyne 2008).

[1] Data for Figure 1 was downloaded on February 11, 2008 from: http://www.mddep.gouv.qc.ca/eau/algues-bv/milieux_affectes/resultats.asp

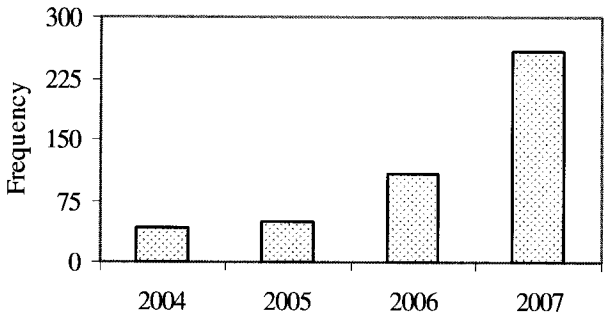


Figure 0.1 Frequency of reported cyanobacterial blooms in Quebec, Canada over four years.

Plankton succession

'Plankton' is derived from the Greek word 'πλανκτος' or 'planktos' meaning drifter or wanderer. Plankton generally are not able to move against a current but may move in calm water (Reynolds 1984b). Viruses, bacteria, phytoplankton, protozoa, and zooplankton make up the planktonic food web. Phytoplankton are microscopic photosynthetic organisms which form the basis of the food chain and can vary in size over three orders of magnitude from picoplankton (0.2 - 2 μm) to microplankton (20 – 200 μm). Six divisions of eukaryotes and one prokaryote, cyanobacteria, make up the phytoplankton. Species from each division can be differentiated based on pigment composition, number and position of flagellum, method of carbohydrate storage and cell wall composition. Although total primary production in temperate lakes is commonly limited by the amount of phosphorus and/or nitrogen in the water column (Dillon and Rigler 1974; Elser et al. 1990), the actual species composition is determined by a number of interacting biotic and abiotic factors (Harris 1986; Reynolds 1998). All phytoplankton species have strategies to stay suspended in the water column, harvest light and nutrients, avoid predation and survive winter conditions (Reynolds 1984). Differences in these strategies give one or several species an advantage under certain conditions (i.e. differences in light, nutrients, turbulence, grazing pressure; Hutchinson 1961).

In temperate lakes, phytoplankton generally follow a recognizable pattern of succession which begins anew during the ice free season (Sommer et al. 1986). In late winter and early spring, light conditions increase and nutrient levels are high leading to an exponential increase in small, fast-growing phytoplankton species. An increase in grazing pressure lags behind this spike in phytoplankton biomass, but first rotifer density and then cladoceran density increase as the spring progresses. Either through nutrient depletion or intensive grazing, phytoplankton biomass decreases in late spring or early summer in a period labeled the "clear water phase". Zooplankton then decrease throughout the summer due to lower food resources and/or unpalatable phytoplankton. Slow growing phytoplankton, such as cyanobacteria that are better adapted to low nutrient conditions and/or are resistant to grazing, increase in density through the summer (Harris 1986). As the water cools in the fall, nutrients are again returned from the hypolimnion to the photic zone and a second period of rapid increase in fast growing species ensues. This description represents a generalized model of plankton succession for deep temperate, stratified lakes.

Though most limnological research on lakes has been conducted on deep stratified lakes (Scheffer 1998), about half of the worlds' lakes are less than 10 meters in depth (Wetzel 2001), Shallow lakes do not stratify for long periods during the summer, rarely for more than a month and typically not for more than a couple of days (Scheffer 1998). In stratified lakes, nutrients are lost from the photic zone during summer with the sedimentation of phytoplankton; however, in shallow lakes, both sedimentation and re-suspension occur at shorter time scales as these processes are related to water depth. In shallow lakes, nutrients are lost by lake flushing and also, for the major nutrients, by binding with iron in the sediment (phosphorus), and by denitrification (nitrogen). If adequate nutrients are available during the summer, light availability commonly becomes the major limiting factor to phytoplankton in shallow lakes (Scheffer 1998).

Bloom-forming cyanobacteria

Cyanobacteria are an ancient group of microorganisms that are thought to have evolved during the Archean period (*ca.* 3465 million years ago; Schopf 1993). Their long history has allowed them to adapt to nearly all habitats on Earth – terrestrial, marine, and freshwater, including some very inhospitable habitats such as deserts, hypersaline lakes and hot springs (Komárek 2003). Although cyanobacteria are prokaryotic, the cells contain flattened vesicles which contain their photosynthetic system. Chlorophyll *a* is the primary photosynthetic pigment and most species contain one or more of four types of accessory pigments termed phycobiliproteins (Reynolds 1984b).

Though cyanobacteria may occur as single cells, the most common bloom-forming species in temperate lakes are colonial (Mur et al. 1999). Those in the class *Nostocacales* include the common genera of *Anabaena, Aphanizomenon, Planktothrix,* and are filamentous. Another common genus is *Microcystis* from the class *Microcystaceae,* which is chrococcoid. Species from the class *Nostocacales,* are capable of fixing atmospheric nitrogen in specialised cells called heterocysts, which provide the anoxic environment necessary for nitrogenase to function (Komárek and Anagnostidis 1989). Filaments in this class also produce akinetes, specialised resting cells for over wintering (Komárek and Anagnostidis 1989). Cell shape, and the location and shape of heterocystes and akinetes are essential for identification in filamentous species (Komárek et al. 2003). Chrococcoid species such as those in the genus *Microcystis* do not produce specialized cells but form distinct colonial patterns with or without colonial mucilage that aids in identification (Komárek 2003).

Bloom-forming cyanobacteria have a number of advantages compared with other aquatic phytoplankton (Hyenstrand et al. 1998). Most species contain gas vesicles which provide bouyancy (Reynolds et al. 1987), an advantage over other species in conditions with high turbidity and low turbulence (Huisman et al. 1999b). Cyanobacteria can actively transport both free CO_2 and bicarbonate across the cell membrane (Espie et al. 1991). This is an advantage under alkaline conditions when the inorganic carbon equilibrium moves away from CO_2 and towards HCO_3^- (Shapiro 1989). The ability of

some species to fix nitrogen gives an advantage in conditions of low nitrogen to phosphorus ratios (Smith 1983), and also in some species, especially those in the genus *Microcystis*, photosynthetic optima occur at high temperatures (Robarts and Zohary 1987). The large size of colonial bloom-forming cyanobacteria and the ability to produce toxins may also limit losses from grazing, though in some studies, zooplankton have been able to control cyanobacterial blooms (Sarnelle 2007).

Microcystins

Most freshwater colonial and bloom-forming cyanobacterial species are capable of producing toxins (Dow and Swoboda 2000; Haider et al. 2003). Cyanotoxins can be categorized by their mode of action: the cyclic peptides microcystin and nodularin, which are hepatotoxins; the alkaloids anatoxin and saxitoxin, which are neurotoxins; and the lipopolysaccharides, which are primarily skin irritants (Sivonen and Jones 1999). These toxins have been reported in brackish and freshwater world wide (Carmichael 1994; Kuiper-Goodman et al. 1999). Of all the toxins, microcystins have been studied best and potentially represent the largest risk to human health as chronic exposure to low dosage can promote tumor growth in the liver (Falconer 1996).

Microcystins are a group of cyclic-septapeptides with over 70 variants due to amino acid substitutions at two points on the peptide ring (Carmichael et al. 1988; Sivonen 1996). The toxicity depends on the variant and ranges from 50 to >1,200 $\mu g\ kg^{-1}$ body weight (LD_{50} as determined by intra-peritoneal injection in mice, Sivonen and Jones 1999). The two most toxic variants are LR and LA, which are also among the most common microcystins detected (Sivonen and Jones 1999). Microcystins are produced by species in the genera *Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon,* and *Anabaenopsis* (Sivonen and Jones 1999). Species in the first three of these genera also commonly form blooms in freshwater systems.

Microcystin production is regulated in a hierarchical fashion; at the genetic level, cellular level and population level (Zurawell et al. 2005). Microcystins are produced

non-ribosomally by peptide synthetases which are encoded by the *mcy* gene cluster (Tillet et al. 2000). Within a species, strains may or may not have this gene cluster and therefore can be categorised as toxic or non-toxic (Kaebernick and Neilan 2001). There is no morphological difference between toxic and non-toxic strains and only through genetic techniques that target the *mcy* gene cluster can the strains be differentiated (Hisbergues et al. 2003). Differences in microcystin toxicity among strains can vary by more than three orders of magnitude (Bolch et al. 1997).

Strains with the *mcy* gene cluster consistently produce microcystins though the amount and the variant of microcystin may change with environmental conditions such as light (Wiedner et al. 2003), temperature, nutrients (Sivonen and Jones 1999; Downing et al. 2005), pH (Song et al. 1998) and exposure to zooplankton (Jang et al. 2007). Cell growth phase can also effect intracellular concentrations (Orr and Jones 1998; Long et al. 2001). Orr and Jones (1998) found that in batch cultures, increases in the total culture microcystin concentration ceased by the end of the log-phase growth and the total microcystin concentration did not change after the onset of the stationary phase. Orr and Jones (1998) hypothesized that there is a positive linear relationship between culture growth rate and the rate of increase in microcystins. This was later validated by Long et al. (2001) who found that at low growth rates in N-limited chemostat culture, cells have a larger volume (about 5x), higher mass and less microcystin and chlorophyll *a* per cell than cells at higher growth rates. Together, the combination of environmental conditions and growth stage regulates microcystins at the cellular level and can lead to changes in concentrations up to four fold (Sivonen and Jones 1999).

Bloom-forming cyanobacterial populations can have high genetic diversity both within and across lakes (Kurmayer et al. 2002; Wilson et al. 2005) and changes in the proportion of toxigenic individuals in a cyanobacterial population is most likely responsible for the large range in microcystin concentrations reported in the literature (Zurawell et al. 2005; Kardinaal et al. 2007a). Microcystins are relatively large molecules and it is expected that the production of these compounds must come at a cost. Non-toxigenic strains may be better adapted to low light and nutrient conditions than toxic strains (Vezie et al. 2002; Kardinaal et al. 2007b).

Though the evolutionary reason for the production of microcystins remains a mystery, there must be an advantage to producing microcystins or they would have low success competing against non-microcystin producing genotypes. Suggestions that MC may have evolved to deter grazing by zooplankton is not supported by phylogenetic evidence (Rantala et al. 2004). Microcystins were likely present in the last common ancestor to the akinete producing genera, *Anabaena, Nostoc* and *Nodularia,* identified in microfossils from the Archean eon (Schopf 1993). As the first eukaryotic microfossils appeared in the Proterozoic eon, microcystins could not have evolved as a defence against grazers (Rantala et al. 2004). However microcystins may provide an advantage in planktonic systems because once ingested, microcystins have been shown to be toxic to *Daphnia* (Chen et al. 2005; Rohrlack et al. 2005).

Thesis overview and objectives

This thesis investigates how, at different temporal scales, toxin-producing, bloom-forming cyanobacteria interact with zooplankton and the potential of biotic and abiotic factors to explain both microcystin seston content and cyanobacterial population dynamics in a shallow lake. To accomplish this objective, a range of methods was employed: meta-analysis, laboratory experimentation and time-series analysis of field observations. Although laboratory experimentation and meta-analysis of past experiments can offer insight into the interaction of a small number of species over short time scales, they cannot address the complexity of natural ecosystems where blooms occur. As a result, detailed field observations at relevant time scales were necessary.

Since cyanobacteria were first hypothesized to be free from grazing pressures and to inhibit the growth of zooplankton (Lampert 1981; Porter and Orcutt 1980a), a large number of laboratory studies on zooplankton-cyanobacteria interactions have been conducted. The results of these studies cover a wide range of responses but the use of different species and experimental conditions make any generalisation difficult. In a first meta-analysis of laboratory studies (Wilson, Sarnelle and Tillmanns 2006), I found that in general, there was a negative effect of cyanobacteria on the population growth rates of

cladocerans and rotifers. Chapter one of this thesis expands on this first meta-analysis to look at differences in zooplankton population growth rates within and across species of zooplankton and cyanobacteria. The mean duration of the experiments was about 3 weeks, the amount of time required to establish the population growth rates of zooplankton exposed to cyanobacteria. An abridged version of this chapter has been published in the *Journal of Fundamental and Applied Limnology* (Tillmanns et al. 2008).

Relationships between species are often assumed to be influenced by community composition or environmental conditions but less frequently by changes in the phenotypic distribution of a species (Holt 2005). Recent studies have found that zooplankton are able to adapt to the presence of microcystins (Hairston et al. 2001; Gustafsson and Hansson 2004), but the adaptive trait(s) has not been identified. The length of time required for adaptive differentiation to occur similarly remains unknown. The second chapter reports on an experiment designed to determine if a population of *Daphnia* is capable of adapting to the presence of different strains of cyanobacteria within a time frame relevant to ecological interactions. In this case the adaptation period was 8 weeks.

Whereas the first two chapters aim to clarify the range of possible zooplankton-cyanobacteria interactions in the laboratory, the last three chapters investigate these relationships in a shallow lake. These chapters are based on data collected from a shallow mesotrophic lake over three summers. For two of the years, sampling frequency occurred at intervals of three days which permitted the use of time-series techniques to identify interactions among the abiotic and biotic components of the lake ecosystem. Time-series analysis is a statistical tool that allows the investigator to probe the dynamics of any variable (Chatfield 2004). It has rarely been used in phytoplankton ecology (Rojo and Alvarez-Cobelas 2000) and is a novel method of investigating the dynamics of bloom-forming cyanobacteria. In chapter four, this method was used to identify biotic and abiotic correlates with microcystin seston content at different time scales and in chapter five to identify correlates with cyanobacterial population dynamics. Although microcystins are the most commonly studied cyanotoxin, standardized methods for

sampling and analysis have not been agreed upon by the scientific community. Chapter three compares these commonly used methodologies and provides recommendations for the adoption of standard methods. This chapter has been published in the *Journal of Environmental Toxicology* (Tillmanns et al. 2007).

It is an interesting time in ecology as, with the inclusion of ideas such as dynamic systems and contemporary evolution, and the increased availability of computing power, ecologists are increasingly able to grapple with the complexities of ecosystems rather than necessarily distilling systems to simpler parts. Though many studies have been conducted on bloom-forming cyanobacteria, both in the laboratory and in the field, the fine temporal scale dynamics of cyanobacteria and toxins have received little attention, as has the adaptive potential of zooplankton when faced with a diet of cyanobacteria. This thesis is the author's attempt to apply the changing views of ecology to the problem of cyanobacterial blooms.

1 Chapter 1 – A meta-analysis of cyanobacteria – zooplankton interactions: taxa-specific responses

1.1 Abstract

Cyanobacteria are a poor food source for zooplankton, but the mediating mechanism, whether intracellular toxic metabolites, large morphology, lack of essential nutrients or interactions among these traits, is still unclear. Results from a previous meta-analysis confirmed that cyanobacteria were a poor food source for herbivorous zooplankton, that there was no clear ecological effect of cyanobacterial toxins on zooplankton, and surprisingly that filamentous cyanobacteria were a better food source than single-celled cyanobacteria for both rotifers and cladocerans. Here the dataset was re-analyzed to determine if these generalizations hold for specific taxonomic groups. The results show that although cyanobacteria were overall a poor food source for zooplankton, most taxa were still able to maintain a positive growth rate. Zooplankton species responded differently to cyanobacteria but the response was not related to genus or mean body length of the species. *Daphnia magna* was the only species that was more negatively affected by the presence of putative toxins in treatment diets. Additionally, there was no difference in mean effect size between strains of *Microcystis aeruginosa* that produced microcystins and those that did not. The addition of a non-cyanobacterial food to treatment diets reduced the negative effect of cyanobacteria for all genera. *Daphnia* were able to maintain positive growth even when fed diets consisting solely of cyanobacteria. Although the mechanisms of toxicity or mechanical interference may be important in some cyanobacteria-zooplankton interactions, the results presented here do not support these hypotheses in a general sense. Further experimentation is necessary to understand the importance of nutrition in zooplankton-cyanobacteria interactions.

1.2 Introduction

Cyanobacterial blooms in freshwater ecosystems are well-studied because some cyanobacteria are able to produce secondary metabolites that may contaminate drinking water for humans and animals (Codd 2000). Multiple hypotheses have been proposed to explain the ability of cyanobacteria to dominate an ecosystem (e.g. Hyenstrand et al. 1998). One central hypothesis is that cyanobacteria are resistant to grazing by zooplankton (Porter and Orcutt 1980b; Lampert 1987; de Bernardi and Giussani 1990). Field observations of the absence of large bodied cladocerans during cyanobacterial blooms (Edmondson and Litt 1982; Infante and Abella 1985) have provided support for this argument and led to numerous laboratory investigations of zooplankton-cyanobacteria interactions. Almost three decades ago, Porter and Orcutt (1980b) proposed three primary hypotheses predicting why cyanobacteria are inadequate food for zooplankton. First, secondary metabolites produced by cyanobacteria may be toxic to zooplankton (toxicity hypothesis). Second, some cyanobacterial genera grow as colonies or filaments that may be inedible to grazers due to their large or irregular size (morphology hypothesis). Finally, cyanobacteria may be deficient in nutrients vital to zooplankton (nutrition hypothesis).

Cyanobacteria produce a suite of secondary metabolites, some of which have been identified as toxic to mammals by mouse bioassays (Carmichael 1994; Carmichael 2001). A number of methods have been used to test the toxicity of cyanobacteria to herbivorous zooplankton. Early studies considered cyanobacteria to be toxic to herbivorous zooplankton if the zooplankton died faster in the presence of cyanobacteria when compared with starvation (Arnold 1971). Using this criterion, multiple cyanobacteria strains have been found to be toxic (Nizan et al. 1986; Ferrão-Filho et al. 2000). However, this method may not always be suitable as some zooplankton stop filtering in the absence of food (Plath 1998) thus reducing their energy requirements compared to the cyanobacteria treatments. Later tests compared growth rates of zooplankton fed toxic or non-toxic strains of the same cyanobacterial species (Reinikainen 1994; Lürling 2003b; Lürling and van der Grinten 2003) or also a toxic strain and its non-toxic mutant (Rohrlack et al. 2001). Although these studies provided some support for the larger

negative effect of the toxic strain on zooplankton growth, the results are questionable because cyanobacterial strains or mutants may differ in more than the ability to produce certain secondary metabolites (Lürling 2003b). In some cases, unidentified secondary metabolites have been found to be more toxic to zooplankton than known cyanotoxins (Jungmann 1992). In addition, some secondary metabolites may act synergistically with cyanotoxins to enhance the toxicity of these compounds to zooplankton (Pietsch et al. 2001; Rohrlack et al. 2005).

Cyanobacterial filaments and colonies may grow too large to be ingested by some herbivorous zooplankton and/or they may interfere with the feeding process (Webster and Peters 1978; Porter 1973; Hawkins and Lampert 1989). Both these processes are thought to hinder large cladocerans more than small cladocerans or rotifers (Webster and Peters 1978; Gliwicz 1990) possibly because large species may be capable of ingesting cyanobacteria colonies and/or filaments and so are more susceptible to toxicity or inadequate nutrients provided by cyanobacteria (Burns 1968). Also, larger species have a larger gape width and larger feeding appendages which increase their susceptibility to interference by filaments (Gliwicz and Siedlar 1980). Mechanical interference by filaments can increase the postabdominal rejection rate (Burns 1968; Webster and Peters 1978; Gliwicz and Lampert 1990) and/or entanglement of the thoracic limbs leading to decreased filtering efficiency and increased respiration (Porter and McDonough 1984; Gliwicz and Siedlar 1980). Large cladocerans are capable of reducing food concentrations below minimum thresholds necessary for smaller species to persist giving large cladocerans a competitive advantage (Gliwicz and Lampert 1990) but the negative impact of cyanobacteria to large species may reverse this competitive advantage when cyanobacteria are present (Gliwicz and Siedlar 1980; Edmondson and Litt 1982; Gliwicz and Lampert 1990). Field observations have reported the exclusion of large cladocerans in the presence of cyanobacterial blooms (Edmondson and Litt 1982; Infante and Abella 1985; DeMott et al. 2001) but coexistence has also been reported (Christoffersen et al. 1993; Epp 1996). Coexistence may occur if large species are capable of reducing their gape size and thereby limiting the effect of interference from filaments (Gliwicz and Siedlar 1980). Or if filaments or colonies are small enough to be ingested by all herbivorous zooplankton then small zooplankton may be affected equally (Gilbert 1994).

Although Wilson et al. (2006) found no relationship between the effect of cyanobacteria on population growth rates and body length for all herbivorous zooplankton, there may be a relationship within finer taxonomic groupings.

Cyanobacteria are deficient in essential highly unsaturated fatty acids (HUFA), which are necessary for zooplankton growth and reproduction, though the extent of deficiency differs between cyanobacterial genera (Ahlgren et al. 1992; DeMott and Müller-Navarra 1997; Muller-Navarra et al. 2000). *Synechococcus*, deficient in two ω3 HUFAs thought essential for *Daphnia* growth, did not support long term growth in *Daphnia* unless fish oil emulsions rich in HUFA were added (DeMott and Müller-Navarra 1997). A later study concluded it was the absence of sterols, not HUFA, that limited zooplankton growth (Von Elert and Wolffrom 2001). Other studies have documented positive growth of cladocerans on diets of only cyanobacteria (de Bernardi et al. 1981; Brett 1993; Repka 1996). In natural populations, however, herbivorous zooplankton ingest a mixed diet of phytoplankton, detritus and bacteria and therefore growth rates measured on a diet composed entirely of cyanobacteria may not be the best indicator of the impact of cyanobacteria in natural seston. Most zooplankton are homeostatic consumers, capable of assimilating a greater proportion of a limiting nutrient than occurs in their food mixture (Andersen and Hessen 1991). Experiments with a mixed diet of cyanobacteria and a secondary food source have demonstrated increased zooplankton growth over a single resource (Rothhaupt 1991; Lundstedt and Brett 1991) highlighting the importance of mixed diets to zooplankton growth (DeMott 1999). Whether mixed diets can compensate for the presence of bacteria, or whether zooplankton in general can maintain positive growth on cyanobacteria alone is uncertain.

A large number of laboratory and field studies have been conducted to directly or indirectly test the importance of each of the three hypothesis described above, however, conflicting results compounded by different experimental conditions across studies have made narrative generalizations difficult (Lampert 1987). In situations such as these, the ability to assess the results through a meta-analysis can provide useful information (Gurevitch and Hedges 1993; Arnqvist and Wooster 1995). A meta-analysis differs from qualitative literature reviews because, by calculating effect sizes for each experiment and

then using these effect sizes to test the over all effect, a quantitative estimation is possible (Gurevitch and Hedges 1993).

In a previous meta-analysis of laboratory studies on the effect of cyanobacteria on herbivorous zooplankton growth, I found that population growth rates of cladocerans and rotifers decreased when fed a treatment diet containing cyanobacteria compared to a control diet of chlorophytes and/or small flagellates (Wilson et al. 2006). In addition, filamentous cyanobacteria had less of an effect than single celled or colonial cyanobacteria and putative toxins had no clear effect on population growth rates. Tests of effect size, however, were made only at the broad taxonomic resolution of cladocerans and rotifers. The results may have been driven by specific taxa. In addition, differences across taxa may help explain the variability in zooplankton response to cyanobacteria.

In this study, the data set used by Wilson et al. (2006) was re-analyzed to determine if the generalizations made for freshwater herbivorous zooplankton hold for specific taxonomic groups. Comparisons of population growth rates on control diets of chlorophytes and/or flagellates and treatment diets containing cyanobacteria were compared for all zooplankton taxa in the data set. Additionally, differences in effect size due to the presence of putative toxins, differences in cyanobacteria morphology, zooplankton body length and interactions between morphology and body length were investigated for the most commonly used zooplankton taxa. Comparisons between treatment diets comprised solely of cyanobacteria and mixed diets were conduced to test if a secondary food source diminished the negative effect of cyanobacteria. And finally, a comparison between all cyanobacterial taxa was done to assess taxa-specific differences in negative impacts on zooplankton.

1.3 Methods

Data were collected by searching the Web of Science (from 1945 to 2004) and Aquatic Sciences and Fisheries Abstract (from 1971 to 2004) databases and the references cited therein, for studies that examined the influence of cyanobacteria on zooplankton population growth rates. Only experiments that calculated population growth rates (r) as a response metric were selected. Population growth rate is a useful endpoint

in toxicological studies as it integrates a number of life cycle attributes (Forbes and Calow 1999). In most cases, *r* (measured as the number of individuals produced per individual per day) was presented in each study, but when necessary, it was calculated using the equation (Gotelli 1998):

$$r = (\ln N_{t+1} - \ln N_t) / \text{time} \qquad \text{Eq 2.1}$$

Selected studies consisted of paired experiments with test populations fed either a control diet of beneficial food such as chlorophytes and/or small flagellates, or a treatment diet consisting of either 100% cyanobacteria or cyanobacteria together with chlorophytes and/or small flagellates. Experiments for cladocerans lasted 6 - 95 days (median =18 days) and for rotifers between 1 to 28 days (median = 8 days). In total 47 research papers were retrieved with data suitable for a meta-analysis giving a total of 378 treatment-control experimental pairs (Appendix 1; Table A1).

For each paired experiment the following variables were recorded: zooplankton species and body length; cyanobacteria species, morphology, and presence of secondary metabolites; the concentration by carbon of the control and treatment diets; and the percentage (by carbon) of cyanobacteria in the treatment diet. Cyanobacterial morphological type was classified as single-celled, filamentous or colonial. The cultures used in experiments did not always correspond with the natural morphology of the genera. In most cases (83%) *Microcystis aeruginosa* cultures were single celled whereas in nature this taxon is largely colonial (Komárek 1991). Most naturally filamentous species (*Anabaena, Aphanizomenon, Planktothrix, Cylindrospermopsis*) used were filamentous in the cultures with a mean length of approximately 226 μm (calculated from the 71% of studies that reported length and by assuming 1 cell was about 4 μm in length; Komárek et al. 2003).

Cyanobacteria were labeled toxic if the strain tested positive via chemical analysis such as HPLC or ELISA, for a previously described metabolite with a demonstrated toxic effect on any organism and non-toxic if the strain tested negative for toxins common to the genus. Secondary metabolites included in this dataset were microcystins (71% of toxic cases), a group of cyclic peptides known to inhibit protein phosphatase I and II

(MacKintosh et al. 1990); anatoxin (15%), a potent neurotoxin, the nucleosides deazaadenosine and glucopyranoside (10%) and pyrrolo [3,2-d] pyrimidines (4%; Sivonen and Jones 1999). Strains were not designated as toxic if the only data available were from biological assays (36 experiments) as these assays often rely on terrestrial or aquatic species unlikely to be in contact with the compound in nature and therefore such results may not be pertinent to freshwater zooplankton (Feuillade et al. 1996). There was not enough information to categorize cyanobacteria as toxic or non-toxic in 105 experimental pairs.

In this study, effect size was calculated for each experimental pair using the equation: $(r_{treatment} - r_{control}) / r_{control}$. This metric normalizes effect sizes by population growth rate on a good food source and makes comparisons across grazer species possible. The effect size is equal to the percent decrease in population growth rate of a zooplankton population fed a diet containing cyanobacteria compared to a control diet. Mean control and treatment r values and mean effect sizes (all with 95% confidence intervals, CI) were reported for zooplankton species and genera and only mean effect sizes were reported for cyanobacterial strains, species and genera. Rather than reporting multiple p-values for each taxa to determine if there was a significant effect of the treatment diet (i.e. the effect size was below zero) the confidence limits were presented graphically. As the likelihood that the actual mean value is within the confidence intervals decreases towards the tails of the confidence intervals, means with confidence intervals that only slightly overlap with zero were considered ecologically meaningful (Di Stefano et al. 2005).

Two-tailed, two-sample t-tests were used to test for differences in mean effect sizes between toxic and non-toxic treatments diets, single and colonial *M. aeruginosa* and microcystin producing and non-producing *M. aeruginosa.* One-way ANOVAs were used to test for differences among more than two groups. The non-parametric Kruskal-Wallis test was used when heteroscedasticity could not be corrected through transformations. Pearson's correlations were used to test for relationships between mean species body length and mean species effect size within each group: cladocerans, rotifers and *Daphnia.* Linear regressions were also used to test for a negative relationship between effect size and percent cyanobacteria in treatment diets for taxa that had a range of at least fifty

percent cyanobacteria in treatment diets. A general linear model was used to test if grazer genera responded differently to an increasing percent of cyanobacteria in treatment diets (percent cyanobacteria x genus interaction). *P* values for significance tests were set *a priori* at 0.05.

1.3.1 Meta-analysis methods

In a meta-analysis, precision differences among studies are accounted for by weighing the effect size by its variance (Gurevitch and Hedges 1999). However in this study, the variance of both control and treatment errors ($\sigma_{\text{treatment error}} = 0.003$; $\sigma_{\text{control error}} = 0.001$) were two magnitudes less than the variance of treatment and control population growth rates ($\sigma_{\text{treatment r}} = 0.205$; $\sigma_{\text{control r}} = 0.206$) indicating that weighted results would not differ substantially from unweighted results. In the previous meta-analysis, a weighted analysis was conducted but few differences between the weighted and unweighted results were found (Wilson et al. 2006). Therefore, in this study weighted results were not calculated.

In a meta-analysis, bias can be introduced in 4 main ways: 1) lack of objective criterion for study selection before data accumulation; 2) differences in measurement error between studies; 3) publication bias of significant results and 4) non-independence of data points due to multiple experiments per study or the use of one control experiment to calculate multiple effect sizes (Gurevitch and Hedges 1993; Gurevitch and Hedges 1999; Gates 2002; Rosenberg 2006). Selection bias was mitigated by clearly defining objective criteria for data selection prior to data collection. A standard practice to reduce the significance of individual results is to use an effect size measure (Gurevitch and Hedges 1999). The one chosen for this meta-analysis accomplished this as well as normalizing the growth rates of various zooplankton (Wilson et al. 2006). As previously mentioned the measurement error for data in this meta-analysis was too low to cause a difference in weighted vs unweighted results and so was not incorporated into this analysis. Publication bias was also not considered a problem for this meta-analysis because non-significant findings – i.e. no effect of a treatment diet containing

cyanobacteria, would be of just as much interest as those studies that did detect an effect. The fourth point of non-independence has been more challenging for the current analysis.

In this meta-analysis, experimental conditions can change dramatically between studies (species and genotypes, diets, temperature, etc.). By dividing experiments among taxa, groups of low sample size were created and as a result, non-independence was a major concern (Gurevitch and Hedges 1999). The influence of multiple experiments per study can be problematic as the test degrees of freedom are inflated (Gurevitch and Hedges 1999). As a first assessment of non-independence the intra-class correlation coefficient (ICC) was calculated to assess the similarity of results within studies (Zar 1999; Haxton and Findlay 2006). Additionally, for each taxa with a significant ICC, a randomized sign test was used to quantitatively assess the effect of multiple experiments per study (Adams et al. 1997; Haxton and Findlay 2006; Manly 2007). This was conducted by first selecting one experiment randomly from each study and calculating the mean effect size. This step was repeated with replacement 1000 times and the overall mean of all samples was calculated to give the test mean. Next, to generate a null distribution, a random number generator (Microsoft EXCEL) was used to assign either a negative or positive sign to the effect sizes of each experimental pair. Experiments with altered signs were then sampled with replacement 999 times. For each resampling exercise the p-value was calculated as the number of values in the null distribution that were further from zero than the test mean and divided by the total number of values in the distribution (in this case, 1000). This process was repeated, each time increasing the number of experiments sampled from each study for both the test and null samples until all the experiments from all studies were included. Randomized methods were also used to test if the originating study influenced the results when comparing toxic and non-toxic groups. For two-sample t-tests the same procedure was used but this time to generate the null distribution, effect sizes were randomly assigned to one of the two categories.

1.4 Results

Mean treatment *r* values for 6 out of 8 rotifer and 15 out of 21 cladoceran species and as well as for three commonly-used genera, *Ceriodaphnia, Daphnia* and *Keratella* were significantly greater than zero, indicating positive population growth rates in the presence of cyanobacteria (Fig 1.1a). Although most cladoceran and rotifer species were able to maintain a positive growth rate, 6 rotifer and 13 cladoceran species had lower population growth rates when fed a treatment diet containing varying proportions of cyanobacteria (1-100% by carbon) relative to growth rates observed on diets containing only chlorophytes and/or small flagellates (mean effect size < 0; Fig 1.1b). Among the cladoceran genera that were negatively affected, *Chydorus* and *Moina* had the largest average decrease in population growth rate when fed treatment diets containing cyanobacteria (-143% and -82% respectively) and amongst the rotifers, *Hexartha* (-194%) and *Brachionus* (-85%) were most negatively affected (Fig 1.1b).

Differences among species could not be explained by zooplankton mean species body length or when grouping by genera (Fig 1.1). There was no relationship between mean species body length and mean species effect size among cladocerans (Pearson's r = 0.087, P = 0.716, n=20), rotifers (r = -0.652, P = 0.161, n=8) or only *Daphnia* species (r = -0.310, P = 0.354, n = 11). There was also no correlation when the data were restricted to experiments using filamentous cyanobacteria (all species, r = -0.465, P = 0.070, n = 16).

Zooplankton taxa showed varied responses to different cyanobacterial morphologies. In general, single-celled cyanobacteria were the most detrimental to zooplankton and caused a negative effect on the majority (18 out of 22) of zooplankton species (Fig 1.2a) relative to diets containing only control foods. Filamentous cyanobacteria had a negative effect on fewer species with half (8 out of 17) of the zooplankton species negatively affected (Fig 1.2b); chroococcoid colonial cyanobacteria had a negative effect on the fewest species tested (3 out of 10; Fig 1.2c). There were differences amongst the cladoceran species when treatment diets contained single-celled ($F_{16, 161}$=5.46, P < 0.001, n= 178; Fig 1.2a) or filamentous ($F_{9, 72}$= 4.10, P <0.001, n=82;

Fig 1.2b) cyanobacteria but not chroococcoid colonial cyanobacteria ($F_{7, 22}$= 0.803, P= 0.593, n=30; Fig 1.2c). Differences amongst rotifer species could only be detected for treatment diets containing single-celled cyanobacteria (single-celled: $F_{4, 30}$=2.92, P=0.037, n=35; filamentous: $F_{6, 34}$=0.076, P=0.603, n=41; chroococcoid colonial: $F_{1, 6}$=0.181, P=0.385, n=8). Filamentous cyanobacteria had less of a negative impact than single-celled cyanobacteria for *Keratella* (Student's t-test; t_{17}=2.51, P=0.023, n=19), *Daphnia* (One-way ANOVA; $F_{2, 209}$=6.27, P=0.002, n=214; Tukey's Post Hoc Test P=0.001), and *Brachionus* ($F_{2, 53}$=11.21, P<0.001, n=56; Tukey's P<0.001). There was no difference between single-celled and chroococcoid colonial cyanobacteria for *Daphnia* (P=0.801) or *Moina* (t_{25}=2.51, P=0.185, n=27) but there was a larger negative effect of chroococcoid colonial over filamentous cyanobacteria for *Brachionus* (P=0.007).

Three genera and seven species of zooplankton were used in both experiments using toxic cyanobacteria as well as experiments using non-toxic cyanobacteria (Fig 1.3). In most cases there was little difference between the toxic/non-toxic effect sizes; only *D. galaeta* and *D. magna* had significantly lower growth on toxic diets (*D. galaeta,* t_{11}= 4.97, *P<0.001.*; *D. magna*, t_{36}=3.2, P=0.003; Fig 1.4). The data for *D. galaeta,* however, included only two observations of growth on non-toxic diets.

There was a negative relationship between effect size and percent of cyanobacteria in the treatment diets in only four of the ten zooplankton species tested and four out of five grazer genera (*Brachionus, Daphnia, Keratella,* and *Moina*; Table 1.1). The response of these four genera differed in slope (percent cyanobacteria × genus interaction; $F_{3, 311}$=14.7, P<0.001). When treatment diets consisting of 100% cyanobacteria were considered, five *Daphnia* species and *Simocephalus vetulus* were able to maintain significantly positive growth rates (Table 1.2). Additionally, the lower confidence intervals of the genera *Daphnia* and *Ceriodaphnia* and the species *D. longispina* and *D. pulex* only minimally overlapped with zero indicating that the actual mean was most likely above zero.

In all there were 26 unique culture collection strains of cyanobacteria from 10 genera represented in the combined dataset but 229 of the 378 treatment-control experimental pairs used *Microcystis aeruginosa* (Fig 1.4). The strain with the largest negative effect was an anatoxin producing *Anabaena flos-aquae* NRC-44-1 (mean ± 95% CI; -1.68 ± 0.92) followed by microcystin producing *M. aeruginosa* PCC 7806 (-1.58 ± 0.06). The cyanobacterial taxa that were the best foods for zooplankton, relative to population growth rates on control diets lacking cyanobacteria, were *Aphanizomenon flexuosum* (0.33 ± 0.47) and the microcystin producing strain, *M. aeruginosa* NPLJ42 (-0.13 ± 0.20). The most commonly used strain was *M. aeruginosa* PCC 7820 (n=52) which produces microcystins and was used predominantly in tests with *D. pulex* (n=31). *D. pulex* fed strain PCC 7820 as part of the treatment diet was more negatively impacted than *D. pulex* fed all other strains of *M. aeruginosa* (PCC 7820: -0.93 ± 0.35, n=31; all other *M. aeruginosa* strains: -0.32 ± 0.17, n=17; $t_{46} = 2.44$, $P = 0.019$).

A comparison of the most common cyanobacterial genera showed that *Microcystis* (comprised of only *M. aeruginosa*) had a larger negative effect on zooplankton than *Anabaena* and *Planktothrix* (One-way ANOVA, $F_{4, 361} = 6.20$, $P < 0.001$; Fig 1.4). Although there were significant differences in mean effect size among strains of *M. aeruginosa* (Kruskal-Wallis, $H_{14} = 28.2$, $P = 0.011$; Fig 1.4) no difference could be detected between *M. aeruginosa* strains that produce microcystins (mean effect size ± 95% CI; -0.72 ± 0.14, n=125) and those that do not (-0.53 ± 0.15, n=27; $t_{150} = 1.19$, $P = 0.237$). There was also no detectable difference in mean effect size between single-celled (-0.83 ± 0.14, n=191) and colonial (-0.91 ± 0.39, n=38) strains of *M. aeruginosa* ($t_{227} = -0.440$, $P=0.661$).

The intraclass correlation coefficient (ICC) revealed that there was an effect of originating study for four of the five zooplankton genera and 8 of the 16 zooplankton species that were used in more than one study (Table A1). Of those with a significant ICC, *Keratella, K. cochlearis,* and *Daphnia carinata* did not show a negative effect of treatment diets containing cyanobacteria when only one experiment was chosen per study (Table A2). The mean effect size for two of the five studies that were used in the meta-analysis for both *Keratella* and *K. cochlearis* were significantly higher than the other

three studies (One-way ANOVA, *Keratella* $F_{4,14}$=5.14, P =0.009; *K. cochlearis* $F_{4,9}$=3.9, P =0.041; Tukey's Post Hoc Test, p<0.05) as was the mean effect size in one of the two studies that used *D. carinata* (two sample t-test, t_6=2.74, P =0.034). Multiple experiments per study did not alter the significant negative effect of putative toxins on *Daphnia magna* (Table A3). Also, regardless of the number of experiments chosen per study there were no differences between toxic and non-toxic or single and colonial *Microcystis aeruginosa* (Table A3).

1.5 Discussion

In this study, I examined variation among zooplankton and cyanobacterial taxa, with respect to cyanobacterial effects on zooplankton population growth and found some major differences among zooplankton taxa. Treatment diets containing cyanobacteria caused a decrease in zooplankton population growth relative to control diets of small chlorophytes/flagellates for the majority of zooplankton species tested (Fig 1.1b) but surprisingly, 6 out of 8 rotifer species and 14 out of 20 cladoceran species maintained positive growth rates when fed a diet containing cyanobacteria (Fig 1.1a). The latter results indicate that populations of some zooplankton genotypes may be able to increase even though their population growth rate is negatively impacted by cyanobacteria (Sarnelle 2007).

Differences in mean effect size among both rotifer and cladoceran species were evident in the experiments using single-celled cyanobacteria (Fig 1.2). Since mechanical interference is not pertinent in this case, differences among grazers must be due to either physiological tolerance to cyanobacteria or a behavioral response (DeMott and Dhawale 1995; Sarnelle and Wilson 2005; Wilson and Hay 2007). Differences in grazer performance on a diet containing single-celled cyanobacteria have been reported previously across grazer genera, species within a genus and genotypes within species (Lundstedt and Brett 1991; Hietala et al. 1995; Nandini and Rao 1998; DeMott 1999; Ferrão-Filho et al. 2000).

Field observations of the lack of large *Daphnia* during cyanobacterial blooms (Edmondson and Litt 1982; Infante and Abella 1985; DeMott et al. 2001) as well as results from some laboratory experiments (Webster and Peters 1978; Porter and Orcutt 1980a; Gliwicz and Lampert 1990) have emphasized mechanical interference as a major mechanism limiting the growth of large bodied zooplankton species during cyanobacterial blooms. However in the present study, I could detect no relationship between mean species effect sizes and mean species body lengths for 20 cladoceran species (body length [BL] range: 0.3 to 3.9 mm; effect size [ES] range: -5.1 to 1), 11 *Daphnia* species (BL: 1.0 to 3.9 mm; ES: -5.1 to 0.3) or eight rotifer species (BL: 0.1 to 0.4 mm; ES: -4.3 to 2.1); nor could I detect a relationship when experiments were limited to those that used filamentous cyanobacteria in treatment diets (12 species, BL: 0.1 to 3.6 mm; ES: -1.2 to 0.3). Also, no difference in mean effect size could be detected between colonial and single celled *M. aeruginosa.* Both of these results suggest that, in general, mechanical interference may not be a major inhibitor of zooplankton growth (as suggested also by Ferrão-Filho et al. 2000). Other factors may be responsible such as susceptibility of zooplankton to unidentified secondary metabolites produced by cyanobacteria (Kurmayer 2001) or differences in susceptibility to cyanobacteria due to grazer life history characteristics (Lynch 1980; Ferrão-Filho et al. 2000).

Comparisons of experiments with treatment diets containing toxic cyanobacteria and experiments using non-toxic cyanobacteria failed to reveal any clear effect of toxicity, which is consistent with findings from our previous meta-analyses (Wilson *et al.* 2006). Of the seven zooplankton species and three genera that were used in both experiments, only *D. magna* and possibly *D. galeata* were more negatively affected by the presence of cyanobacteria containing known intracellular toxins (Fig 1.3). For both species, the most frequently identified cyanobacterial secondary metabolites (about 80%) were microcystins. Also, no difference in mean effect size could be detected between *M. aeruginosa* that produced microcystins and those that did not. Non-toxic cyanobacteria may, however, contain yet unidentified toxins (Rohrlack *et. al.* 2004, Wilson & Hay 2007) or may not have been tested for all known toxins, only those most common to the genus.

Though there appears to be no overall effect of known cyanobacterial toxins, except in the case of *D. magna,* these metabolites may nevertheless have an effect on some genotypes. For example, *D. pulicaria* taken from a lake with documented cyanobacterial blooms was less susceptible to toxic cyanobacteria than *D. pulicaria* taken from a less eutrophic lake (Wilson and Sarnelle 2005). In a direct test of toxicity, Wilson and Hay (2007) found microcystin- LR added to freeze dried *Chlorella* and fed to zooplankton significantly reduced the population growth rate of one clone of *D. pulicaria* while having no effect on a second *D. pulicaria* clone. Also, Rohrlack et al. (2001) found a mutant strain of *M. aeruginosa* not capable of producing microcystins to be less toxic to five clones of common *Daphnia* species than the microcystin-producing wildtype. However, the effects of toxins may be fundamentally altered when the toxin is separated from its normal biochemical setting within a cyanobacterium (Wilson and Hay 2007), and knocking-out the genetic sequence necessary to produce microcystins may alter the strain in other undocumented ways.

Nutritional inadequacy of cyanobacteria may be responsible for limiting zooplankton growth (DeMott and Müller-Navarra 1997). Cyanobacteria have reduced amounts of highly unsaturated fatty acids compared with green algae (Ahlgren et al. 1992; DeMott and Müller-Navarra 1997), and the addition of a second algal species with sufficient fatty acids to treatment diets has been shown to decrease the negative effect of cyanobacteria (Reinikainen et al. 1994; Hietala et al. 1997a), though this result may be due to the addition of sterols rather than fatty acids (Von Elert et al. 2003). Here I found a negative relationship between effect size and percent of cyanobacteria in treatment diets for only four of the ten species that were fed a range in percent cyanobacteria (Table 1.1). Though other mechanisms such as the presence of harmful bioactive compounds could cause this negative relationship, the absence of a relationship in six of the ten species suggests a supplementary food source was not necessary to maintain population growth. Additionally, six species and to some extent the genera *Daphnia* and *Ceriodaphnia,* were capable of maintaining a positive growth rate even when fed 100% cyanobacteria (Table 1.2). Thus earlier findings that showed a negative relationship between effect size and percent cyanobacteria in the treatment diet (Wilson et al. 2006) may not hold for all zooplankton – cyanobacteria interactions. Other studies (de Bernardi et al. 1981; Brett

1993; Repka 1996) have also reported positive growth rates of zooplankton species on diets of solely cyanobacteria.

In general, the genus *Microcystis* was a poorer food source for zooplankton than *Anabaena* or *Planktothrix*, but the effect size was strain specific and did not depend on the strain's capacity to produce microcystins (Fig 1.4; Nizan et al. 1986; Ferrão-Filho et al. 2000; Lürling 2003b). Microcystin-free extracts of *M. aeruginosa* can still be detrimental to herbivorous zooplankton (Kurmayer 2001; Rohrlack et al. 2004). The cyanobacterial strain with the largest negative effect on zooplankton was *Anabaena flos-aquae* NRC-44-1 which produces anatoxin-*a* (Porter and Orcutt 1980a; Starkweather and Kellar 1983). Although there were insufficient data to statistically compare toxic and non-toxic *Anabaena* strains, it is interesting to note that the one non-toxic *Anabaena* strain, *A. flos-aquae* UTEX 1444, was also the only strain that did not cause a clear negative effect on zooplankton grazers. Although our results and those previously reported (Wilson *et al.* 2006) suggest no clear effect of putative cyanobacterial toxins in general, further research on the effects of anatoxin-*a* on grazer growth rates is needed.

Daphnia pulex (n=95) and *Daphnia magna* (n=46) were the most commonly used species in this meta-analysis, and because of the large sample sizes for these two species it is particularly interesting to note the differences. Both *D. pulex* and *D. magna* were affected by treatment diets containing cyanobacteria but only *D. pulex* could maintain a positive growth rate when fed diets containing cyanobacteria. *D. magna* was affected by treatment diets containing putative toxins while *D. pulex* was not (Fig 1.3) which is congruent with the fact that there was a negative effect of percent cyanobacteria on effect size for *D. magna* but not *D. pulex* (Table 1.2).

Though multiple experiments from one study does reduce the independence of effect sizes (Gurevitch and Hedges 1999), the results of the re-sampling procedures (Table A2 & A3) showed that even when only one effect size was chosen from each study effect size was still negative for all but three zooplankton taxa. Two studies that used *K. cochlearis,* Gilbert (1990) and Gilbert and Durand (1990), took this taxon from the same pond near Hanover, NH. The *Keratella* in this pond were well adapted to

cyanobacteria. Similarly, *D. carinata* used by Chen and Xie (2004) were also well adapted. The limnological details in both papers were scarce so little is known of the characteristics of these ponds but for these three taxa, *Keratella, K. cochlearis* and *D. carinata,* the range of responses indicates that cyanobacteria may not always negatively affect their population growth rates.

This meta-analysis and the previous study (Wilson et al. 2006) have provided quantitative generalizations across laboratory studies of zooplankton population growth rates when fed treatment diets containing cyanobacteria. Neither toxins nor colonial morphology appear to have specific negative effects in general across zooplankton species. The nutritional hypothesis could not be addressed directly as it is difficult to separate the possible effects of unidentified bioactive cyanobacterial metabolites from nutritional inadequacies. However, the finding that numerous zooplankton species could maintain a positive growth rate when fed diets of only cyanobacteria implies that some cyanobacterial genotypes contain adequate nutritional value to maintain population growth of some zooplankton species, at least for the duration typical of laboratory studies.

The majority of studies summarized by this meta-analysis used single clones, typically from laboratory cultures, making it difficult to extrapolate these results to natural systems. This meta-analysis generalizes the results of these studies which used a range of treatments (cyanobacteria species, concentrations, morphology - including filament length and concentration), and genetic strains of both zooplankton and algae. These findings do not negate the results of individual studies but suggest that many of the hypotheses put forward may not adequately explain why cyanobacteria are of poor food quality to zooplankton. Furthermore, as this meta-analysis was conducted at the scale of species, it does not consider the importance of intra-specific variation which, given the large amount of variation in population growth rates among studies compared to within studies as well as demonstrated differences in responses between zooplankton clones (Repka 1996, Sarnelle and Wilson 2005), may be quite high. A qualitative review of the literature would find many examples and exceptions to each of the generalizations made in this meta-analysis. However, the strength of a meta-analysis is to draw quantitative

generalizations across multiple studies which used different conditions and different strains of organisms to assess if the hypotheses found prevalently in the literature are supported by the available data.

In general, our results support the conclusion that cyanobacteria are a poorer food resource for zooplankton than small chlorophytes and/or flagellates, though some species were able to maintain positive growth rates on diets containing cyanobacteria. More importantly, our results highlight the high degree of variation in species-specific responses of zooplankton to cyanobacteria. For example, some strains of cyanobacteria were particularly detrimental to some species of zooplankton while other zooplankton species were capable of reproducing even on diets composed entirely of cyanobacteria. The effects of cyanobacterial morphology and known toxins were not general or strong enough to stand out over variation among strains and species. Although a meta-analysis can provide a summary of available data, experiments using multiple clones isolated from multiple water bodies with documented limnological history are necessary to document the range of traits within each species. Only once this range is established can I begin the process of understanding variation in the interaction between any two species (Holt 2005).

Acknowledgments

I would like to thank Alan Wilson for compiling and checking the data set used in this analysis and also for extensive comments on the first version of this chapter. I would also like to thank Whitney Wilson for paper retrieval, digitizing graphs and data entry.

1.6 Tables

Table 1.1 Results of linear regressions which test for the dependence of effect size on percent cyanobacteria in treatment foods for zooplankton genera and species. Ranges for percent cyanobacteria in treatment diets are also given. Only taxa with a range of at least fifty percent cyanobacteria across treatment diets were considered suitable for a linear regression model. Genus entries give mean values for all species in the specified genus.

Zooplankton taxa	n	range	slope	*P* value
Brachionus	56	1 - 100	-0.018	<0.001
B. calyciflorus	45	1 - 100	-0.020	<0.001
B. rubens	11	1 - 100	-0.019	0.138
Ceriodaphnia	34	2 - 100	-0.002	0.606
C. cornuta	28	5 - 100	-0.004	0.400
Daphnia	214	1 - 100	-0.004	0.001
D. ambigua	5	2 - 90	-0.011	0.018
D. carinata	8	6 - 100	-0.010	0.075
D. galeata	16	20 - 100	-0.008	0.001
D. longispina	12	23 - 100	-0.008	0.191
D. magna	46	5 - 100	-0.012	<0.001
D. pulex	95	1 - 100	-0.003	0.159
Keratella	19	2 - 60	-0.011	0.003
Moina	27	5 - 100	-0.017	0.001
M. micrura	20	5 - 100	-0.006	0.050

Table 1.2 Population growth rates (*r*) of zooplankton taxa fed treatment diets consisting solely of 100% cyanobacteria. *r* values with lower confidence intervals (CI) that are above zero maintained positive growth even when fed diets of 100% cyanobacteria. Genus entries give mean values for all species in the specified genus.

Zooplankton taxa	n	Mean	Lower CI	Upper CI
Brachionus	20	-0.32	-0.57	-0.07
B. calyciflorus	18	-0.27	-0.52	-0.02
B. rubens	2	-0.78	-1.81	0.25
Ceriodaphnia	12	0.12	0	0.24
C. cornuta	9	0.09	-0.07	0.25
C. dubia	3	0.21	0.19	0.24
Chydorus sphaericus	2	-0.05	-0.17	0.07
Daphnia	75	0.04	-0.01	0.09
D. carinata	4	-0.05	-0.2	0.1
D. cucullata	2	0.14	0.11	0.18
D. galeata	11	0.12	0.1	0.14
D. hyalina	3	0.24	0.15	0.33
D. laevis	2	0.09	0.07	0.11
D. longispina	8	0.15	-0.01	0.3
D. magna	18	-0.13	-0.25	0
D. obtusa	3	0.33	0.13	0.53
D. pulex	22	0.05	-0.04	0.13
Hexartha mira	4	-0.54	-1.33	0.24
Moina	10	-0.30	-0.52	-0.08
Moina macrocopa	7	-0.33	-0.58	-0.07
Moina micrura	3	-0.25	-0.74	0.24
Simocephalus vetulus	4	0.27	0.25	0.3
Scapholeberis kingi	4	0.07	-0.12	0.25

1.7 Figures

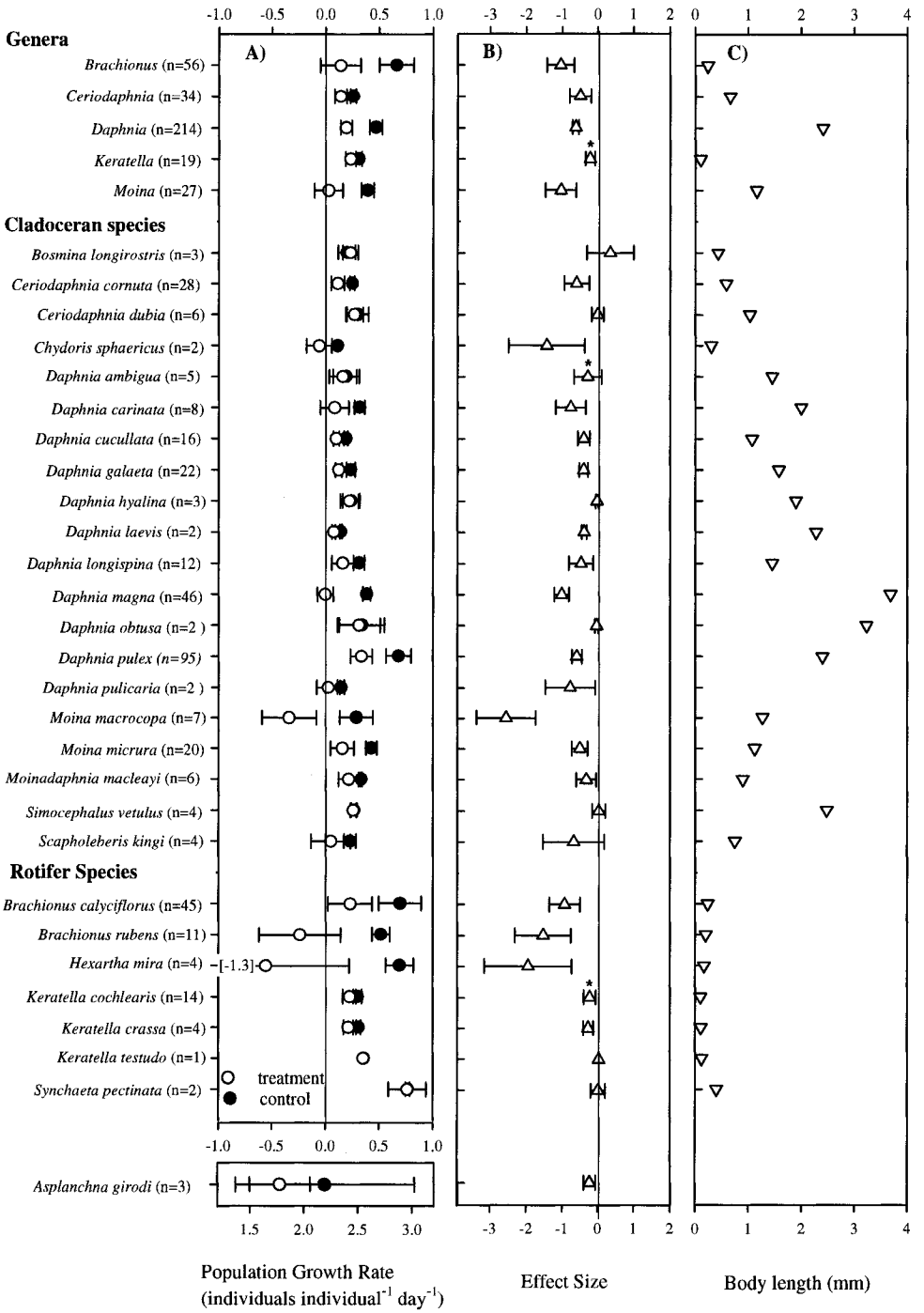


Figure 1.1 Panel A: A comparison of mean population growth rates (± 95% CI) of herbivorous zooplankton fed a control diet of small chlorophytes and/or flagellates and a treatment diet containing small chlorophytes and/or flagellates with the addition of cyanobacteria (1-100%). Panel B: Mean effect sizes (± 95% CI) for zooplankton taxa calculated from control and treatment population growth rates (see methods). Panel C: Mean body length (± 95% CI) of zooplankton taxa. Sample size for each zooplankton taxon is given in parenthesis. Underlined entries give mean values for all species in the genera. * These effect sizes were not significantly different from 0 when one effect size was chosen per study.

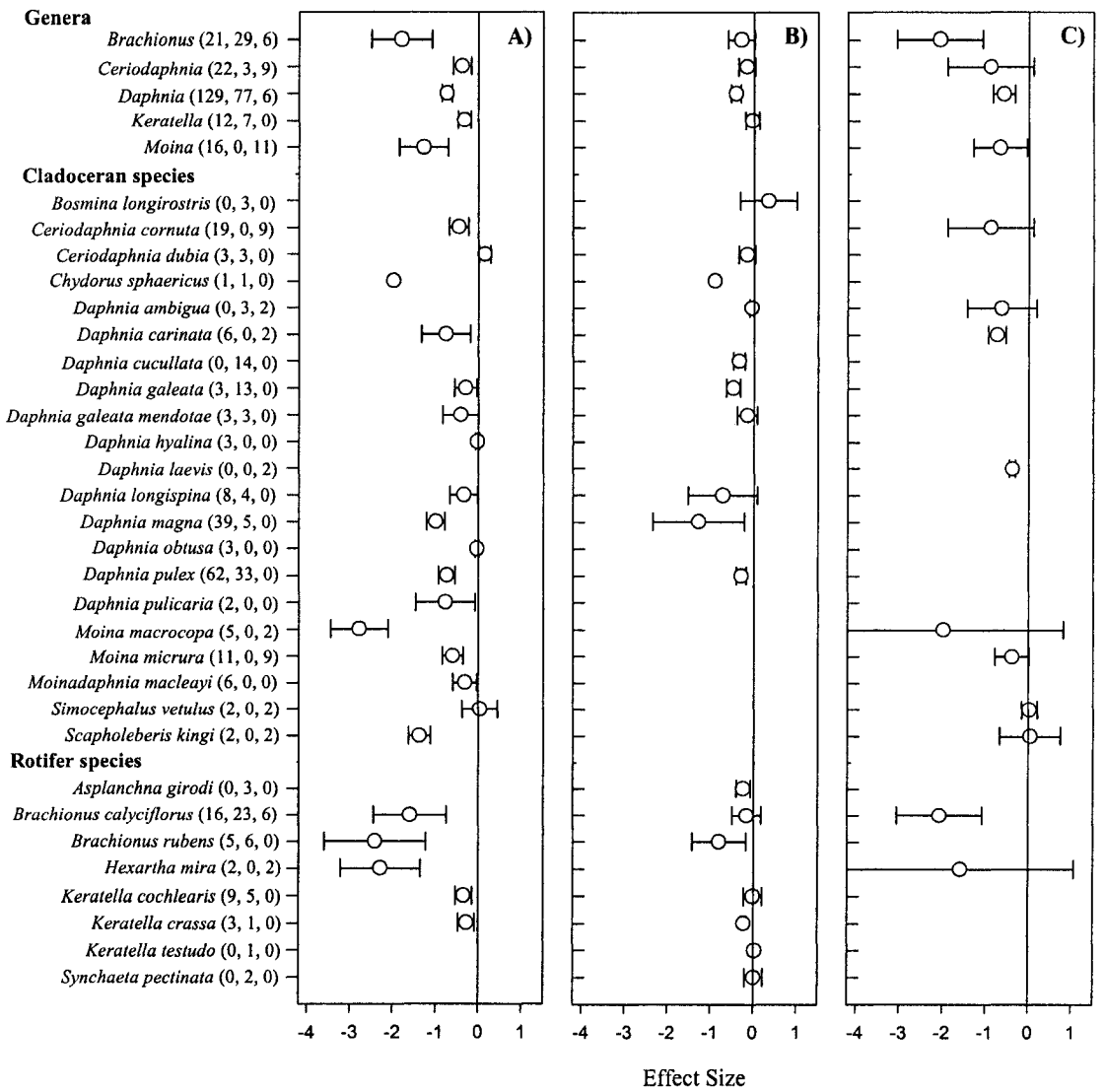


Figure 1.2 A comparison of mean effect sizes (±95 % CI) for taxa fed a treatment diet containing single celled (A), filamentous (B), and colonial cyanobacteria (C). Effect size was calculated from population growth rates for paired experiments of grazers fed a control diet of chlorophytes and or flagellates and a treatment diet containing cyanobacteria. There is no difference between genera means that are sharing the same vertical line on right (One-way ANOVA & Tukey's post hoc test, $P<0.05$).

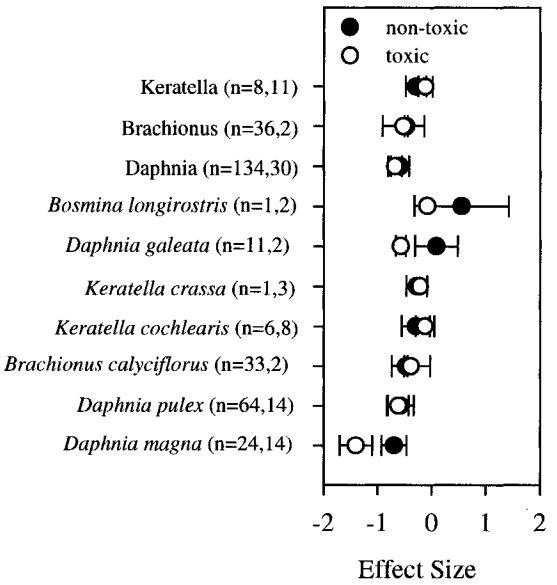


Figure 1.3 Mean effect sizes (±95 % CI) for zooplankton taxa fed treatment diets of cyanobacteria which produce putative toxins (n is given as first number in parenthesis) and those that do not (n is second number in parenthesis).

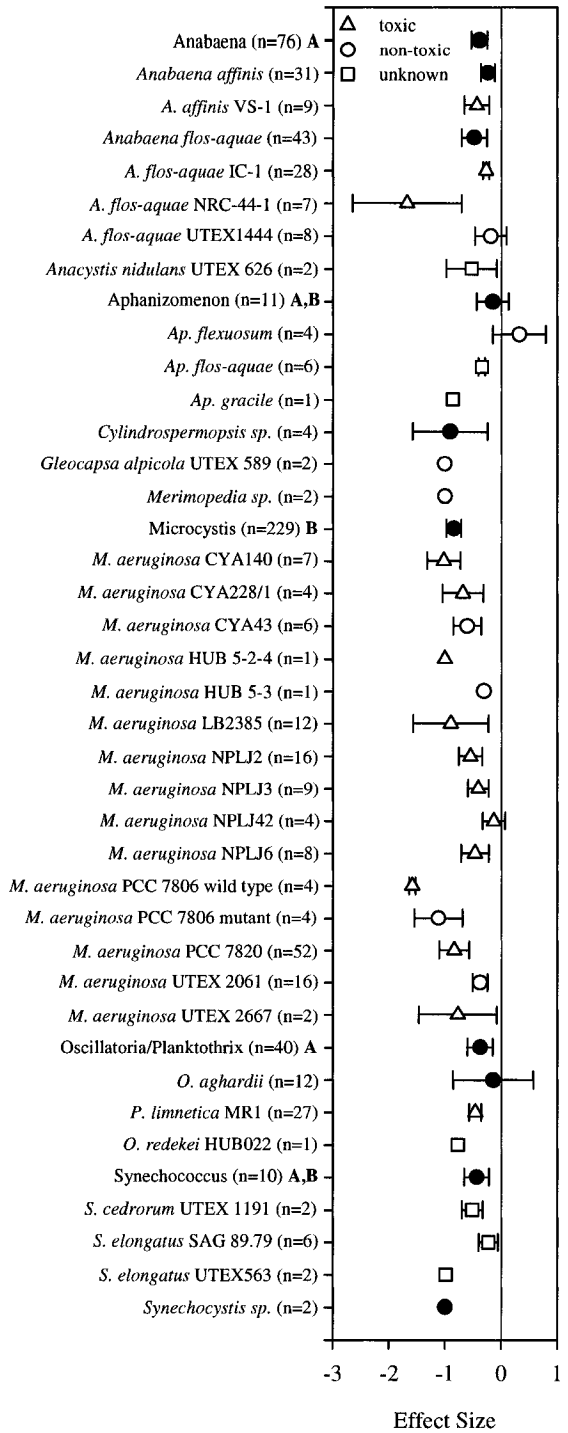


Figure 1.4 A comparison of mean effect sizes (±95 % CI) for cyanobacterial taxa used in treatment diets of herbivorous zooplankton. Black symbols denote mean values for all species in a specified genus. Black symbols also give means of a species when strains were not identified. Differences among cyanobacterial genera were tested by one-way ANOVA. There is no difference in means between genera sharing the same letter (Tukey's Post Hoc Test, $p<0.05$). Error bars are presented for all taxa with $n>1$ but may be smaller than symbol.

2 Chapter 2 - Acclimation in a *Daphnia* population leads to altered cell ratio in experimental diet

2.1 Abstract

Recent evidence suggests that *Daphnia*, a non-selective planktonic filter feeder, can acclimate to food resources that contain toxic compounds such as microcystins but the adaptive trait, whether physiological or behavioral, remains unknown. In this study, the cell ratios of two algal species remaining after short term feeding experiments were used to determine if *Daphnia* pre-exposed to toxic cyanobacteria could avoid ingesting toxic cells. The results show that *Daphnia ambigua* was capable of altering the proportion of a cyanobacterium (*Microcystis aeruginosa*) to a chlorophyte (*Chlorella kessleri*) of similar size and shape in short-term feeding experiments. Also, *D. ambigua* populations acclimated for eight weeks to a toxic strain of *M. aeruginosa* were more successful at avoiding this strain than populations acclimated to *C. kessleri* or a non-toxic strain of *M. aeruginosa.* A modeling exercise showed that although the different settling rates of *Microcystis* and *Chlorella* could explain the ability of *Daphnia* to alter the cell ratio in most acclimated population × diet treatments, differential settling could not explain results for the population acclimated to toxic *M. aeruginosa* which required either differential gut passage or selective feeding to simulate the results. The ability of *Daphnia* to acclimate to different strains of cyanobacteria in an ecologically relevant time period could influence the dominance of cyanobacterial strains in natural populations.

2.2 Introduction

Daphnia, a genus of planktonic freshwater crustaceans, is capable of reducing phytoplankton biomass leading to the spring clear water phase in lakes (Sommer et al. 1986), and also of altering phytoplankton community composition (Sarnelle 1993). The mechanisms underlying this process, however, are not well understood. *Daphnia* is widely considered a non-selective filter-feeder based on previous experiments of food quality (Lampert 1974; DeMott 1982) and taste (DeMott 1986) but some studies have provided evidence for feeding selectivity. For example, *Daphnia pulicaria* was able to differentiate between cyanobacteria filaments (Epp 1996) and *D. magna* and *D. pulex* selected individual *Cryptomonas* cells over filaments of *Anabaena* (Kirk and Gilbert 1992). These results suggest that *Daphnia* may have some means to choose their food. Generally, cyanobacteria are considered poor food for zooplankton due to nutritional deficiencies, large size and/or toxicity (Porter and Orcutt 1980a). Cyanobacteria produce a suite of secondary metabolites, the most commonly studied of which are microcystins (MC), a group of cyclic peptides that inhibit protein phosphatases (MacKintosh et al. 1990). MC producing species usually have both MC and non-MC producing strains and the genotype composition of cyanobacteria populations can fluctuate over a season (Kardinaal et al. 2007a). The mechanism controlling the proportion of each strain, however, is unknown; a question of considerable societal relevance given the toxicity of microcystins to humans and other animals (Sivonen and Jones 1999).

Even though in general cyanobacteria may be a poor food resource for zooplankton (Wilson et al. 2006), some studies have reported positive growth in zooplankton when fed toxic cyanobacteria (de Bernardi et al. 1981; Brett 1993; Repka 1996). These observations have led to the investigation of the adaptive potential of *Daphnia.* Recent experiments suggest that *Daphnia* can acclimate to toxic cyanobacteria (Hairston et al. 2001; Gustafsson et al. 2005; Sarnelle and Wilson 2005; Guo and Xie 2006). For example, *Daphnia* from eutrophic lakes exhibited higher juvenile growth rates than those from less nutrient rich lakes when fed a diet of toxic *Microcystis* (Sarnelle and Wilson 2005). The best example demonstrated increased growth rates in *Daphnia* as a response to three decades of cultural eutrophication. Hairston et al. (2001) hatched *Daphnia* from

diapausing eggs collected from the sediment over a three periods in Lake Constance, Germany: prior to eutrophication, the height of eutrophication when the phytoplankton community became dominated by cyanobacteria, and during the recovery phase when nutrient levels were reduced. Genotypes hatched from the eutrophication period had higher juvenile growth rates than other time periods and had less of a decrease in growth rates when fed toxic *Microcystis* in combination with *Scenedesmus* compared to *Scenedesmus* alone.

The higher growth rates in *Daphnia* that have been pre-exposed to cyanobacteria may be due to detoxification enzymes (Pflugmacher et al. 1998; Gustafsson et al. 2005) and/or increased tolerance to toxins (DeMott and Dhawale 1995). Thus far, however, a behavioral response has not been considered. Daphnia populations that acclimated by increasing detoxification enzymes or tolerance would not necessarily affect the composition of strains in populations of cyanobacteria. If, however, *Daphnia* acclimated behaviorally and were able to avoid assimilating certain strains *Daphnia* would influence the genotype composition of cyanobacterial populations.

In the following experiment I tested the hypothesis that *Daphnia ambigua* can acclimate over a short period (8 weeks) to toxin producing strains of *Microcystis aeruginosa*. Given that acclimation towards food selection may occur in *Daphnia* (Epp 1996; Sarnelle and Wilson 2005), I predicted that the animals from populations subjected to toxic *M. aeruginosa* in their diets would be more successful at avoiding toxic *M. aeruginosa* cells than those previously unexposed. I used short-term feeding experiments and measured avoidance of *Microcystis* by *Daphnia* as the ratio of remaining cells at the end of the experiments.

2.3 Methods

2.4 Experimental organisms –

Daphnia ambigua was collected by vertical net haul from a depth of 2 m with a plankton net (64 μm pore-size) from the middle of Constance Lake, a shallow (mean

depth = 3 m) mesotrophic lake (annual mean TP is about 30 μg L^{-1}) west of Ottawa, Ontario on 10 June 2005. In the laboratory, *D. ambigua* was maintained at 20 ± 1 °C on a 16: 8 hour light: dark cycle in aquariums containing dechlorinated water gently bubbled with air. *Daphnia* populations were initially fed RotiRich, a commercially prepared aquatic invertebrate food (WARD's natural science). After four months the *Daphnia* were divided into separate 4 L Erlenmeyer flasks and fed the following acclimation diets: population 1 was fed only a good quality food (GF) mixture (50% *Chlorella kessleri* strain UTCC266 and 50% *Pseudokirchneriella subcapitata* UTCC66 (Viviberg 1989); population 2 was fed GF plus non-toxic *Microcystis aeruginosa* strain UTCC124 (no detectable microcystins by HPLC (Aranda-Rodriguez et al. 2005); and population 3 was fed GF plus toxic *M. aeruginosa* UTCC300 (1.3 mg g^{-1} dry weight of total microcystins with approximately half being microcystin-LR). The initial concentration of *M. aeruginosa* for populations 2 and 3 was 0.5 % of total carbon and increased every week by 0.05 % to a maximum of 3 %. This low value was chosen because, under experimental conditions, even concentrations of toxic *Microcystis* as low as 5% mg C L^{-1} can be lethal to *Daphnia* (Hietala et al. 1997). A preliminary study showed nearly 100% mortality when *Daphnia* were fed 30% mg C L^{-1} toxic *Microcystis.* All food concentrations were maintained at a constant concentration of 0.26 mg C L^{-1} to mimic the conditions of a mesotrophic lake (Hietala et al. 1997a). Every two weeks about half of the population was removed and clean water was added to the flasks. This ensured that the populations did not become over crowded and begin sexual reproduction. It also reduced population density at random, allowing individuals with higher fitness under the acclimation diet to out-compete well-adapted genotypes. The total acclimation period was 8 weeks.

All algal cultures were maintained in batch culture in a growth chamber at 25±0.1°C under 24 hour light at 9 μmol m^{-1} sec^{-1} PAR. *C. kessleri* was cultured in Bold's Basal (SIGMA) and both strains of *M. aeruginosa* were grown in BG11 (SIGMA). Media for *P. subcapitata* was made by mixing 1 mL of each of the following stock solutions in 1 L of distilled water: 1) $NaNO_3$ (12.75g/500mL), 2) $MgSO_4 \cdot 7H_2O$ (7.35g/500mL), 3) K_2HPO_4 (0.522g/500mL), 4) $NaHCO_3$ (7.5g/500mL), and 5) a micronutrient stock solution containing: $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, H_3BO_3,

$MnCl_2 \cdot 4H_2O$, $ZnCl_2$, $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $Na_2MoO_4 \cdot 2H_2O$, $FeCl_3 \cdot 6H_2O$, and (7) $DTH \cdot 7H_2O$. Once mixed, this solution was filtered through a polycarbonate 0.2 μm pore-size filter with a central PIAB filtration system at −400 mm Hg. Green algal cultures were kept on an orbital shaker at 70 rpm and *Microcystis* was swirled once daily. New cultures were started approximately every 10 days.

2.4.1 Experimental setup –

A pilot feeding experiment indicated that a food supply of 30 % *M. aeruginosa* by carbon caused high mortality in *D. ambigua* but that there was no mortality in a 10 % treatment. Therefore, to ensure all animals survived the feeding experiment, only 10 % *M. aeruginosa* by carbon treatments were used. After the acclimation period, feeding experiments were conducted on daphnids from each population with each of the following experimental diets: 100 % *C. kessleri* (CHL); 90 % C + 10 % non-toxic *M. aeruginosa* UTCC124 (NTM); and 90 % C + 10 % toxic *M. aeruginosa* UTCC300 (TM) at a constant food concentration of 0.26 mg C L^{-1}. Both *C. kessleri* and *M. aeruginosa* (NTM and TM) grew as spherical, single cells under their culturing conditions and were similar in size (~ 4 μm diameter).

Feeding experiments were conducted at low light for 6 hours in 24 mL glass scintillation vials with four daphnids per vial and eight replicates per treatment (balanced 3×3 factoral design) plus controls (no daphnids added) yielding 96 vials in total. The duration of the experiment and number of daphnids per vial were determined by estimating the filtering rate for each daphnid (Mourelatos and Lacroix 1990). Algal cultures used in the feeding experiment were rinsed and stored in dechlorinated water in the light for 36 hours to reduce variability due to cell division. During the experiment, the vials were gently inverted once every hour to prevent settling of algal cells. Hourly inversion was determined adequate given the settling velocity of single cells of *Chlorella* (~ 3 mm hr^{-1} calculated from Stokes equation; Reynolds 1984b) and buoyancy of *Microcystis* (~ 0.5 mm hr^{-1}; Reynolds et al. 1987).

At the end of the experiment each replicate was preserved with 1% by volume paraformaldehyde and refrigerated at 4°C. Aliquots (10 ml) of each replicate were concentrated by filtration (0.2 μm polycarbonate filter) and a minimum of 300 cells was counted for each cell type in each replicate using epifluorescence microscopy (Zeiss Jenamed 2; Pick 1991). As the *Chlorella* and *Microcystis* cells are similar in size and dimension the easiest way to differentiate between them was based on pigment composition: *Microcystis*, like other cyanobacteria, contains phycocyanins which fluoresce red under green excitation, whereas both *Chlorella* and *Pseudokirchneriella* contain predominantly chlorophyll a which fluoresces red under blue excitation. Feeding rates were calculated using the equations of Peters (1984) and avoidance was determined from selectivity coefficients calculated as the ratio of *Microcystis: Chlorella* feeding rates (DeMott 1982). A one-way ANOVA was used to test for differences between the control and the *Daphnia* treatments. Two-way ANOVAs were conducted to test the effect of acclimation diet, experimental diet and their interactions on the selectivity coefficients and the feeding rates of *Chlorella* and *Microcystis*. Further comparisons were tested using two-tailed t-tests. All statistical tests were carried out using SYSTAT vs 11. When necessary to meet the assumption of normality, data were log transformed. Alpha values were set *a priori* at 0.05.

2.4.2 Model description –

As a follow-up, I created a model to determine if the different settling rates of *Chlorella* and *M. aeruginosa* could explain the experimental results. Although *Microcystis* and *Chlorella* are of similar size and shape, they differ in cell density and therefore settling velocity (Reynolds 1984). If *Daphnia* fed near the bottom of the vial, they would experience a changing cell ratio as more *Chlorella* cells entered the feeding zone and *Microcystis* cells floated upwards. The following model was developed to simulate changes in cell ratio due to feeding by *Daphnia*:

$$Chl_{t_x} = Chl_{t_{x-1}} + z_{Chl} - f_{Chl} \qquad \text{Eq. 2.1}$$

$$Mic_{t_x} = Mic_{t_{x-1}} - z_{Mic} - f_{Mic} \qquad \text{Eq. 2.2}$$

where *Chl* or *Mic* is the cell concentration at time *t*; z is the number of cells entering (*Chl*) or leaving (*Mic*) the feeding zone at the bottom of the vial; f is the feeding rate calculated from experimental results as the average feeding rate of all four *Daphnia* on *Chl* or *Mic* for each acclimated population × diet combination. z was calculated based on the settling and buoyancy rates calculated from Stoke's Law and the proportion of the bottom of the vial in which *Daphnia* were feeding (feeding zone). Cell densities were taken from Reynolds (1984b) and Reynolds et al. (1987). Initial cell concentration for both *Chl* and *Mic* was taken as the average cell concentration in each control for experimental diet 2 and 3. Total feeding rate was constant but feeding rate on *Chl* and *Mic* was calculated each time step based on the ratio of cells in the bottom proportion of the vial in which *Daphnia* were assumed to be feeding. To simulate experimental mixing which occurred every hour, the total *Chl* and *Mic* in the vial was calculated every 60 minutes (initial cells - cells fed upon) and the cells in the bottom portion of the vial was reset to reflect the new concentration.

By altering the proportion of the vial *Daphnia* fed in and feeding rates, it was possible to determine if different settling velocities could explain the different cell concentrations. The model was considered to be a good fit if the final simulated *Chlorella* and *Microcystis* cell concentrations were within the 95% confidence interval calculated from the experimental replicates.

2.5 Results

All *Daphnia* survived the 6 hour feeding experiment and actively ingested a significant number of cells relative to control scintillation vials (Fig 2.1). *Daphnia* fed only *Chlorella* ingested more cells than those fed diets containing *Microcystis* (One-way ANOVA $F_{2,69} = 32.9$, $P < 0.001$; Fig 2.1). Regardless of experimental diet, there was an increase in the ratio of *Microcystis* to *Chlorella* cells compared to the control for all three

populations of *Daphnia ambigua* (One-way ANOVA $F_{3,60} = 82.6$, $P < 0.001$; Fig 2.2a). *Daphnia* selectivity was affected by the acclimation diet ($P < 0.001$) and the response to experimental diet was dependent on previous acclimation (acclimated population × experimental diet, $P < 0.001$; Table 2.1). There was no difference in selectivity among populations when fed an experimental diet containing non-toxic *Microcystis* (Fig 2.2b). When the experimental diet contained toxic *Microcystis,* however, the selectivity of the populations diverged, but the only population that decreased its selectivity on *Microcystis* in the presence of a toxic strain was the population previously acclimated to toxic *Microcystis* (Fig 2.2b).

Chlorella feeding rates were highest for the population previously acclimated to toxic *M. aeruginosa* (Fig 2.3a). This population also had a lower feeding rate on toxic *M. aeruginosa* than non-toxic *M. aeruginosa* and a lower feeding rate on toxic *M. aeruginosa* than either of the other two populations (Fig 2.3b). The population acclimated only to green algae had a higher feeding rate on toxic compared to non-toxic *M. aeruginosa* (two-tailed t-test; $t_{14} = 2.41$, P=0.031) but there was no difference in feeding rates between toxic and non-toxic *M. aeruginosa* by the population acclimated to non-toxic *M. aeruginosa* ($t_{14} = 0.84$, P=0.414).

The model was sensitive to changes in both the volume of the feeding zone and the feeding rates. Intermediate feeding rates had the largest changes to the remaining cells ratio because at higher feeding rates the cell concentration in the feeding zone was exhausted before the hourly mixing occurred (Fig 2.4a). The smaller the feeding zone at the bottom of the vial the greater the change in cell ratio as *Microcystis* cells float upwards and *Chlorella* cells settle (Fig 2.4b). From literature values of cell density, 19 *Microcystis* cells left and 288 *Chlorella* cells entered the 3mm feeding zone at the bottom of the vial every minute. The model was less sensitive to changes in the number of *Microcystis* cells floating out of the feeding zone than *Chlorella* cells sinking. Changes in *Microcystis* resulted in small range in remaining cells ratio from 0.5 to 0.6 *Microcystis: Chlorella* (Fig 2.4c). An increase in the number of *Chlorella* cells beyond 288 did not alter the resultant cell ratio because feeding rates became saturated. Lower numbers of

sinking *Chlorella* cells, however, decreased the final *Microcystis: Chlorella* ratio (Fig 2.4d).

The model was successful at reproducing the experimental results of most of the acclimated population × experimental diet combinations (Table 2.2) by changing only the feeding zone or the proportion of the bottom of the vial in which *Daphnia* fed. The exception was the population acclimated to toxic *Microcystis* and fed an experimental diet of toxic *Microcystis*. By altering only feeding rate or feeding zone, the model could not reproduce mean final cell concentrations. Another parameter was added to the model, differential gut passage, to produce results that fit the experimental results. There was no need to define a feeding zone when modeling differential gut passage as this mechanism alone could reproduce the experimental results but it was necessary to increase the feeding rate above the rate calculated from the experimental results.

2.6 Discussion

All *Daphnia ambigua* populations, regardless of acclimation diet, were capable of increasing the ratio of *Microcystis* to *Chlorella* cells, of similar size and shape, in this short-term feeding experiment. This result seems to suggest that *Daphnia* are capable of avoiding *Microcystis* cells either during the feeding process or following ingestion. A model developed to determine if the different settling velocities of *Microcystis* and *Chlorella* could explain this result showed that if *Daphnia* fed in the bottom 3 mm of the vial, the change in final cell ratio would occur just due to the changing cell concentrations in the feeding zone. Although at first this may imply that experimental setup was flawed, the results suggest that through behavioral changes, *Daphnia* were capable of selectively consuming *Chlorella* cells and that the behavioral response differed with acclimation treatment.

If avoidance of *Microcystis* is an evolved response for contending with toxic resources, then previous studies using bacteria and diatoms (Lampert 1974), green algae and bacteria (DeMott 1982), or green algae and polystyrene beads (DeMott 1986) would

not have triggered this response. Also, the *Daphnia* used in this experiment had a history of exposure to toxic cyanobacteria since the lake from which it was isolated has had potentially toxic cyanobacteria for over a decade (Hamilton et al. 2005). Thus the ability to avoid cyanobacteria was likely already present in the population and initiation of our experiment with a population rather than a single clone, which is the norm for feeding experiments (Lampert 1974; DeMott 1982; DeMott 1986), allowed for broader genetic variation in the population and potential to acclimate to different food resources.

The mechanism proposed to explain the results is the movement of *Daphnia* in vertical space to a zone where the ratio of *Microcystis: Chlorella* cells is lower. In this experimental set-up, this condition existed near the bottom where buoyant *Microcystis* cells float upwards and *Chlorella* cells sink downwards. *Daphnia* have been documented to move vertically to avoid predation by fish and also to avoid cyanobacterial bloom material (Berthon and Brousse 1995). In lakes, under calm conditions, *Daphnia* and other zooplankton could take advantage of differential settling rates of phytoplankton species and find pockets of suitable prey. This may be even easier for grazers to locate *in situ* where *Microcystis* and most other cyanobacteria occur in colonial form which rise through the water column more quickly than single celled forms (Reynolds et al. 1987).

This behavioral avoidance mechanism, however, does not explain the differences in experimental results among acclimated populations. I found that the population previously exposed to toxic *Microcystis* also digested the most *Chlorella* cells relative to *Microcystis*, resulting in a higher *Microcystis: Chlorella* remaining cells ratio than any of the other acclimated population × experimental diet combinations. Previous experimental work has described the adaptive potential of *Daphnia* to single celled microcystin producing *M. aeruginosa* (Sarnelle and Wilson 2005; Hairston et al. 2001). Production of a detoxification enzyme or increased tolerance to microcystins has been typically proposed as the mechanism (Pflugmacher et al. 1998; Gustafsson and Hansson 2004; Guo and Xie 2006) but both these hypotheses assume that single celled *Microcystis* was digested by *Daphnia*. Our results show that the toxic *Microcystis* cells were not digested in the same numbers as non-toxic *Microcystis* or *Chlorella* cells. In addition, the

experimental results could not be replicated in the model unless an additional parameter for differential gut passage or selectivity was included.

Of these two mechanisms, differential gut passage or selectivity, differential gut passage is the less controversial explanation. Differential gut passage has been documented in *Daphnia* when fed diets of P-limited green algae (Van Donk and Hessen 1993). *Microcystis aeruginosa* can increase intracellular toxin concentrations in response to zooplankton infochemicals and it is plausible that they also alter cell membrane characteristics to avoid digestion by zooplankton (Jang et al. 2007). Rohrlack et al. (2005) documented the process of toxicity after *Daphnia* ingested and subsequently digested *M. aeruginosa* cells. In this latter study, as in the current study, the algal diet was neither nutrient limited nor was it pre-exposed to zooplankton chemicals and therefore it was unlikely to evoke any anti-grazing strategies.

The alternative possibility, that *Daphnia* avoided ingesting toxic *Microcystis* has not been as widely investigated. This is largely based on the fact that *Daphnia* are consistently assumed to be non-selective feeders based on the currently accepted model of *Daphnia* feeding by use of fine setae to sweep the water column (Peters 1984; Wetzel 2001). Critics of this model point out that the low Reynolds number environment experienced by *Daphnia* would not allow filtration of particles by the gaps in the setae and suggest that the setae push food particles towards the mouth where food is selected (Gerristen and Porter 1982; Gerristen et al. 1988; Hartmann and Kunkel 1991) but see (Fryer 1987; Fryer 1991). In addition, phenotypic plasticity of feeding appendages in response to cyanobacteria has been documented (Ghadouani and Pinel-Alloul 2002; Bednarska and Dawidowicz 2007) though the implications for feeding selectivity has not been investigated.

Regardless of the feeding mechanism, our results demonstrate that *Daphnia* were capable of altering the composition of their food resource, even when algal particles were the same size and shape (but of different cell density). The population acclimated to non-toxic *M. aeruginosa* did not differ in selectivity between experimental diets containing non-toxic and toxic *M. aeruginosa* suggesting that *Daphnia* previously acclimated to

toxic *M. aeruginosa* responded to the presence of microcystin or some other factor associated with the toxic strain. Also of interest, the population of *D. ambigua* acclimated to toxic cyanobacteria had the highest grazing rates on *Chlorella,* supporting the hypothesis that genotypes with high growth rates are selected in the presence of toxic cyanobacteria (Hairston et al. 2001).

Daphnia are known to migrate vertically in the water column to avoid predation and our results suggest that *Daphnia* also use this behavior to avoid cyanobacteria. Our results further suggest that *Daphnia* acclimated to toxic cyanobacteria were capable of avoiding toxic *Microcystis* above levels that could be attained by movement alone. The simplest explanations are differential gut passage and/or feeding selectivity. Both these mechanisms could lead to altered strain ratios in natural populations. Here I show the potential of a population *Daphnia ambigua* to acclimate over the course of 8 weeks to the presence of toxic cyanobacteria, a much shorter period than previously considered (Hairston et al. 2001). At this time scale, acclimation in *Daphnia* could alter the dominance of cyanobacterial strains in natural populations.

2.7 Tables

Table 2.1 Results of two-way ANOVAs to test the effect of acclimation diet and experimental diet on different measures of feeding selectivity of *D. ambigua* on *Chlorella kessleri* and toxic and non-toxic *Microcystis aeruginosa* following a 6 hour feeding experiment.

Dependent variable	Factor	df	MS	*F*	*P*
Selectivity coefficients	Acclimation diet (A)	2	0.06	9.69	<0.001
	Experimental diet (B)	1	0.02	2.03	0.161
	A x B	2	0.06	9.53	<0.001
	Error	42	0.01		
Chlorella feeding rate	Acclimation diet (A)	2	0.86	83.06	<0.001
	Experimental diet (B)	2	1.43	138.87	<0.001
	A x B	4	0.37	36.27	<0.001
	Error	63	0.01		
Microcystis feeding rate	Acclimation diet (A)	2	0.03	3.33	0.046
	Experimental diet (B)	1	0.02	1.60	0.212
	A x B	2	0.21	22.91	<0.001
	Error	42	0.01		

Table 2.2. A comparison of experimental and model results for each acclimated population (acc pop) × experimental diet (exp diet) combination. Confidence intervals of *Chlorella* (*Chl.*) and *Microcystis* (*Mic.*) concentrations (cells ml^{-1}), and mean feeding rates (f. rates; cells $individual^{-1}$ $hour^{-1}$) have been calculated from experimental replicates (n=8). Simulated final cell concentrations and ratios were also calculated. Model parameters include: feeding rates (f. rates), proportion of the bottom of the vial that *Daphnia* fed (f zone), selectivity coefficient (s) and differential gut passage coefficient (d). Acclimation diets: GF – 50% - *Chlorella kessleri* and 50% *Pseudokirchneriella subcapitata*, NTM – non-toxic *M. aeruginosa* and TM – toxic *M. aeruginosa*. Experimental diet: CHL – 100% *Chlorella kessleri*; NTM – 10% non-toxic *Microcystis aeruginosa* and 90% CHL; TM – 10% toxic *M. aeruginosa* and 90% CHL.

		Experimental Results					Model parameters				Model Results		
Acc.	Exp.	mean	95% C.I. *Chl.*		95% C.I. *Mic.*			f.					
pop.	diet	f. rates	lower	upper	lower	upper	f. rates	zone	s	d	*Chl.*	*Mic.*	ratio
GF	CHL	993	5248	5710									
GF	NTM	578	4586	5463	2875	3284	578	0.08	1	1	5444	2900	0.53
GF	TM	694	3858	4455	2574	2924	694	0.09	1	1	4391	2670	0.61
NTM	CHL	924	6127	6914									
NTM	NTM	629	4103	4955	2685	2929	629	0.09	1	1	4801	2720	0.57
NTM	TM	624	4768	5627	2714	2790	624	0.09	1	1	4801	2720	0.57
TM	CHL	1162	2760	3140									
TM	NTM	673	3830	4176	2625	2739	750	0.09	1	1	4112	2632	0.64
TM	TM	649	4003	4641	3122	3401	649	0.09	1	1	4391	2670*	0.61
TM	TM	649	4003	4641	3122	3401	649	1	0.3	1	4156	3124	0.75
TM	TM	649	4003	4641	3122	3401	850	1	1	0.25	4037	3271	0.81

* This value is outside the experimental confidence intervals.

2.8 Figures

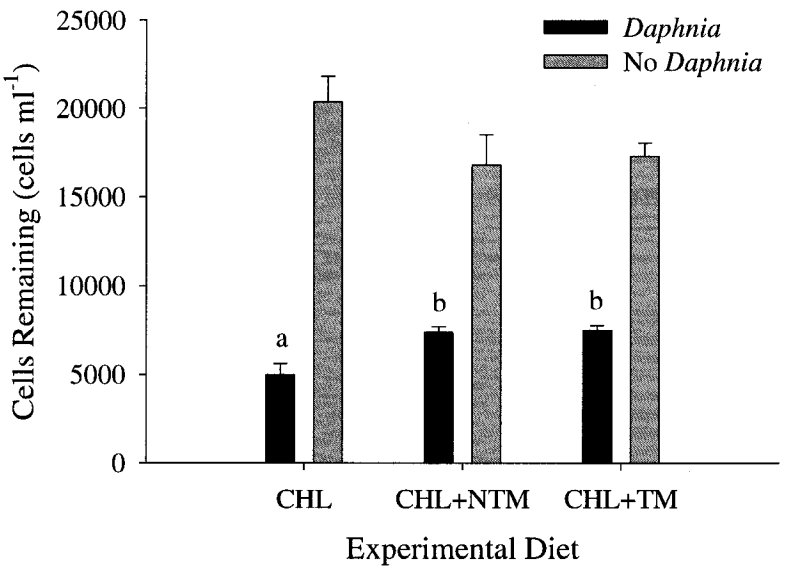


Figure 2.1 The mean remaining total cells for control (no *Daphnia*) and all acclimated populations (control n=8, treatment n=24; error bars are 95% CI). A one-way ANOVA was used to test for differences among experimental diets with *Daphnia* (diets sharing same letter are not significantly different; Tukey's post hoc test, $p<0.05$). Experimental diet: CHL – 100% *Chlorella kessleri*; NTM – 10% non-toxic *Microcystis aeruginosa* and 90% CHL; TM – 10% toxic *M. aeruginosa* and 90% CHL.

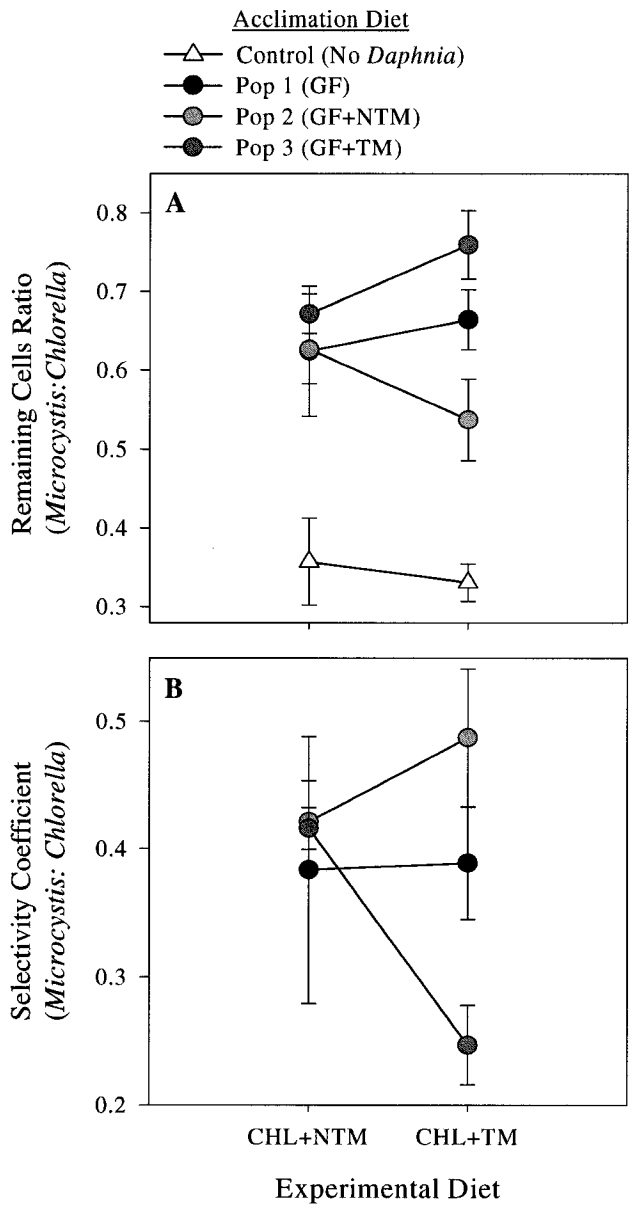


Figure 2.2 Cell ratio of *Microcystis: Chlorella* (A) and selectivity coefficient (B) following a 6 h feeding experiment using three populations of *Daphnia ambigua* that were first acclimated to one of three diets and then fed an experimental diet (n=8, error bars are 95% CI). Experimental diet acronyms are the same as Fig 2.1. Acclimation diets: GF – 50% - *Chlorella kessleri* and 50% *Pseudokirchneriella subcapitata,* NTM – non-toxic *M. aeruginosa* and TM – toxic *M. aeruginosa.*

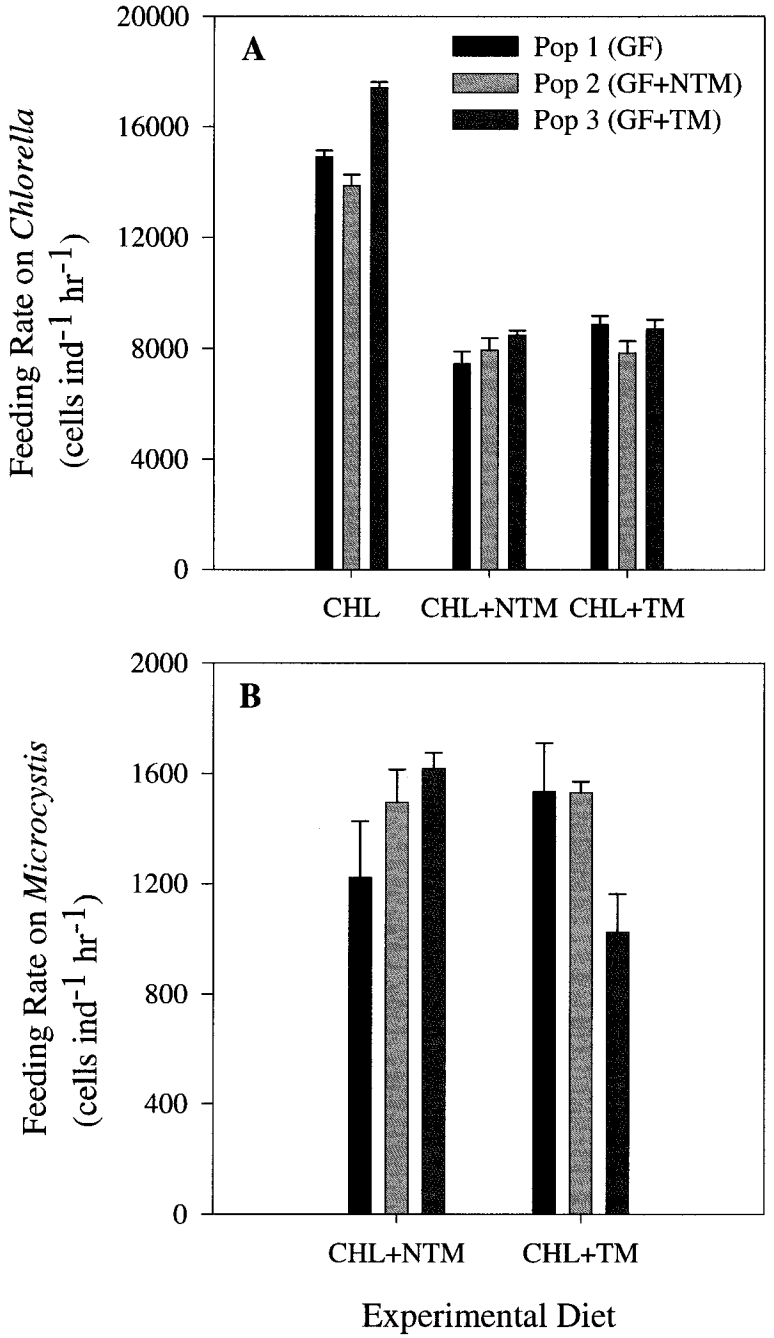


Figure 2.3 Feeding rate of *Daphnia ambigua* on *Chlorella* (A) and *Microcystis* (B) following a 6 h feeding experiment using three populations of *Daphnia ambigua* that were first acclimated to one of three diets and then fed an experimental diet (n=8, error bars are 95% CI). Experimental and acclimation diet acronyms are the same as Fig 2.1 and Fig 2.2.

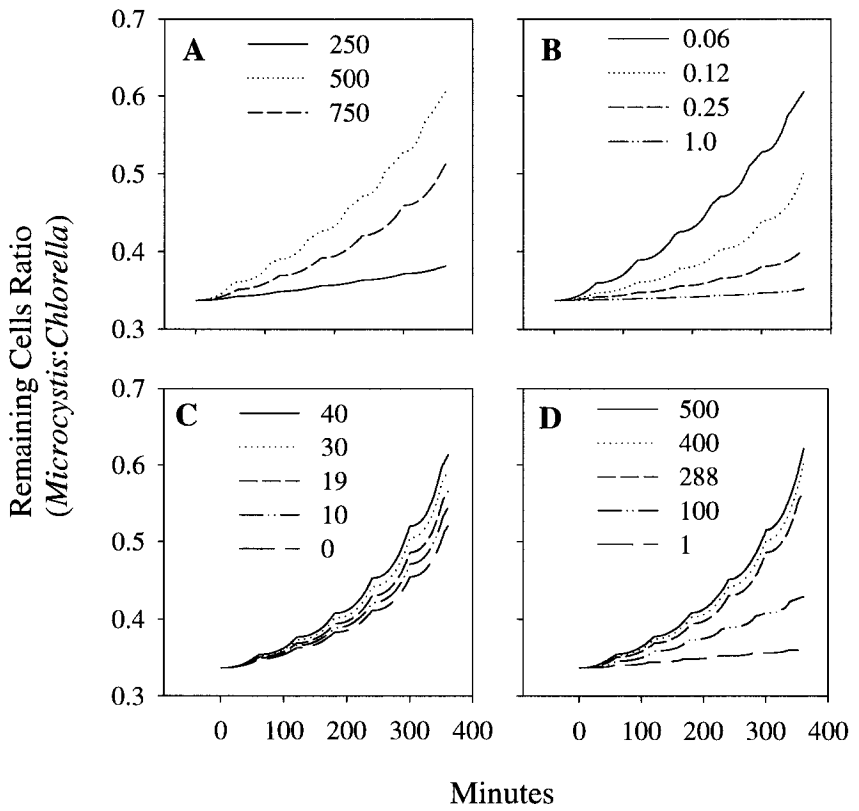


Figure 2.4 Model simulations that show the change in cell ratio as a function of *Daphnia* feeding rate, the proportion of the bottom of the vial that *Daphnia* actively filter (feeding zone), the number of *Chlorella* cells sinking into the feeding zone and the number of *Microcystis* cells leaving the feeding zone. Panel descriptions, units and default values: A) feeding rates (641 cells individual^{-1} hour^{-1}); B) bottom proportion of the vial in which *Daphnia* fed (feeding zone; 0.06); C) *Microcystis* cells leaving the feeding zone (19 cells min^{-1}); D) *Chlorella* cells entering the feeding zone (288 cells min^{-1}).

3 Chapter 3 - Sampling and analysis of microcystins: Implications for the development of standardized methods

3.1 Abstract

Microcystins (MC), a group of cyanotoxins, have been found in lakes and rivers worldwide. One goal of MC research is to develop models which predict MC concentrations but these efforts have been hampered by a lack of standardized methods necessary for comparing data across studies. Here, I investigate the effect of chemical analysis (HPLC-PDA and ELISA), sample collection (whole water, plankton tow and surface scum) and choice of normalizing parameter (volume, dry weight and chlorophyll *a*) on reported MC concentrations. Samples were collected over three years from a temperate mesotrophic, shallow lake with episodic blooms of cyanobacteria. I found that microcystins were up to 4 times higher in lake samples when analyzed by ELISA relative to HPLC-PDA and that MC concentration measured by HPLC explained less than half of the variation in MC concentrations measured by ELISA. Also, samples collected by plankton tow gave consistently higher concentrations than whole water samples. An additional HPLC analysis of two chlorophyte cultures revealed the presence of compounds with a similar UV absorbance spectrum to MC-LR, suggesting that identifying MC based solely on UV absorbance is not valid. Our results document the discrepancy in MC concentrations that can arise by using different methods throughout all stages of sampling, analysis and reporting of MC concentrations.

3.2 Introduction

Toxic cyanobacterial blooms are a growing environmental and human health concern (Codd 2000; Carmichael 2001; Haider et al. 2003). The most common toxins produced by cyanobacteria are microcystins (MC), a class of mono-cyclic heptapeptides (Rinehart et al. 1994). Over 70 different microcystin variants have been identified, distinguished by an amino acid substitution or modification, spanning a 100 fold change in toxicity (Sivonen and Jones 1999). Microcystins have been found in lakes and rivers world-wide (Amé et al. 2003; Shen et al. 2003; Graham et al. 2004; Briand et al. 2005) and individual jurisdictions as well as the World Health Organization have responded with guidelines for maximum allowable concentrations in drinking and recreational waters (National Health and Medical Research Council 1996; Health Canada 2002; World Health Organization 2004).

Since recognition of MC as a potential health risk, research has been focused on characterizing patterns of MC concentration and composition in lakes and rivers with the dual purposes of informing guidelines and identifying limnological triggers of MC production (Kotak et al. 2000; Graham et al. 2004; Giani et al. 2005). Progress on the latter goal has been slow, possibly due in part to the lack of standardization. Without standard methods it is difficult to compare results between studies; such comparisons can often lead to new insight (e.g. Downing et al. 2001).

Determination of microcystins can be done by chemical methods such as liquid chromatography (HPLC, LC) coupled with UV, photodiode array (PDA) and/or mass spectrometer (MS) detectors and also by immunochemical methods such as enzyme linked immunosorbent assay (ELISA) and the protein phosphatase inhibition assays (PPA; Harada et al. 1999; Msagati et al. 2006). The method recommended by the ISO (ISO 20179, 2005) includes HPLC-PDA and is often considered the "gold" standard for MC analysis. However, the small number of available commercially purified MC variants relative to the high number of known MC variants limits the ability to quantify all MC present in the sample.

One method to overcome this limitation is to use LC-MS which has the advantage of unequivocal identification of microcystins. The main drawback, however, is again the lack of certified reference materials to establish the necessary response factors. In this case it is not only the limited number of MC commercially available but also the uncertain purity of these variants (Kubwabo et al. 2004). An advantage of LC-MS is that even without the necessary variants, possible MC can be characterized and compared with published descriptions (Hummert et al. 2001; Bogialli et al. 2006). A second, untested method used by some researchers is to rely solely on the characteristic UV spectrum of MC (Lawton et al. 1994) to identify total MC in a sample (Wirsing et al. 1999; Sabour et al. 2002; Janse et al. 2005; Jayatissa et al. 2006).

In contrast to HPLC, both ELISA and PPA are sensitive to multiple microcystin variants. These assays are typically a thousand times more sensitive than HPLC and so do not require a pre-concentration step. However, neither ELISA nor PPA can identify or quantify individual variants (An and Carmichael 1994; Fischer et al. 2001) and therefore these tests cannot give an accurate estimate of toxicity, (but see Mountfort et al. 2005). In addition, ELISA test kits manufactured by different companies can give different results (Metcalf et al. 2002).

Differences in sampling methodology may also have an effect on resultant MC concentrations. Field sample collection for MC follow one of three main protocols (Utkilen et al. 1999): 1) collecting water at a specific depth or integrating water samples over multiple depths (whole water method); 2) taking a vertical or horizontal plankton net tow (plankton tow method) and 3) skimming off accumulated surface scums. Plankton tows are useful for collecting large quantities of cyanobacterial colonies but MC concentrations can be underestimated if net efficiency is not considered (Wetzel and Likens 1990). In addition, plankton tows are not suitable for measuring both particulate and soluble fractions which is required for the WHO guideline (World Health Organization 2004).

Once collected and analyzed, environmental concentrations of MC can be expressed in terms of seston dry weight, water volume or chlorophyll *a*. Early studies

reported concentrations per unit dry weight (Scott 1991; Watanabe et al. 1992). More recently, Welker et al. (2003) advocated the use of volume because it is consistent with the WHO guidelines (expressed as $\mu g\ L^{-1}$), and it is also how other limnological information such as nutrient concentrations are reported. Still it is unclear how the choice of standardizing variable will affect the resultant MC concentrations and whether or not there is an interaction with sampling method.

The main purposes of the following study were therefore to document the methodological steps currently used to measure microcystins from freshwater sources and to assess the possibility for each step to cause discrepancies in microcystin measurements. To accomplish these goals I used a combination of original data collected from Constance Lake and published literature. In addition, I used algal cultures to determine if other extracted algal compounds can interfere with MC quantification by HPLC.

3.3 Methods

3.3.1 Literature Review -

Using the Web of Science, I reviewed literature published between 2002 and June 2006 which reported microcystin concentrations measured in freshwater bodies. I recorded the sample method, the analysis method, the purified MC variants, and the variable used to normalize MC concentration.

3.3.2 Sample Collection

Samples were collected from Constance Lake, a mesotrophic (annual mean total phosphorus = 30 $\mu g\ L^{-1}$), shallow lake (mean depth 3 m) located near Ottawa, Ontario (45° 24' N, 75° 59' W), Canada. Constance Lake contains populations of microcystin producing cyanobacteria including species of *Microcystis, Anabaena, Planktothrix,* and *Aphanazomenon*. The lake also contains the subtropical species *Cylindrospermopsis raciborskii* that presumably invaded more recently (Hamilton et al. 2005).

Samples were collected once every three days from the end of May to the first week in November in 2003 (n = 55) and from the middle of April to the middle of June in 2004 (n = 9) using a tygon tube to integrate the sample to a depth of 2 m (whole water samples – ww). For the latter portion of June to the end of October in 2004 (n = 25) samples were collected every six days by combining water from four vertical net hauls, each from 2 m (net diameter = 19.8 cm; mesh size = 64 μm; net efficiency = 31%; plankton tow samples - pt). In 2005 I used the same vertical net haul method as in 2004 but sampled bi-monthly from mid May until mid October (n = 9). In each case water for toxin analysis was transferred to an amber glass jar to minimize loss of MC due to adherence to the container wall (Utkilen et al. 1999).

3.3.3 Algal Cultures -

Two diatoms (*Stephanodiscus hantzschii* UTCC 267 and *Fragilaria crotonensis* UTCC 269) and three chlorophytes (*Chlorella kessleri* UTCC 266, *Selenastrum capricornutum* UTCC 66 and *Scenedesmus quadricauda* UTCC 158) were obtained from the University of Toronto Culture Collection. *Cylindrospermopsis raciborskii,* isolated in Florida, was obtained from Green Water Laboratories, Palatka Florida. These species were chosen because they are representative of the taxonomic diversity commonly found in Constance Lake. Chlorophytes and *Cylindrospermopsis* were grown under continuous irradiance (35-45 μMol/m^2/s) at 20°C. Diatoms were grown under a 12:12 light dark cycle (50 μMol/m^2/s) at 18°C. Between 60 to 150 ml of each culture in late exponential growth was filtered (dry weight 3.3 to 18.2 mg) on a Whatman GF/D filter and frozen until chemical analysis as described below.

3.3.4 Chemical Analysis

To determine microcystin concentration, a known volume of water (1 - 4 L for whole water (ww); 0.35-0.45 L of concentrated sample for plankton tow (pt)) was filtered onto pre-ashed and weighed Whatman GF/D (~2.7 um) glass fiber filter papers and dried over night at 60°C. Filter papers were then re-weighed, re-hydrated with distilled water and stored at -20°C until analysis (Harada et al. 1999).

Microcystin extraction from cells was carried out by pressurized liquid extraction using an ASE 200 (Dionex, Canada) with 75% MeOH at 60°C and 2000 psi (Aranda-Rodriguez et al. 2005). The extract was evaporated to dryness using ultra high purity nitrogen (UHPN) in a TurboVap® and then resuspended in 50% MeOH for analysis. HPLC analysis was conducted using a Zorbax SB-C18 column [250 nm x 4.6 mm (i.d.), 5 μm particle size] and a gradient program with two mobile phases consisting of 0.05% (v/v) trifluoroacetic acid (TFA) in water and 0.05% (v/v) TFA in acetonitrile. UV spectra were collected between 200 and 300 nm using a PD40 photodiode array detector and concentrations were calculated from the absorbance at 238 nm. Sample chromatograms were compared against purified extracts for four microcystin variants: MC-LR, MC-RR (Sigma-Aldrich, Ontario, Canada), MC-YR and MC-LA (Calbiochem-Novabiochem Co.). HPLC detection limit was calculated (Harris 2003) at 0.079 ppm but I reported lower concentrations if the retention time was identical to a purified extract, the maximum absorbance was at 238 nm and the absorption spectrum matched MC-LR. I found no MC with tryptophan in Constance Lake.

Samples with MC determined by HPLC as described above, were chosen to investigate the use of UV absorbance as the primary means of identifying MC. I identified chromatogram peaks with a UV spectrum (200-300 nm) that matched LR and that fell between the estimated retention times of MC-RR and MC-LW. I then estimated the concentration of these peaks at 238 nm based on the LR standard curve. The resultant sum of potential MC was compared with ELISA results.

ELISA tests were conducted using an Envirologix QuantiPlate ™ kit (Envirologix, Portland USA) for microcystins. As this test does not distinguish between the microcystin variants, values were expressed as MC LR equivalents. The detection limit of ELISA was 0.16 ppb according to the manufacturer and was a thousand times more sensitive than our detection limit for HPLC. Methanol interferes with the functioning of the ELISA (Metcalf et al. 2000) so samples were diluted at least by 1:3 with Milli-Q water and quite often were diluted further to ensure the MC concentration fell within the range quantifiable by the kit (0.16-2.5 ppb). Duplicates of each sample were done and CV values >15% were re-analyzed based on the manufacturer's recommendations.

3.3.5 Normalizing Parameters and Statistical Analysis -

Microcystin(s) in each sample were normalized by volume of water sampled, seston dry weight and chlorophyll *a* (chl *a*). For whole water samples, volume was the amount of water filtered per sample. For plankton tow samples, volume was the total amount of water filtered by the plankton net hauls and corrected for net efficiency (Wetzel and Likens 1990). Water for chl *a* analysis was collected in exactly the same manner as the corresponding toxin sample (ww or pt), filtered on Whatman GF/D glass fiber filters and immediately frozen at -20°C until extraction with dimethyl sulphoxide (Burnison 1980). The equations of (Jeffrey and Humphrey 1975) were used to calculate concentration of chl *a*.

Spearman's rank correlation, a non-parametric test which is powerful in cases where there is uncertainty around values of close rank, was used to test for correlations between ELISA and HPLC results (Zar 1999). A linear regression was then fit to determine the amount of variation explained by HPLC in ELISA results. Paired t-tests were used to compare MC content by dry weight measured with ELISA and HPLC. Standard t-tests were used to compare the means of samples collected by whole water and plankton tows and when comparing limnological variables between years. When necessary, variables were log or square root transformed. It was not possible to transform HPLC results due to the high number of samples below detection. Although the standard t-test is known to be robust when sample size is large (Zar 1999), I still checked the results using a Wilcoxon rank sum test. All data were analyzed using S-PLUS 6.0.

3.4 Results

3.4.1 Literature Review -

Of the 42 studies published between 2002 and 2006 that reported MC concentrations from freshwater bodies (Table 3.1), HPLC was the most commonly used analytical method (31 studies) and HPLC was often coupled with an immunochemical assay (13 studies). Ten studies, however, relied solely on ELISA or PPIA. Samples were

collected by the whole water method in 26 studies, by plankton tow in 9 studies, from surface scums in 3, and 4 studies used a combination of collection methods. About two thirds of the authors chose to normalize MC concentrations by volume, a third by dry weight and two reported MC concentrations by both volume and dry weight.

3.4.2 Constance Lake Background Limnology -

During the three years of sampling Constance Lake, chl *a* ranged from 2.4 to 17.6 $\mu g\ L^{-1}$; total phosphorus from 20 to 50 $\mu g\ L^{-1}$ and total nitrogen from 510 to 850 $\mu g\ L^{-1}$. For each of these three variables the mean values were greater in 2003 than 2004 (mean ± s^2 in $\mu g\ L^{-1}$; Chl *a*, 2003: 10.6±3.5, 2004: 8.9±2.8, t_{117}=2.39, P =0.018; TP, 2003: 31.9±7.7, 2004: 28.3±5.39, t_{117}=2.59, P=0.012; TN, 2003: 760±57.6, 2004: 689±84.6, t_{117}=5.18, P <0.001). Mean values were not compared with 2005 because sample size was much smaller in 2005 (2003: n=54; 2004: n=63; 2005: n=10).

3.4.3 MC Results by HPLC and ELISA -

Filter papers spiked with three concentrations of MC-LR (0.1, 0.5 and 1 ppm) and subsequently extracted as described above, gave similar results for HPLC and ELISA (R^2 = 0.996). Of the four microcystin variants I had available (LR, LA, LW, RR) only LA and LR were detected in Constance Lake. Dissolved MC (< 0.45 µm) was measured but was always below the detection level of HPLC, even after concentration (0.5 - 1 L) by solid phase extraction.

In general, regardless of sampling methodology, total MC in Constance Lake samples was greater when measured by ELISA than by HPLC (mean ± s^2: ELISA = 41.4 $\mu g\ g^{-1}$ ± 54.5, HPLC = 18.8 $\mu g\ g^{-1}$ ± 27.8; paired-sample t-test: n=94, t=5.1, P <0.001). When I removed sample days below the HPLC detection limit, mean ELISA values were still greater than HPLC (mean ± s^2: ELISA = 64.3 $\mu g\ g^{-1}$ ± 63.22, HPLC = 35.34 $\mu g\ g^{-1}$ ± 29.6; n=50, t=3.77, P <0.001) suggesting that either other MC were present or there were other compounds that interfered with the ELISA.

When samples were separated by collection method and normalized by dry weight (Fig 3.1), ELISA results were greater than HPLC for both whole water and plankton tow

but not for surface scum (paired t-test; ww: n = 58, t = 3.9, P <0.001; pt: n=33, t=3.6, P =0.001; sc: n=5, t=4.58, P =0.67). The latter result could be due to low sample size.

For all samples combined, total MC determined by ELISA was correlated with both LR and LA measured by HPLC (LR, Spearman's ρ = 0.616, P <0.001; LA, Spearman's ρ = 0.586, P <0.001; Fig 3.2). The amount of MC-LR or MC-LA explained a little more than a third of the variation in ELISA results (LR, R^2 = 0.37; LA, R^2 = 0.31). MC-LA and LR added together explained the most variation in ELISA values (LA+LR, R^2 = 0.49). When all peaks with a UV spectrum matching MC-LR (including those identified as LA and LR) were used as total MC measured by HPLC, the amount of variation in MC measured by ELISA dropped dramatically (R^2 = 0.004; Fig 3.2d). However, when whole water and plankton tow samples were compared separately, plankton tow ELISA samples were correlated with the sum of all peaks (Spearman's ρ = 0.4906, P =0.033, R^2 = 0.15).

3.4.4 Effect of Sampling Strategy and Choice of Normalizing Parameter -

The mean concentration of MC expressed per dry weight or by chl *a* determined by both ELISA and HPLC was greater when collected by plankton tow compared with whole water (Table 3.2). This difference remained even when values below detection were removed (data not shown). However, when MC was normalized by volume, whole water sample means were greater when measured by ELISA and there was no difference when measured by HPLC

A comparison of MC values normalized by volume and dry weight or volume and chl *a* (Fig 3.3), revealed that in all cases the slope was not significantly different from 1. For whole water samples, there was a better relationship between values normalized by volume and chl *a* (ELISA, R^2=0.87, Fig 3.3c; HPLC, R^2=0.62, Fig 3.3d) than by volume and dry weight (ELISA, R^2=0.78, Fig 3.3a; HPLC, R^2=0.31, Fig 3.3b). The opposite was true for plankton tow samples which displayed a better relationship between values normalized by volume and dry weight (ELISA, R^2=0.52, Fig 3.3a; HPLC, R^2=0.88, Fig 3.3b) than volume and chl *a* (ELISA, R^2=0.37, Fig 3.3c; HPLC, R^2=0.70, Fig 3.3d).

3.4.5 Surface Scums -

Two surface scums were detected in 2003, four in 2004 and none in 2005. All were in the months of September and October except for one on May 25th of 2004. Fall samples had colonies of both *Microcystis* and *Anabaena* genera, whereas the May 2004 sample contained mostly *Microcystis aeruginosa.* Fall samples ranged from 12.4 to 42.2 $\mu g\ g^{-1}$ MC LR eq. (ELISA) and below detection to 44.9 $\mu g\ g^{-1}$ by HPLC (excluding a high HPLC value of 78.93 $\mu g\ g^{-1}$ that was not analyzed by ELISA). The fall values were all within an order of magnitude even between years whereas the one spring scum was dramatically higher with concentrations of 5.59 $mg\ g^{-1}$ by ELISA and 6.2 $mg\ g^{-1}$ by HPLC. If the fall samples are expressed volumetrically their range spanned an order of magnitude (0.011 to 0.43 $\mu g\ L^{-1}$ by ELISA).

3.4.6 Interference by Algal Compounds -

All algal cultures tested were below the ELISA detection limit indicating that, as expected, there was no MC present. When these cultures were analyzed by HPLC there were chromatogram peaks that had a similar absorbance spectrum to MC-LR though none of the retention times matched the four MC variants (Fig 3.4). In many cases the peaks were very weak or the absorbance was one or two nm off of the expected maximum at 238 nm. However, the UV absorbance spectrum of peaks observed from *S. quadricauda* (UTCC 158) and *S. capricornutum* (UTCC 66) could not be distinguished from MC LR.

3.5 Discussion

In all but one of the 98 samples collected from Constance Lake over three years, MC could be detected by ELISA. HPLC values, however, were below the detection level on 45 sample days. An advantage of using ELISA is the ability to detect low concentrations of MC and therefore the presence of toxigenic taxa and the potential for higher MC concentrations later in the season.

MC concentrations measured by ELISA were up to 4 times and on average 2.4 times greater than MC concentrations measured by HPLC. This is low compared to (Conti et al. 2006) who found ELISA to be on average 7.4 times greater and much lower than Frank (2002) who found ELISA to be 88 times greater than HPLC for environmental samples. These two methods give similar results for MC-LR standard (this study) but for environmental samples, the difference between ELISA and HPLC will depend on the number of variants being produced *in situ* and the number of MCs that can be positively identified by HPLC.

Even though ISO methods by HPLC are available (ISO 2005), there is a range of analysis methodologies as well as different sampling and reporting methods in use (Table 3.1). Numerous reviews have documented the different analytical techniques developed to measure microcystins (Reynolds et al. 1984; Metcalf and Codd 2003; McElhiney and Lawton 2005; Msagati et al. 2006) and others have directly compared two or more of these methods ((Mathys and Surhold 2004; Mountfort et al. 2005). Fastner et al. (2002) reported that the different analysis methods in combination with a lack of certified reference material can lead to a low degree of agreement between laboratories. It remains unclear if any one of the analytical methods will best suit all situations, however, standardized reference material including an increase in the number of commercially available MC variants will aid in making results comparable.

Our results suggest that the best approach to determining MC concentration is to use a combination of chemical and immunochemical/enzyme methods. Chemical methods such as HPLC or LC-MS allow the determination of common MC variants which are commercially available. However, the sum of all microcystin variants in a sample will remain unknown if only a chemical approach is taken. This information can be supplied with the addition of an immunochemical/enzyme method.

In samples from Constance Lake with identified MC, I quantified all unknown peaks with UV absorption matching MC-LR and found that the sum concentration of these peaks was not correlated with the ELISA results. There was a correlation, however, when plankton tow samples were tested alone (Fig 3.2d). In addition, I found peaks with

an UV absorbance spectrum similar to MC-LR in the chlorophyte cultures, but MC was not detected by ELISA. Therefore, without validation by a purified MC, or a secondary analytical method such as mass spectrometry (Harada et al. 1996), classification of unknown peaks by UV absorbance spectrum alone can give spurious results. This may be especially true when working with diverse algal communities typical of environmental samples. Extraction methods for MC may also extract compounds with similar chemical properties, increasing the risk of misidentification. If, however, the sample is known to contain almost exclusively cyanobacterial colonies, then the use of UV spectrum can give at least a first estimate of the concentration of MC in the sample.

Interestingly, when MC was normalized by volume, whole water MC means were greater than plankton tow MC estimates (Fig. 3.3a). There are two possible explanations for this. First, the net efficiency correction may have been too high. I calculated net efficiency only once during the summer but net efficiency can change with the size of plankton which in turn varies seasonally so one estimate may not be representative of the year (Lathrop 1999). A second plausible explanation, with some support in the literature, is that there is a significant amount of MC produced by cyanobacterial colonies < 64 μm in diameter. For example, MC concentrations in the range of 0.08 to 3.7 μg g^{-1} have been detected in picocyanobacteria (Domingos et al. 1999) and (Giani et al. 2005) found that when *Aphanocapsa* was included amongst the MC producers there was a stronger correlation between MC and toxigenic biomass. In contrast, (Kurmayer et al. 2003) showed a positive correlation between toxigenic genotypes and colony size in *Microcystis sp.* and (Graham et al. 2006) found that net planktonic chlorophyll (>35 μm) was correlated to MC values whereas nano planktonic chlorophyll (<35 μm) was not. When I compared analysis method and normalizing parameter (Fig 3.3) I found that for whole water samples, the best relationship was between MC values normalized by volume and MC values normalized by chl *a* when analyzed by ELISA (Fig 3.3c). And for plankton tow samples, the best relationship was between MC values normalized by volume and MC values normalized by dry weight when analyzed by HPLC (Fig 3.3b). This suggests that large colonial species are the main producers of MC LA and LR in Constance Lake and that that if small species are important contributors to MC then they likely produce other variants.

Although the choice of normalizing parameter did not greatly change the relative concentration between sampling dates in our study it is important to note that normalization by different parameters can greatly change the concentration. In Constance Lake samples, the use of dry weight resulted in MC concentrations in the order of parts per million whereas the same sample when normalized by volume gave concentrations in the order of parts per billion. Normalization by dry weight is appropriate for comparing samples with different cyanobacteria density; for example between planktonic samples and surface scums. In addition, dry weight can be measured directly with little error and its use removes the need to correct for net efficiency. Although dry weight does include other particles such as zooplankton, this may not be a disadvantage because MC can accumulate in zooplankton (Kotak et al. 1996; Thostrup and Christoffersen 1999). I therefore favor the use of dry weight to normalize MC concentrations for reporting. In this way, MC is associated with biomass (with an unavoidable error associated with abiotic seston) which can then be compared across studies. I recognize, however, that to assess the risk of exposure through drinking water, local water quality managers will need to re-express this concentration volumetrically.

The challenges of MC in freshwater ecosystems have brought together researchers from analytical chemistry, limnology and public health. Agreeing on standard methods across disciplines can be difficult as each group of researchers approaches the phenomenon with a different question. Nevertheless, ensuring that data are comparable between studies will help to increase our understanding of MC production *in situ*. Thus far most literature reviews and comparisons have been conducted on analytical methodologies. As our results have demonstrated, the choice of analytical procedure is not the only step that can cause discrepancies in reported MC; sampling method and reporting should also be considered. Standardized methods for measuring microcystins in freshwater will have to include all steps of the process to ensure that data between studies are comparable.

3.6 Tables

Table 3.1 Comparison of studies published between 2002 and June 2006 which report microcystin (MC) concentrations in freshwater samples.

Study	Sample[a] method	Mesh Size (μm)	Analysis[b] method	MC [c] variants	Max MC Value μg L^{-1}	μg g^{-1}
Giani et al. 2005	pt	35	1,4	1	1.9	
Graham et al. 2004	pt	64	2	1,2,3	4.5	
Jones and Jones 2002	pt	64	2	1,2,3		3300
Jurczak et al. 2004	pt	20	**1,5**[d]	1,2,3,5,6,7,8,9		1687
Kemp and John 2006	pt	25	1	1,2,3	8428.6	
Lindholm et al. 2003	pt	25	**1**,3,7	1,2,5,6,7,8,9	6.4	
Ozawa et al. 2005	pt	40	**1**,2	1,2,3		3284
Rolland et al. 2005	pt	30	4	NR	4.3	
Willame et al. 2005	pt	20	1	1,2,3,5,6,9		2231
White et al. 2003	**pt**,ww,sc	NR	1	NR	1000	
Cuvin-Aralar et al. 2002	sc		**1**,6	NR		4049
Sabour et al. 2002	sc		1,**2**	NR		0.8
Shen et al. 2003	sc		2	1		97.3
Ballot et al. 2003	ww		**1**,6	1,2,3,4,5,6,8	3.3	19800
Ballot et al. 2004	ww		**1**,2	1,3,5,6,8		4593
Ballot et al. 2005	ww		1	1,2,3,4,5,6,8		39
Briand et al. 2005	ww		**1**	1,2,3,4	6.7	
Conti et al. 2006	ww		2,**8**	1,2,3	662	
Frank 2002	ww		**1**,2,3	NR	7020	
Hotto et al. 2005	ww		1,**3**	1,2,3	3.4	
Maatouk et al. 2002	ww		1,**4**	1	0.074	
Mankiewicz et al. 2005	ww		**1**,3	1,2,3,5,6,7,9	12.1	
Moreno et al. 2004	ww		**2**	1	21.7	
Moreno et al. 2005	ww		1,2	1,2,3	6	
Nasri et al. 2004	ww		**4**,6	NR		28346
Padilla et al. 2006	ww		**1**,8	1,2,3	1,000	
Znachor et al. 2006	ww		1	1,2,3,5,6,7,9	128	
Jayatissa et al. 2006	ww, **sc**		1	1		0.3
Amé et al. 2003	ww^{l}	NR	1	1,2		2401
Baldia et al. 2003	ww^{l}	30	1	NR		886
Vieira et al. 2005	ww^{l}	20	2	NR	1.25	
Wang et al. 2002	ww^{l}	10	1	1,2,3	1.45	
Graham et al. 2006	ww^{lp}	20	2	1,2,3	2.5	
Jann-Para et al. 2004	ww^{lp}	12	1	2	3.3	14.1
Albay et al. 2003	ww^{p}		**1**,2,3	1	3.7	
Billam et al. 2006	ww^{p}		**2**,4	1	5.8	
Briand et al. 2002	ww^{p}		1,**4**	1	4.1	
Janse et al. 2005	ww^{p}		1	1	109	
Sekadende et al. 2005	ww^{p}		1	NR	1	
Welker et al. 2003	ww^{p}		1	1,2,3	3	
Johnston and Jacoby 2003	**ww^{p}**,sc		2	NR	3.8	
Murphy et al. 2003	**ww^{p}**,sc		1,2	1,2	0.4	

[a] Sample method: pt = plankton tow; ww = whole water; sc = surface scum; ww^{l} = whole water with post collection concentration by plankton net; ww^{p} = water collected at a depth > 0.5 m. [b]Analysis method: 1) HPLC; 2) ELISA; 3) PPIA; 4) PP2A; 5) LC/ESI-MS; 6) MALDI-TOF; 7) TR-FIA; 8) LC/MS.
[c]Purified MC variants: 1) LR; 2) RR; 3) YR; 4) LA; 5) LW; 6) LF; 7) LY; 8) dm LR; 9) dm RR.
[d]Bolded entries indicate the method corresponding to the maximum MC recorded when more than one method was used. NR = data not reported.

Table 3.2. A comparison of microcystin concentrations sampled by whole water and plankton tows from Constance Lake (2003 – 2005) normalized by dry weight (μg g^{-1}), sample volume (μg L^{-1}) and chlorophyll *a* (μg μg^{-1}) for both total microcystin determined by ELISA and HPLC. Mean sample volume, dry weight and total chlorophyll are also given.

	units	Whole water (mean)	Plankton[a] tow (mean)	df	t[b]	P
MC ELISA	μg g^{-1}	22.07	77.06	89	-5.18	<0.001
	μg L^{-1}	0.080	0.016	89	3.08	0.003
	μg Chl a^{-1}	0.012	0.085	85	-7.44	<0.001
MC HPLC	μg g^{-1}	6.49	40.11	89	-6.69	<0.001
	μg L^{-1}	0.027	0.009	89	1.46	0.14
	μg Chl a^{-1}	0.004	0.046	85	-7.84	<0.001
Volume	L	1.85	31.50	85	-109.4	<0.001
Dry weight	mg	7.37	7.35	85	-0.71	0.48
Chl *a*	μg	15.01	8.42	185	8.5	<0.001

[a]Plankton tows were taken by vertical net haul from 2 m using a 64 μm mesh net.

[b]Means were compared using standard t-tests.

3.7 Figures

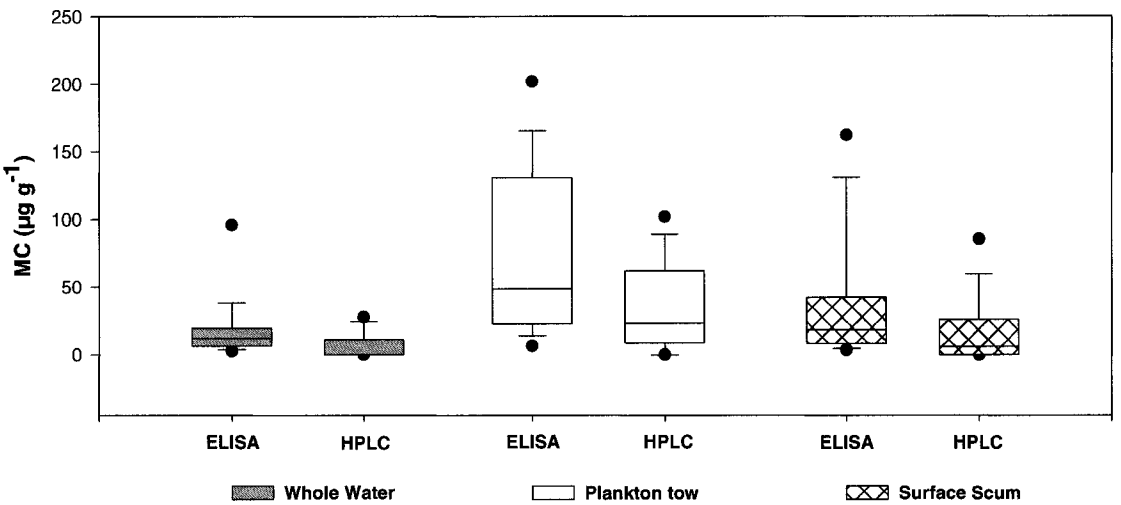


Figure 3.1 A comparison of mean microcystin concentrations measured by ELISA and HPLC on samples collected in three different ways (whole water, n=57; plankton tow, n=34; surface scum, n= 5) over three ice-free seasons from Constance Lake, Ontario, Canada. ELISA and HPLC were conducted on the same sample but samples collected using different methods were taken on different days. The central line is the median, the box edges are the 25th and 75th percentile and the whiskers represent the 10th and 90th percentiles. Minimum and maximum values are represented as points.

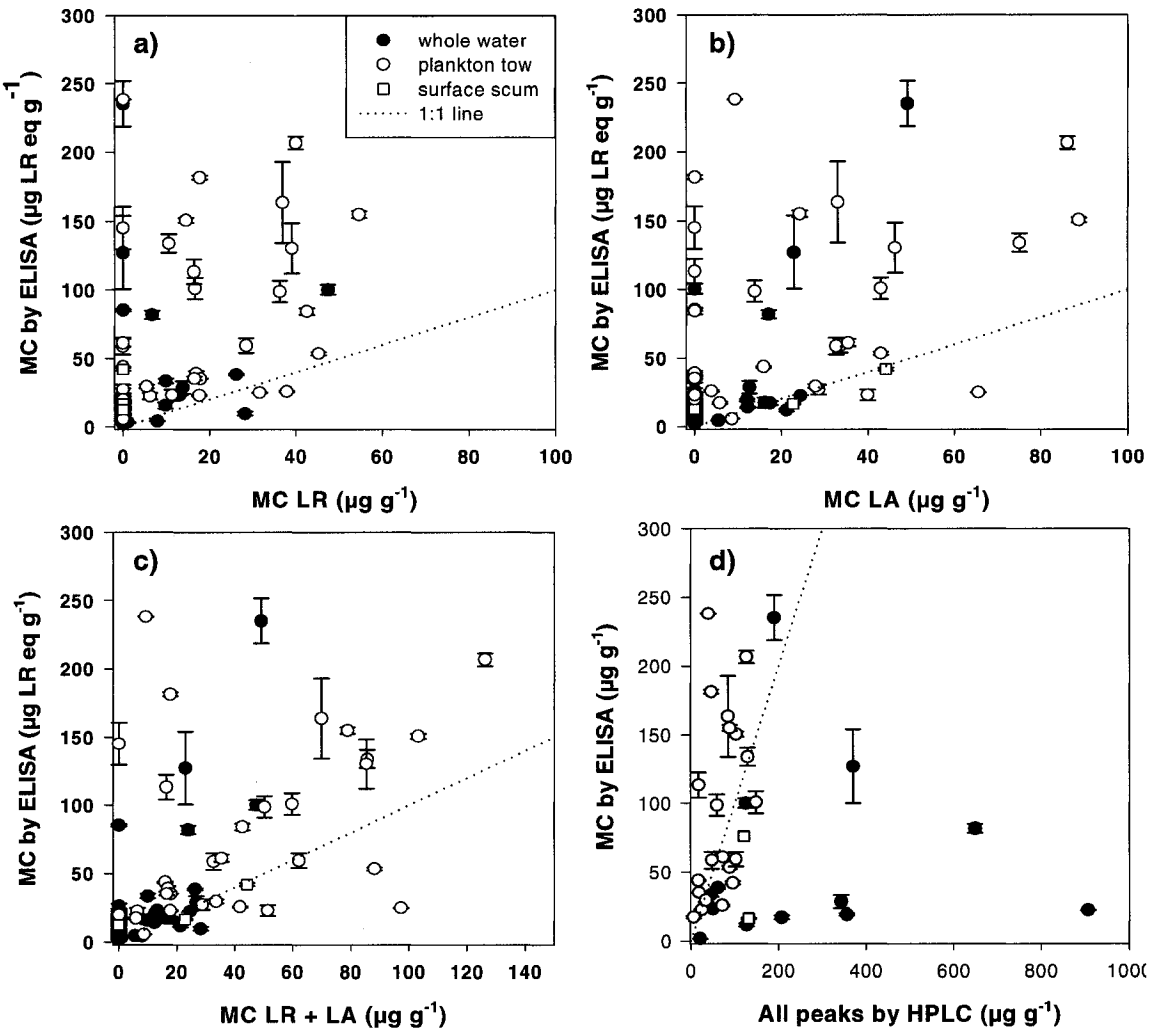


Figure 3.2 Relationships between total MC analyzed by ELISA and MC-LA and LR analyzed by HPLC: a) LR vs ELISA, b) LA vs ELISA, c) LA+LR vs ELISA and d) Sum of all peaks in HPLC that have a UV absorbance spectrum that matches MC LR vs ELISA. In each graph data points are distinguished by sampling method: whole water (○), plankton tow (●) or surface scum (□). Dotted line represents 1:1 line.

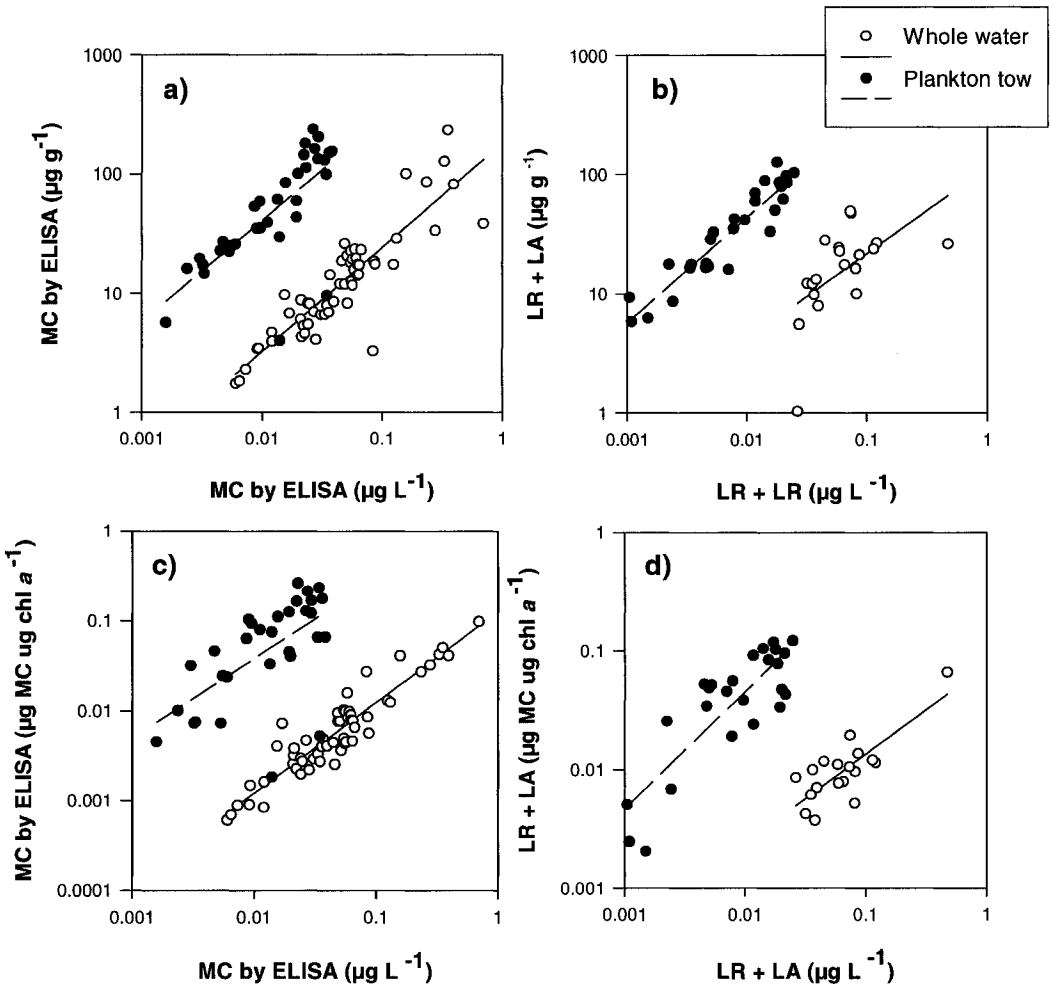


Figure 3.3 The effect of normalization parameter on concentration of microcystins measured by ELISA and HPLC. Panel a) ELISA results normalized by sample volume and dry weight, b) LA+LR (HPLC) normalized by sample volume and dry weight, c) ELISA results normalized by volume and chlorophyll *a* and d) LA+LR (HPLC) normalized by volume and chlorophyll *a*. Data are separated according to sampling method (whole water (○), plankton tow (●)). Lines represent best fit using linear regression for each sample method.

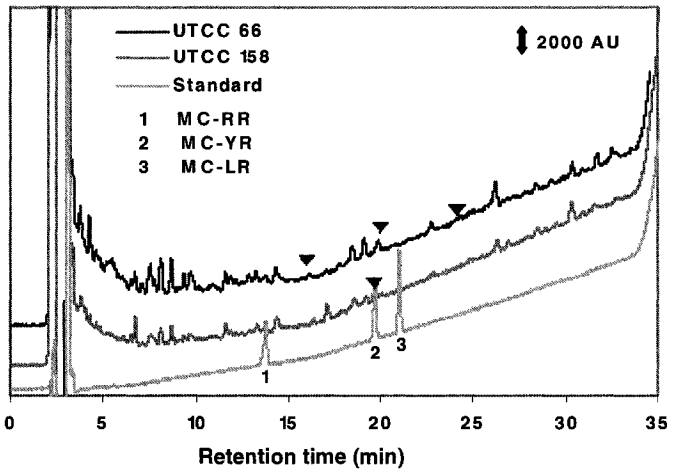


Figure 3.4 Chromatograms (at 238 nm detection) of cell extracts for two chlorophytes (UTCC 158 and UTCC 66) and a standard mixture containing MC-RR, MC-YR and MC-LR. Arrows indicate unknown peaks that had the same absorbance spectrum (200-300 nm) as MC-LR. Both cell extracts were negative when tested with ELISA.

4 Chapter 4 - Temporal variability of the cyanotoxin microcystin in relation to algal biomass, zooplankton and environmental factors

4.1 Abstract

Microcystins (MC), a class of cyanobacterial toxins, were measured in a shallow mesotrophic lake over three years to determine if sampling frequency affected correlations between MC and environmental factors and if the factors that explain MC variation across lakes are the same as those that explain temporal variability within a lake. The within year variability of MC was almost an order of magnitude higher than that of the abiotic variables measured and that of algal biomass (chl *a*). No correlations were detected at the seasonal scale, but at the monthly scale pH and temperature were positively correlated with MC. At the weekly scale, reactive phosphate (at a lag of 12 days) was correlated with MC. Our results show that correlations between MC and environmental factors are dependent upon the temporal sampling scale. Also, the factors most often associated with microcystins in among lake comparisons (i.e. total phosphorus, nitrogen and light attenuation) did not explain MC variation within the lake suggesting that across lake studies may be detecting correlations related to changes in phytoplankton community composition rather than any direct effects of nutrients on MC dynamics.

4.2 Introduction

Beyond the negative aesthetics of algal blooms, the toxins associated with cyanobacterial blooms are a public health problem affecting water for drinking and recreation as well as fish consumption (Carmichael 1994; Kuiper-Goodman et al. 1999). The hepatotoxic microcystins (MC) are the most widely studied of the cyanotoxins (Kotak et al. 1995; Fastner et al. 1999). Regional surveys of lakes have found that MC concentrations are best explained by the same factors that predict algal biomass: total nitrogen, total phosphorus and underwater light conditions (Kotak et al. 2000; Graham et al. 2004; Giani et al. 2005). It is unclear, however, whether these conditions are also important for predicting within-lake MC concentrations.

Seasonal patterns in MC concentrations are not easily generalized. Yearly maximum MC values may occur between early spring and late fall and inter-annual comparisons have reported high variability in seasonal patterns (Kotak et al. 1995; Park et al. 1998; Jones and Jones 2002; Ozawa et al. 2005). Though environmental conditions may be responsible for this variability, so far a comparison of results among studies only reveals inconsistencies. For example, Graham et al. (2006) found no relationship with soluble reactive phosphate yet Jacoby et al. (2000) found a positive relationship. And where Graham et al. (2006) also found a positive relationship with temperature, Billam et al. (2006) found no relationship with temperature in one lake and a negative relationship in another lake. One explanation for these differences may be the comparison of results across different temporal scales.

In the studies described above, sampling frequency ranged from 4 to 17 times per year. Such different sampling frequencies may explain these disparate conclusions since different factors operate at different time scales (Reynolds 1990). For example, seasonal means in abiotic conditions can help explain the phytoplankton assemblage present, but not the specific species (Reynolds 1984a). Higher frequency fluctuations in abiotic conditions, such as those that occur below the monthly scale, may then select species based on physiological characteristics such as nutrient uptake kinetics (Tilman et al. 1981). Similarly, community processes such as grazing and competition may also occur

at different time scales dependent upon the generation time of algae and zooplankton and/or the time necessary to adapt to changing resources (Jones 1978; Zurawell et al. 2005; Kardinaal et al. 2007a).

To inform lake management strategies, which are applied at the scale of individual lakes, it is important to understand how the choice of scale affects observed correlations between microcystins and environmental conditions. The objectives of this study were to determine if the choice of temporal scale (seasonal, monthly or weekly) affects the correlations between MC and environmental factors including zooplankton density and to determine whether the factors that explain variation across lakes are the same as those that explain temporal variability at the within-lake scale. Comparisons were made with similar analysis for algal biomass (chlorophyll *a*) since a great deal is known about its dynamics both within (Marshall and Peters 1989) and across lakes (Dillon and Rigler 1974). The lake chosen for the study was a mesotrophic shallow lake that exhibits periodic cyanobacterial dominance (Hamilton et al. 2005) and lies within the range of lake trophic state where the risk of cyanobacterial blooms abruptly increases (Downing et al. 2001).

4.3 Methods

4.3.1 Study site, sampling and laboratory analyses –

Constance Lake is a mesotrophic, shallow lake (mean depth 3 m) in the west end of Ottawa, Ontario (45° 24' N, 75° 59' W). It is located on the flood plains of the Ottawa River and the underlying substrate is predominantly limestone and dolomite (Williams et al. 1984). Additional information on the lake can be found in Hamilton et al. (2005). Water for nutrient and chlorophyll analyses was collected every three days using an integrated tube sampler to a depth of 2 m. Samples were collected between 1000 h and 1200 h from the same location near the middle of the lake. On each sampling day, Secchi depth was recorded and turbidity measured on water samples in the laboratory using a LaMotte 2020 turbidimeter. Average water column temperature and pH were calculated

from measurements made at 0.25 m intervals with a Hydrolab multisensor probe. Water was analyzed for total phosphorus (TP), reactive phosphate (RP), total nitrogen (TN) nitrate+nitrite [$NO_3^- + NO_2^-$], ammonium [NH_4^+], and silica [SiO_2] by the City of Ottawa laboratory following standard methods of the Ontario Ministry of Environment (1983). For chlorophyll *a*, water was filtered through a GF/F filter and immediately frozen at -20°C until extraction with dimethyl sulphoxide (Burnison 1980; Jeffrey and Humphrey 1975). When net hauls were used to collect samples for microcystin analysis (*see below*), a sub-sample of the net concentrate was also analyzed for chlorophyll *a*.

From 29 May to 7 November in 2003 and from 28 May until 21 June in 2004, 1.5 to 2 L of water from an integrated tube sample was used for toxin analysis every three days. After 21 June in 2004, seston was concentrated for toxin analysis by vertical net haul from 2 m (mesh size of 64 μm) every 6 days. In 2005, samples were collected by vertical net haul bi-monthly. In all cases water was filtered through a pre-ashed and weighed GF/D filter, dried at 60°C overnight and then frozen at -20°C (Harada et al. 1999). Before extraction, samples were defrosted and rehydrated using distilled water and again frozen at -20°C for at least 24 hours (Harada et al. 1999; Ortea et al. 2004). Samples were extracted using accelerated solvent extraction (Dionex ASE 2000) with 75% MeOH, evaporated to dryness and resuspended in 250 μl of 50% MeOH (Aranda-Rodriguez et al. 2005). Extracts were analyzed using reverse-phase high pressure liquid chromatography equipped with a PD40 photodiode array detector (Dionex, Canada). UV spectra were acquired at 200 to 300 nm and data were recorded at 238 nm and compared against MC standards (Aranda-Rodriguez et al. 2005). MC-LR and MC-RR were obtained from Sigma-Aldrich (Ontario, Canada) and MC-YR and MC-LA from Calbiochem-Novabiochem Co. (La Jolla, CA, USA). Total microcystin concentration was determined by enzyme linked immune sorbent assay (ELISA kit from Envirologix, Canada). Fourty-six percent of samples analyzed by HPLC were below detection (*see results*) so statistical analyses were conducted primarily on total MC from ELISA. Microcystin analytes, standardized by total dry weight, were used in statistical analyses but descriptive statistics were also calculated for MC standardized by chlorophyll *a* (collected by the same sample method as MC) and for 2003, volume.

Cladoceran samples were collected by vertical net haul from 2 m with a conical zooplankton net (diameter = 19.8 cm, mesh size = 64 μm). Samples were preserved in the field with 40% formalin buffered with sucrose (Haney and Hall 1973). In the laboratory, zooplankton were counted in subsamples of 5 mL, until either 20 mL of the sample or 200 organisms were counted. Water for rotifer samples was collected by integrated tube sampler and then concentrated by screening through a 35 μm mesh. Sub-samples were counted at 150X magnification using a Wild M40 inverted microscope. Zooplankton were identified to genus with the aid of texts by Thorp and Covich (2001), Eddy and Hodson (1961), and Balcer et al. (1984).

4.3.2 Statistical analysis –

Pearson's correlation coefficient was used to test for correlations in seasonal and monthly means and maxima between MC or chlorophyll *a* and environmental variables. Single monthly maxima were used but the maximum seasonal values were taken as the mean of sample dates with MC or chlorophyll *a* levels that were above the 90th percentile for each season. To delineate seasons, two approaches were compared. In the first approach absolute dates were chosen to divide the year into three seasons: spring – sampling start until 1 July; summer – 1 July to 10 September; and autumn – samples collected after 10 September. The second approach was to use the inflection points of a 3rd order polynomial fit to chlorophyll *a* values for each year which made the season's ranges within each year unique from one another. A one-way ANOVA was conducted using both groupings and the first approach to delineating season explained a larger proportion of variation in MC and so was used in subsequent models.

Analyses at a temporal scale of 6 days were conducted using cross-correlation analysis for time-series data (Chatfield 2004). Missing values in MC (2003 n=7; 2004 n=1) were fit by linear interpolation and where sampling interval was 3 days, only alternate days were used. To prepare data for analysis, a number of transformations were necessary. For all analyses, data were first screened for normality using quantile-quantile (Q-Q) plots and histograms. If the distribution of the data series was not normal, variables were transformed using log (MC, chl *a,* Secchi depth), log+1 (cladoceran

density, rotifer density, TP, NH_4^+) or square root (silica). Variables were then first-order differenced to remove the presence of autocorrelation (Chatfield 2004). Some of the differenced data series were still auto-correlated so an auto-regression model (AR1) was fit and the residuals were used in subsequent analyses. Cross-correlation plots were then used to identify significant instantaneous and lagged correlations up to two time steps (12 days).

Variables with significant lagged or instantaneous cross correlations were used in a lagged regression model which took the following form (Pace et al. 1998):

$$\text{MC or Chlorophyll } a = \alpha + \beta_1 X_{1\,t} + \beta_2 X_{2\,t-z} + \beta_3 X_{3\,t-z} + \varepsilon \qquad \text{Eq. 4.1}$$

where t is time, X_1 is a predictor variable at t=0, X_2 and X_3 are predictor variables with a lag = z in days, β are fitted coefficients of the model, and ε is a series of uncorrelated residuals. Backwards selection was used to choose the best regression model but where variables were co-linear, further refinement of the model was conducted by removing variables with low standard coefficients and comparing adjusted R^2 values and residual plots of subsequent models. Autocorrelation in residuals was tested using the Durbin-Watson statistic. Data were analyzed using SYSTAT version 11.

A random-blocked, re-sampling protocol was used to determine if correlations detected for monthly means and maxima were applicable at lower sampling frequencies. Using all data, for each month, 1, 2, 3 or 5 samples were chosen without replacement (or if n<5 for a particular month then n -1 samples were chosen; if n=1 then that sample was included in all iterations). This was then repeated for a total of 250 iterations. As the autoregressive relationship in the dependent variables (MC and chl *a*) was significant for two time steps, samples were chosen so consecutive samples were not within two time steps of the previous sample chosen. To further account for autoregression, samples chosen were re-ordered so the first sample chosen from each month was grouped followed by the next sample from each month and so on. As a final failsafe, the dependent variable was included in the model at a lag of one time step (6 days). Mean coefficients were calculated as well as mean *t*, *F* and R^2 values. Confidence intervals (95 %) for model statistics were compared with the critical *t* and *F* values. Optimal sample

sizes for estimating means were calculated for confidence intervals of 20, 40, 60 and 80 $\mu g\ g^{-1}$ of microcystin seston content at 95% confidence intervals using the equation and critical t values given in Zar (1999).

4.3.3 Standardizing MC –

In this study, samples for MC were collected by two methods: integrated tube sample and vertical plankton haul both to a depth of 2 m. A plankton haul sample concentrates cyanobacterial colonies compared to an integrated tube sample that collects a more diverse phytoplankton assemblage. The potential for error caused by including these two methods in our analyses was mitigated by standardizing MC values by total dry weight. Each standardizing variable (volume, chl *a,* and total dry weight) has its limitations. For example, volume does not account for differing cell density; chl *a* differs with phytoplankton community composition (Reynolds 1984b); and total dry weight will include non-organic particles. However, I chose total dry weight because it represents most closely the MC in the seston as experienced by zooplankton grazers or water purveyors. A regression of MC standardized by total dry weight against MC standardized by volume revealed that the slope was not significantly different from one for either net haul samples or whole water samples so a systematic error was not introduced by standardizing by total dry weight (Tillmanns et al. 2007). Differences between the two methods due to absolute concentration were minimized in the time-series analysis by using the first order difference of the logged series. In addition, as the two methods were used consecutively in two blocks of time rather than interspersed, all but one data point in the analysis represents the relative difference between two dates sampled by the same method. Also, the time-series analysis was focused on cross-correlations with environmental factors, for which the sampling method remained constant through out the entire study period.

4.4 Results

In Constance Lake, microcystins (MC) LR and LA were detected in all three years but MC-YR and MC-RR were not detected (Fig 4.1). The maximum MC seston content measured by ELISA was 238.4 $\mu g\ g^{-1}$ and occurred in August of 2005. Maxima in 2004 also occurred in August (206.9 $\mu g\ g^{-1}$) but in 2003 the maximum MC occurred in June (235.4 $\mu g\ g^{-1}$ or 0.353 $\mu g\ L^{-1}$). Maximum MC measured by ELISA did not necessarily coincide with maximum values of MC-LR or MC-LA as measured by HPLC (Fig 4.1). This is not surprising given that other microcystin congeners for which standards are not available are likely present in environmental samples.

The within year variability in MC was much higher than most abiotic variables (50 times higher than pH and 3 times higher than ammonium), about five times higher than chlorophyll *a,* three times higher than rotifer density but about the same as cladocerans (Table 4.1; Figure 4.1). MC standardized by chl *a*, also showed high variability within years as did MC standardized by volume in 2003 (Table 4.1). Chlorophyll *a* was highest during the summer with maxima ranging from 14.4 $\mu g\ L^{-1}$ in 2004 to 17.6 $\mu g\ L^{-1}$ in 2003 (Fig 4.2). TN and TP had low temporal variability; yearly CV ranged from 8 to 12 % for TN and 17 to 24 % for TP (Table 4.1) and mean TN: TP ratios ranged from 14 to 43 but predominantly were between 19 (10^{th} percentile) and 30 (90^{th} percentile). Ammonium and silica concentrations showed much higher temporal variability than TN or TP (Table 4.1). Nitrate and nitrite were always below detection (minimum detectable level: nitrate - 0.02 $mg\ L^{-1}$; nitrite - 0.02 $mg\ L^{-1}$).

At the seasonal scale, there was no correlation between seasonal mean or maximum MC content and environmental variables (Table 4.2) but algal biomass (chl *a*) was positively related to TN and silica concentrations and negatively related to ammonium. In contrast, at the monthly scale, average MC was positively correlated with mean pH ($P < 0.005$) and maximum MC was positively correlated with maximum values of pH and temperature ($P < 0.05$; Table 4.2). At this same scale, monthly average chl *a* was positively correlated with TP, TN and silica, and negatively correlated with ammonium and Secchi depth while maximum monthly chl *a* values were positively

correlated with TP and negatively correlated with MC (Table 4.2). At a scale of 6 days, MC was positively correlated with RP at t-12 days (Table 4.3). Chlorophyll *a* was instantaneously correlated with TP and silica and cross-correlated with temperature at t-6 days (Table 4.3). A linear regression revealed that RP explained a significant amount of variation in MC (n=47, P =0.001, R^2=0.14). For chl *a,* only TP was necessary to explain the largest amount of variation in the dataset (n=51, P =0.001, R^2=0.19).

The results of the re-sampling process revealed that only some of the variables were significant when random sampling within months with decreasing sampling frequencies (Table 4.4). MC was related to pH when five or three samples per month were sampled but not when less than three samples were chosen. Neither RP nor temperature was significant at this lower sampling frequency. Regardless of the sample frequency, chl *a* was always strongly dependent upon TP (Table 4.4). As with most environmental variables, the variance in MC was positively related to the mean (Figure 4.3a). When samples across seasons were combined, the standard deviation decreased with sample size (Figure 4.3b). However for months, where the maximum sample size was 10, there was a wide range in standard deviations. Given this wide range in standard deviations for different seasons and months, a consistent sample size will give a range in precision when estimating the mean (Figure 4.3b).

4.5 Discussion

The results presented here demonstrate that the choice of temporal scale had an effect on the correlations detected between MC and environmental variables. At the monthly scale, mean MC was correlated with pH and at shorter time scales (6 days), MC was correlated with reactive phosphorus at a time lag of 12 days. Previous studies that sampled at a frequency higher than once per month also detected a positive correlation with pH (Kotak et al. 1995; Kotak et al. 2000; Jacoby et al. 2000) and a positive correlation was detected with soluble reactive phosphate in those studies with sampling frequencies greater than 10 per year (May to Oct; Kotak et al. 1995; Jacoby et al. 2000). Though differences in sampling scale may be responsible for some of the discrepancies

among studies, at least one study with frequent sampling did not find these same correlations (Rolland et al. 2005). At a seasonal scale, I could detect no correlations between MC and environmental variables suggesting that regional studies with infrequent sampling may be observing relationships associated with lake trophic status rather than variables affecting within lake MC fluctuations.

The correlation between MC and pH detected at a monthly scale was also detected by regional studies which have shown both cyanobacterial biomass and MC to be positively correlated with pH (Kotak et al. 1995; Kotak et al. 2000; Reynolds and Petersen 2000). Cyanobacteria in general are known to have an effective CO_2 concentrating mechanism (Espie et al. 1991; Miller et al. 1991), which provides them with an advantage over other algae at high pH when concentrations of dissolved CO_2 are low. The fact that pH was not cross-correlated with MC at a scale of 6 days or at the seasonal scale suggests that the processes that relate pH to MC occur at time scales longer than 6 days but shorter than seasons. This is the same scale over which within-species genotype succession (toxic vs. non-toxic strains) in cyanobacterial blooms is thought to occur (Kardinaal et al. 2007a). In addition to light (Kardinaal et al. 2007b), nutrients (Vezie et al. 2002) and susceptibility to grazers (Tillmanns et al. 2008), pH may also influence the competition between toxic and non-toxic genotypes. Alternatively, changes in pH could increase the intracellular production of MC (Song et al. 1998). However, different patterns in MC-LA and MC-LR compared with total MC measured by ELISA suggest that there were probably changes in strain dominance at the monthly scale (Figure 4.1).

Aside from the lagged relationship with RP, neither total phosphorus nor total nitrogen were important for explaining fluctuations in MC at any scale in Constance Lake. Regional surveys of lakes have found TP, TN and underwater light conditions to be the best predictors of both MC concentrations (Kotak et al. 1995; Kotak et al. 2000; Graham et al. 2004; Giani et al. 2005) and phytoplankton biomass (Reynolds 1984b). Regional studies, however, typically covered an order of magnitude in TP and TN concentrations. Generally, the proportion of cyanobacteria biomass increases with overall phytoplankton biomass (Downing et al. 2001), so correlations of MC with TP and TN are

likely due to a higher probability of finding toxigenic cyanobacteria in eutrophic conditions (Reynolds et al. 2000). Regional correlations between MC and chl *a* may also be due to the trophic status of lakes rather than any causal association between MC and chl *a* (Downing et al. 2001). Indeed in this study, in contrast, MC and chl *a* were either not correlated or were negatively rather than positively correlated, depending on the temporal scale (Table 4.2). Similar to the original TP - chl *a* relationship (Dillon and Rigler 1974), correlations with nutrients have helped managers identify lakes that may be at risk of MC production. However, major nutrients do not seem important when considering a lake that changes little in overall nutrient concentrations either seasonally or across years (this study; Jacoby et al. 2000; Graham et al. 2006).

The correlation between MC and reactive phosphorus was only detected at a weekly scale with a lag time of 12 days. In 2003 and 2004, RP underwent periodic increases of between 3 and 6 $\mu g\ L^{-1}$, likely the result of internal loading episodes (Hamilton et al. 2005) providing brief periods of phosphorus availability. Common bloom-forming cyanobacteria, and especially *Microcystis,* can quickly uptake phosphorus even when cellular levels are not depleted and can store phosphorus for use during low nutrient conditions (Kilham and Hecky 1988; Krivtsov et al. 2005). The 12 day time lag likely indicates a period between cellular uptake and population increase in toxicogenic strains rather than a change in cellular production of MC which would occur more rapidly.

Correlations detected at monthly and weekly scales were based on data collected every 3 or 6 days (bi-monthly in 2005). The results of the re-sampling process address the likelihood of detecting these relationships when sampling less frequently. Although both monthly mean and maximum pH and maximum temperature were correlated with MC, only pH remained important when sampling frequency was decreased. RP was also not related to MC when sampling frequency was reduced to five times per month. The relationship between MC and pH at a monthly scale explained more variance in MC at a lower sampling frequency than the relationship between RP and MC when sampling every 6 days (R^2 monthly = 0.25 vs R^2 weekly = 0.14).

Compared with MC, the effect of temporal scale was not as important when testing for correlations between algal biomass and environmental factors. Four of the six environmental factors correlated with chlorophyll *a* were significant at more than one scale compared to MC where both pH and RP were only significant at one time scale. The variability associated with chl *a* was also about a quarter of the variability in MC. This may be expected as chlorophyll *a,* a surrogate for algal biomass, represents a high level property of aquatic ecosystems which is the sum of multiple species with compensatory dynamics (Tilman and Downing 1994; Harris 1994). MC, however, is produced only by those genotypes that have the *mcy* gene cluster (Tillet et al. 2000) which is present in several species across a number of genera (Sivonen and Jones 1999). The results presented here suggest that the dynamics of toxicogenic genotypes may be highly variable and high frequency sampling will be necessary to track changes in strain dominance over time.

Developing an empirical model between MC and environmental factors at the within lake scale would be of useful to water managers. Given that all microcystin producing strains have evolved from a common MC producing ancestor (Rantala et al. 2004), it may be possible to develop a direct empirical model for MC rather than beginning with community composition, which remains difficult to predict (Reynolds et al. 2002). Selection of the appropriate time scale must first be determined to frame future studies and also to ensure comparisons across studies can be made. The appropriate temporal scale will need to minimize variance due to environmental stochasticity but not lose variation due to the response of these genotypes to changing environmental and biotic conditions. Here I show that relationships developed at a monthly scale provided the best potential for describing fluctuations in MC.

4.6 Tables

Table 4.1 Means, ranges and coefficients of variation (expressed as a percentage) of biological, chemical and physical variables measured in Constance Lake, Canada. MC could not be standardized by volume in 2004 and 2005 because net efficiency was not calculated simultaneously with sample collection. Sample sizes of all variables except MC are: 2003, n=55; 2004, n=52; 2005, n=10. Sample size of MC: 2003, n=48; 2004, n=33; 2005, n=8. Clad – cladoceran; Rot – rotifer.

	2003			2004			2005		
	Mean	Range	%CV	Mean	Range	%CV	Mean	Range	%CV
MC ($\mu g\ g^{-1}$)	22.3	1.8 - 235.4	174	66.6	1.7 - 206.9	91	49.7	5.7 - 238.4	157
MC ($\mu g\ \mu g$ chl a^{-1})	0.004	0 - 0.021	116	0.033	0 - 0.101	87	0.012	0.002 - 0.053	146
MC ($\mu g\ L^{-1}$)	0.032	0.003 - 0.144	106	NA	NA	NA	NA	NA	NA
Chl *a* ($\mu g\ L^{-1}$)	10.5	3.9 - 17.6	33	9.4	3.8 - 14.4	22	9.6	2.5 - 15.3	39
Clad (no. L^{-1})	11	0 -113	168	4	0 - 41	163	23	0 - 85	119
Rot (no. L^{-1})	1995	601 - 7149	61	2003	68 - 5580	63	2103	300 - 6525	85
TP ($\mu g\ L^{-1}$)	32	20 - 50	24	29.1	16.5 - 37.5	17	32	20 - 40	21
RP ($\mu g\ L^{-1}$)	6	3 - 10	28	7.3	1.5 - 11.5	31	8	4 - 11	30
TN ($\mu g\ L^{-1}$)	750	600 - 900	8	720	539 - 829	9	690	540 - 790	12
NH_4^+ ($\mu g\ L^{-1}$)	10	2 - 37	81	10.8	3.5 - 26	51	13	4 - 36	71
Silica ($mg\ L^{-1}$)	2.9	0.7 - 5.4	47	1.4	0.003 - 2.3	45	0.62	0.1 - 1.7	84
Secchi (m)	1.5	0.7 - 2.4	26	1.5	0.9 - 2.8	26	1.6	1.1 - 2.6	31
Temp (°C)	19.1	5.3 - 27	33	19.5	8.1 - 25	23	21.9	13.4 - 28	21
pH	8.2	7.7 - 8.7	3	8.7	8.1 - 9.2	4	9.1	8.8 - 9.5	3

Table 4.2 Pearson's product moment correlations of seasonal means and maxima (n=8) and monthly means and maxima (n = 15-16) for microcystin seston content (MC) and chlorophyll *a* concentrations (Chl *a*) with environmental variables. Years were divided into three seasons: spring – sampling start until 1 July; summer – 1 July to 10 September; and autumn – samples collected after 10 September. Seasonal maxima were calculated as the average of values greater than the 90th percentile. Season-years or month-years that were only represented by one data point were not included in this analysis.

	Seasonal Ave		Seasonal Max		Monthly Ave		Monthly Max	
	MC	Chl *a*	MC	Chl *a*	MC	Chl *a*	MC	Chl *a*
MC	-	ns	-	ns	-	ns	-	-0.77*
Chl *a*	ns	-	ns	-	ns	-	ns	ns
Cladoceran no.	ns	ns	ns	ns	ns	ns	ns	ns
Rotifer no.	ns	ns	ns	ns	ns	ns	ns	ns
TP	ns	ns	ns	ns	ns	0.83**	ns	0.78*
RP	ns	ns	ns	ns	ns	ns	ns	ns
TN	ns	0.82*	ns	0.91*	ns	0.84**	ns	ns
NH_4^+	ns	-0.93**	ns	ns	ns	-0.61*	ns	ns
Silica	ns	0.83*	ns	0.95**	ns	0.79**	ns	ns
Secchi	ns	ns	ns	ns	ns	-0.84*	ns	ns
Temp	ns	ns	ns	ns	ns	ns	0.682*	ns
pH	ns	ns	ns	ns	0.821**	ns	0.618*	ns

* $P<0.05$; ** $P<0.005$

Table 4.3 Cross-correlations of microcystin seston content and chlorophyll *a* concentrations with environmental variables in Constance Lake, Canada. Data used in this analysis was at a frequency of every 6 days in between May and November in 2003 and 2004. The number of days the environmental variable is lagged is given in parentheses. Only lagged cross-correlations up to a maximum of 12 days were included (2 time steps; n=51).

	MC-Total	Chl *a*
Chl *a*	ns	-
Cladoceran no.	ns	ns
Rotifer no.	ns	ns
TP	ns	0.436(0)**
RP	0.375(12)*	ns
TN	ns	ns
NH_4^+	ns	ns
Silica	ns	0.317(0)*
Turbidity	ns	ns
Temp	ns	0.370(6)*
pH	ns	ns

* $P<0.05$; ** $P<0.005$

Table 4.4 Model parameters and confidence intervals from 250 iterations of multiple regression models. Each set of 250 iterations was conducting by sampling without replacement 5, 3, 2 or 1 sample(s) from each month. Slopes for each variable were considered significant if the confidence intervals of the mean *t*-values did not over-lap with the critical *t*-value. Intercepts were included in all models as well as the dependent variable (MC or Chl *a*) lagged by one time step to account for any remaining autocorrelation. Only coefficients for β_1 were included in the table below as it was the only coefficient that in some models differed significantly from zero.

Model	No./ Month	n	Mean β_1 (95% CI)	Mean *t* (95% CI)	Mean *F*-value (95% CI)	Mean R^2
$log\ MC = \beta_1 pH + \beta_2 temp + \beta_3 log\ MC_{t-6}$	5	52	0.473 (0.471 to 0.475)	2.256 (2.245 to 2.267)**	5.669 (5.634 to 5.704)*	0.25
	3	38	0.466 (0.463 to 0.469)	1.982 (1.968 to 1.996)*	5.398 (5.354 to 5.443)*	0.30
	2	28	0.451 (0.449 to 0.454)	1.701 (1.689 to 1.713)		
	1	17	0.459 (0.455 to 0.463)	1.637 (1.623 to 1.651)		
$log\ MC = \beta_1 RP_{t-12} + \beta_2 log\ MC_{t-6}$	5	52	39.846 (39.428 to 40.264)	1.031 (1.02 to 1.042)		
log Chl $a = \beta_1 log\ (TP+1) + \beta_2 log$ Chl a_{t-6}	5	52	31.739 (31.679 to 31.799)	6.374 (6.354 to 6.394)**	21.836 (21.699 to 21.972)**	0.45
	3	38	30.992 (30.901 to 31.083)	5.054 (5.031 to 5.078)**	14.963 (14.818 to 15.109)**	0.43
	2	28	32.237 (32.122 to 32.351)	4.47 (4.444 to 4.495)**	11.99 (11.849 to 12.132)**	0.44
	1	17	44.764 (44.603 to 44.925)	4.061 (4.041 to 4.082)*	9.766 (9.679 to 9.852)*	0.53

* $P<0.05$; ** $P<0.005$

4.7 Figures

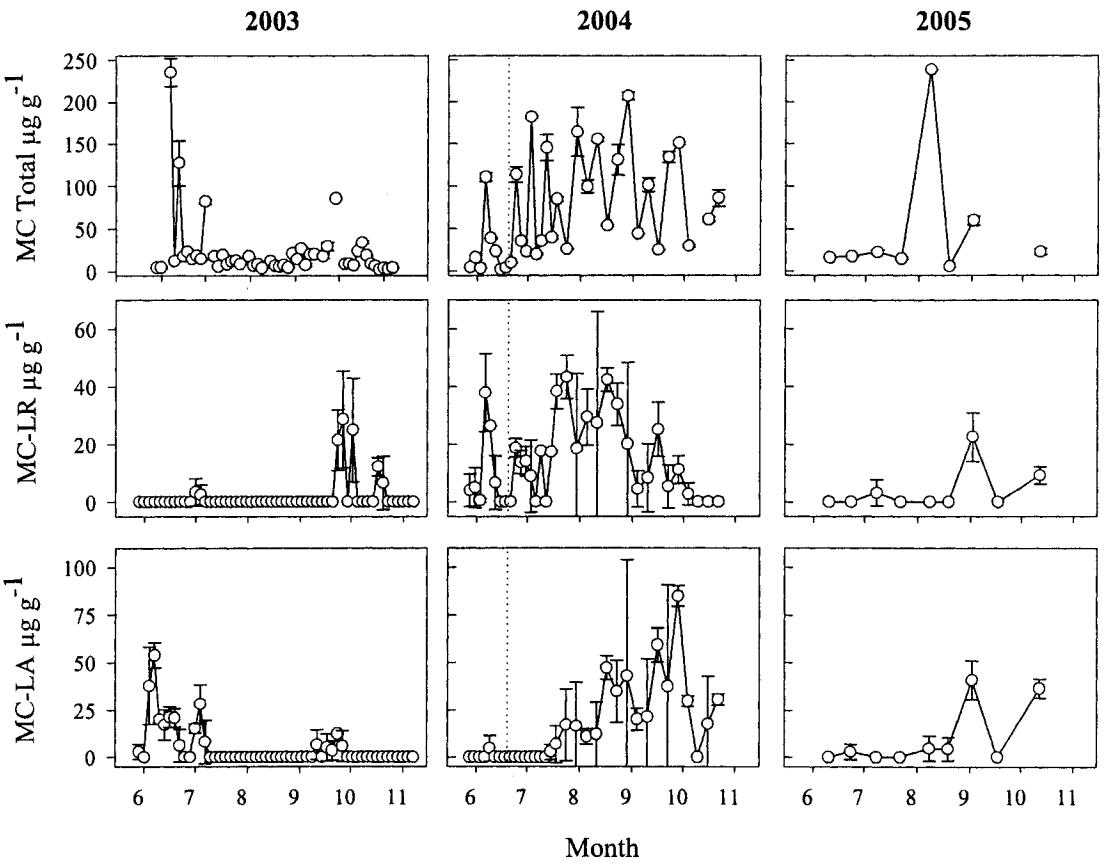


Figure 4.1 Microcystin content of seston measured in Constance Lake by ELISA (top row), and HPLC (bottom two rows) for 2003-2005 (mean ± s^2, n=2). Samples collected before 23 June 2004 were collected by integrated tube sampling from a depth of 2 m. Samples collected after 23 June 2004 were collected by vertical net haul from 2 m (mesh size 64 µm). 23 June 2004 is marked by a horizontal line in 2004. Samples collected by integrated tube sampling were collected at an interval of 3 days while samples collected by vertical net haul were collected every 6 days in 2004 and bi-monthly in 2005.

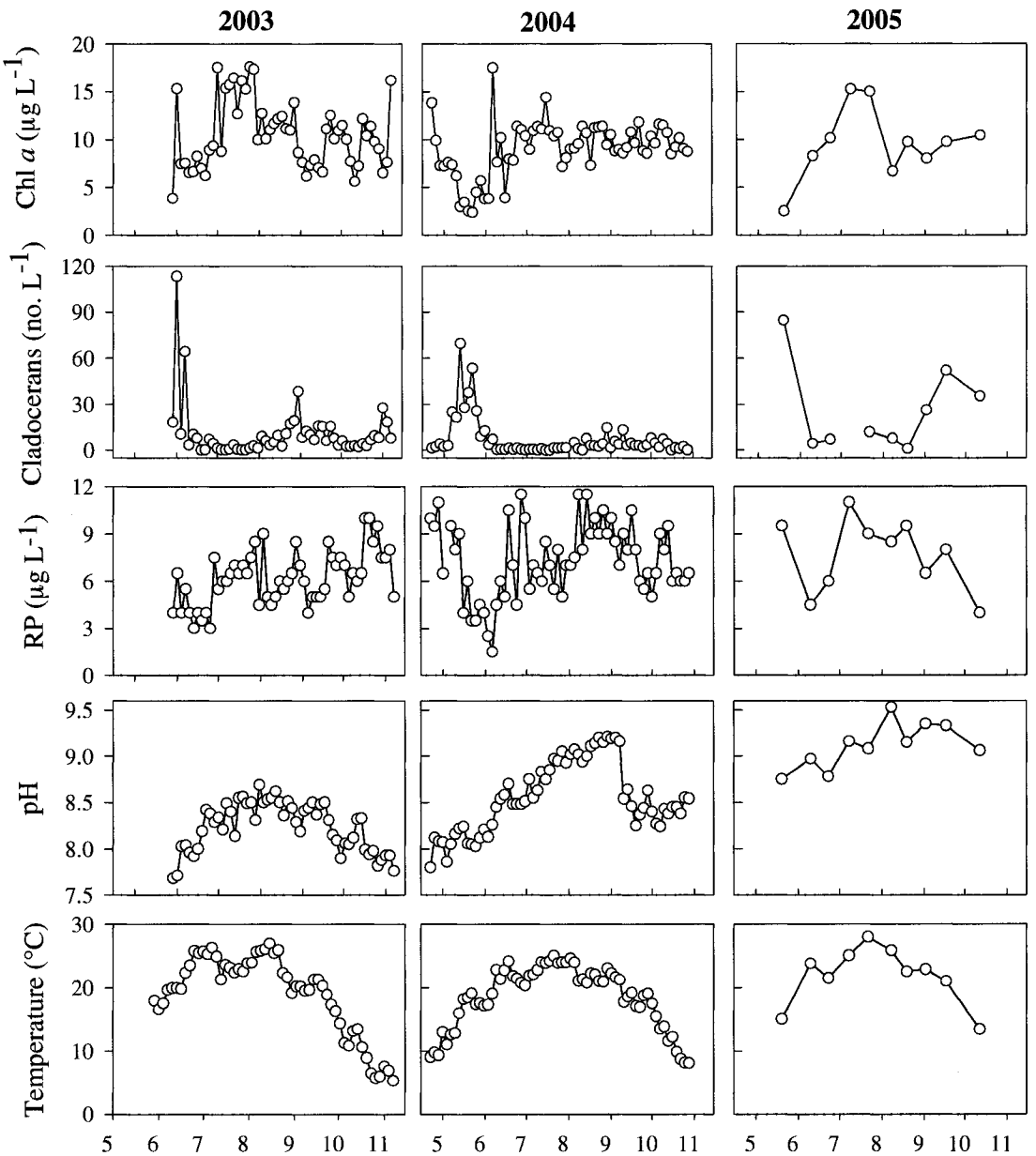
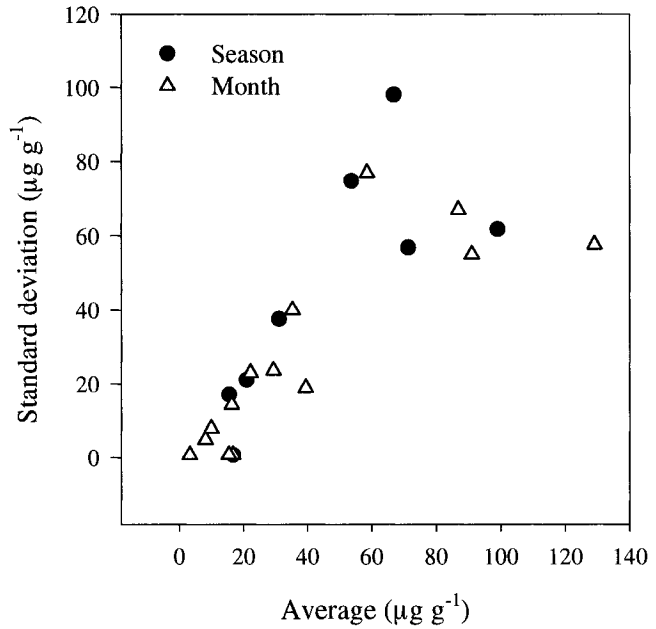


Figure 4.2 Chemical and physical measurements made in Constance Lake in 2003-2005. Samples were collected at an interval of 3 days in 2003 and 2004 and bi-monthly in 2005.



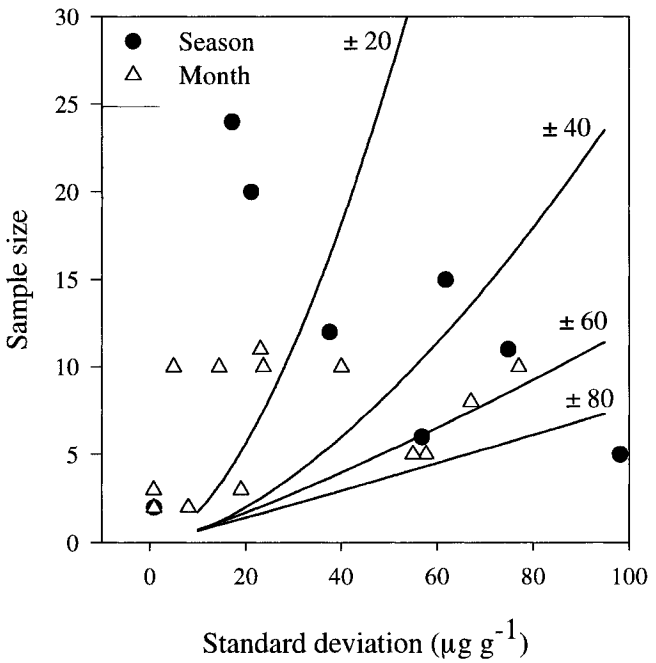


Figure 4.3 The relationship between seasonal and monthly mean, standard deviation and sample size for microcystin seston sampled in Constance Lake. The lines in panel B show the sample sizes necessary to estimate means with 95% confidence for each confidence interval (20, 40, 60, 80 µg g^{-1}) given the variance of the sample.

5 Chapter 5 - Regulation of colonial cyanobacterial populations by abiotic and biotic factors during non-bloom years

5.1 Abstract

Cyanobacterial blooms occur when one or a few species of cyanobacteria dominate an ecosystem. In this study, abiotic factors and zooplankton density were measured in a shallow lake every three days over two growing seasons to test for correlates with cyanobacterial growth. Although populations of *Anabaena, Microcystis, Woronichinia,* and *Aphanizomenon* were present none of these genera reached bloom levels. Abiotic limits to growth were expected as positive correlations between changes in abiotic factors and changes in cyanobacterial growth rates. Grazing pressure and competition between cyanobacteria were expected as negative correlations. The results showed that most correlations with zooplankton and between cyanobacteria taxa were positive suggesting that facilitative relationships may be more important in explaining cyanobacterial population dynamics than grazing pressure or competition. Inclusion of time lagged variables in regression models doubled the variance explained for some cyanobacterial taxa. For all cyanobacterial taxa, biotic interactions explained more variation than abiotic variables suggesting that more emphasis should be placed on studying community dynamics as a means of predicting cyanobacterial blooms.

5.2 Introduction

Freshwater cyanobacteria, especially a few species of colonial cyanobacteria, undergo occasional dramatic increases in population abundance. These population outbreaks are known as water blooms when one or a small number of species dominate the pelagic zone, (Reynolds and Walsby 1975). There are a number of societal consequences of bloom events, especially if the dominant species produce toxins (Codd 2000; Zurawell et al. 2005). Ecologically, cyanobacterial blooms can disrupt normal ecosystem functions by reducing energy transfer through the food chain, phytoplankton diversity and nutrient recycling rates in the pelagic zone (Mehner et al. 2002; Sunda et al. 2006). Blooms are thought to occur when one or a few species proliferate under ideal abiotic conditions and/or in the absence of competition or grazing (Hyenstrand et al. 1998). Time lags may also be important in detecting these relationships as lags in population response time to environmental changes can result in large fluctuations (May 1976).

Cyanobacteria have a number of physiological adaptations that give them an advantage over other phytoplankton species under specific abiotic conditions (Hyenstrand et al. 1998). These adaptations include the ability to regulate buoyancy, which is beneficial under low turbulence (Reynolds et al. 1987; Miller et al. 1991; Shapiro 1997; Huisman et al. 1999c; Johnston and Jacoby 2003), and the ability of some species to fix atmospheric nitrogen, which may explain the dominance of cyanobacteria in lakes with low nitrogen to phosphorus levels (Smith 1983; Pick and Lean 1987). Many cyanobacterial species reach an optimal photosynthetic capacity at water temperatures of 25°C (Robarts and Zohary 1987), and have the ability to store phosphorus (Kilham and Hecky 1988). Not surprisingly, blooms are commonly found when temperature and phosphorus levels are high (McQueen and Lean 1987; Downing et al. 2001). In each of the adaptations listed above, the response time may not be immediate or immediately detectable. In batch cultures, for example, cells transferred from nutrient deficient conditions to nutrient rich conditions undergo a lag phase while the cell readies itself for cell division (Fogg and Thake 1975).

Cyanobacteria have a number of features, such as the large size of colonies and the production of secondary metabolites, which are thought to deter grazers (Lampert 1987). But although cyanobacteria may not be an ideal food source for zooplankton, zooplankton populations can increase when fed a diet consisting solely of cyanobacteria and the responses of zooplankton to cyanobacteria are highly variable within species (Chapter 1, Tillmanns et al. 2008). This genetic diversity of zooplankton populations provides the basis for adaptation to cyanobacteria (Hairston et al. 2001; Sarnelle and Wilson 2005; Gustafsson et al. 2005). Individuals may adapt within one lifespan (Ghadouani and Pinel-Alloul 2002; Sarnelle and Wilson 2005) and then pass this on to subsequent generations (Gustafsson et al. 2005) or adaptation may occur over a longer period (Hairston et al. 2001; Chapter 2). Adaptation of a zooplankton population to cyanobacteria may cause a lag between changes in zooplankton population growth and cyanobacterial populations.

Competition is expected to be strongest among species with strongly overlapping resource use (Hardin 1960). Bloom-forming cyanobacteria share similar physiological adaptations and therefore competition among cyanobacteria should be apparent. Competition may explain why blooms consist of one or a small number of species when multiple cyanobacterial species were present and why summer assemblages typically have low diversity (Lindenschmidt and Chorus 1998). Competitive exclusion under different light and nitrogen regimes has been documented for cyanobacterial species *in vitro* (De Nobel et al. 1998; Huisman et al. 1999a). Competition may also be mediated by allelopathy (Keating 1977; Keating 1978; Gross 2003). Succession occurs among phytoplankton groups over the course of a season (Lindenschmidt and Chorus 1998; Reynolds et al. 2002), but little is known about the strength of competition among cyanobacterial species.

The importance of top down (grazer mediated) vs. bottom-up (abiotic conditions) factors on the growth of cyanobacteria and phytoplankton in general has been long debated in the literature (Power 1992; Gliwicz 2002). Most studies have focussed on abiotic controls of cyanobacteria due to the collection of evidence that found zooplankton, especially cladocerans, to be inhibited by the presence of colonial

cyanobacteria (DeMott et al. 2001; Ghadouani et al. 2003). However, in some situations top down control of cyanobacteria has proved effective (Mehner et al. 2002) and a recent paper has shown that *Daphnia* are capable of invading an established population of *Microcystis* (Sarnelle 2007). Most studies recognize that both processes are important but the relative importance of each for describing the variability in cyanobacterial population dynamics has not been resolved (Elser 1999; Gliwicz 2002).

As highlighted above, a great deal is known about the conditions that favour cyanobacterial population growth but still the factors that lead to a bloom, especially within a mesotrophic lake, remain elusive (Downing et al. 2001). If during bloom years, a set of ideal conditions lead to a bloom, it is reasonable to surmise that during non-bloom years, some of these factors must be limiting cyanobacterial growth. Time lags between abiotic and biotic factors and cyanobacteria growth rates may lead to complex dynamics which periodically lead to bloom events. In this paper, population dynamics of cyanobacterial species sampled *in situ* over two years, were analysed using time-series methods to determine if cyanobacterial population growth 1) is regulated by abiotic and/or biotic factors during non-bloom years; 2) is influenced by lagged interactions and 3) regulated more by biotic or abiotic factors.

5.3 Materials and Methods

5.3.1 Sample Collection and Analysis

Samples were collected from Constance Lake, a mesotrophic lake in the west end of Ottawa, every three days between 4 June and 7 November in 2003 and between 22 April and 28 October in 2004. In 2005, samples were collected bi-weekly between 20 May and 12 October. For each sampling date, water was collected by integrated tube sampler to a depth of 2 m for water chemistry, chlorophyll, colonial cyanobacteria, and rotifers. Macrozooplankton samples were collected by vertical net haul from 2 m with a conical zooplankton net (diameter = 19.8 cm, mesh size = 64 μm) and preserved immediately with 40% formalin buffered with sucrose (Haney and Hall 1973). A

Hydrolab multisensor probe was used to record water temperature and pH every 0.25 m. In the lab, 2 L of water collected by integrated tube sample was screened through a 35μm Nitex mesh and preserved in formalin for later enumeration of rotifers and colonial cyanobacteria. Water chemistry analyses were conducted by City of Ottawa laboratory (Ontario Ministry of the Environment 1983). Further details on laboratory methods are given in Chapter 2 and 4.

Cyanobacteria and rotifer density was estimated using a Wild M40 inverted microscope at 150X and 300X magnification. After inverting collection vials at least 10 times, sub-samples of between 0.05 and 0.4 mL (corresponding to 5 to 35 mL) were pipetted from the vial directly to an Utermöhl counting chamber. A couple of drops of diluted (10%) Lugol's iodine solution was added to help identify the cyanobacteria and the chamber was left to settle for at least two hours. The entire chamber was counted. Cyanobacteria colonies >35 μm were identified to species using the following taxonomic references: Komárek and Anagnostidis 1998; Komárek 1999; Komárek 2003; Komárek et al. 2003; Li et al. 2000; Stücken and Wiedner 2008. For each sample a minimum of 23 individuals of each common cyanobacterial species was counted. This has an associated error of ±30% (Laslett et al. 1997) and was deemed an acceptable trade off given the large number of samples that needed processing. Methods for enumerating macro-zooplankton were explained in Chapter 4. Only colonial cyanobacterial species found in greater than 50% of all samples (2003 - 2005) and zooplankton genera in greater than 30% of samples were reported in this paper.

5.3.2 Statistical Analysis

Time-series analysis was conducted on growth rates of cyanobacteria and zooplankton calculated using the equation (Gotelli 1998):

$$r = \frac{\ln N_t - \ln N_0}{t} \qquad \text{Eq. 5.1}$$

where N is the density of cyanobacteria cells, zooplankton individuals or concentration of chlorophyll and t is time in days. Environmental factors were log+1 transformed to

adjust for normality and first order differenced. As growth rates are the population series that are the first order difference of ln transformed series divided by a constant, both of these calculations resulted in creating a stationary data series.

A first examination of the calculated growth rate series revealed large fluctuations due to the highly variable nature of the population series and zero values. Both of these events are likely a consequence of random sampling error rather than actual fluctuations in growth rates. Sampling error is introduced through algal counts, estimated at ± 30% (Laslett et al. 1997), and also by the occasional patchy distribution of cyanobacteria and zooplankton within a lake (Verhagen 1994). To minimize this sampling error, population growth rates were calculated from population density series that were first smoothed using a moving average filter over a three-sample window (Chatfield 2004). Remaining zeros were then filled with a random number between 0 and half the minimum value of the complete series (all years combined).

Before correlations between variables could be identified, data series were first pre-whitened to remove autocorrelation (Chatfield 2004). This was accomplished by fitting an autoregressive model to each growth rate and differenced environmental series, where autocorrelation was identified from correlograms, and using the residuals from this model in all subsequent analyses (Chatfield 2004). Next, cross-correlations were calculated among cyanobacteria genera (*Anabaena, Microcystis, Woronichinia,* and *Aphanizomenon*) as well as among cyanobacteria species (*Anabaena flos-aquae, A. lemmermanii, Microcystis aeruginosa, M. wesenbergii, Aphanizomenon flos-aquae* and *Woronichinia sp.*) and with each of these taxa and zooplankton population growth rates and changes in environmental conditions. In the case of *Woronichinia* and *Aphanizomenon,* these genera are represented by only one species and were tested for correlations with both cyanobacterial species and genera.

One of the problems with conducting multiple correlation tests is the inflated probability of detecting significant results purely by chance. The most common practice to correct for this is the Bonferonni correction where the significance threshold is reduced by dividing the alpha value (0.05) by the total number of correlation tests (Zar 1999).

The likelihood of detecting correlations in pre-whitened data series is already reduced and reducing the significance level further would make a type II error likely (Nakagawa 2004). Rather than correct the alpha value, the total number of correlation tests was limited by 1) reducing the number of taxa used in the analysis; 2) limiting the number of time lags considered and 3) omitting tests for delayed relationships (t+z). Delayed correlations with zooplankton may occur if a period of time is necessary for zooplankton to increase in population after grazing upon cyanobacteria. Delayed relationships, however, will not aid in the development of predictive models and will increase substantially the number of correlation tests conducted. Cyanobacteria taxa were limited to include all colonial and potentially toxic bloom-forming genera and the two *Microcystis* and *Anabaena* species with the highest densities (*Anabaena flos-aquae, A. lemmermanii, Microcystis aeruginosa, M. wesenbergii*). Calanoid copepods, *Ceriodaphnia* and *Diaphanosoma* were eliminated from time-series analysis due to low densities. For cross-correlations among cyanobacteria species, a time lag of 3 steps or 9 days was considered. This would allow for about three generations of a given cyanobacterial taxa to respond to changes in the growth rates of a second taxa (Reynolds 1984b). Similarly, correlations with environment variables were limited to 9 days. Correlations with rotifers were limited to 12 days, *Bosmina* to 21 days and *Daphnia* and cyclopoids to 27 days. In each case this would cover approximately three generations of each zooplankton taxa (Gillooly 2000).

To determine the total amount of variation explained in cyanobacterial population growth rates by abiotic and biotic variables, all variables that were significantly cross-correlated were used in a linear regression. Two models were considered – the first one using only variables that were instantaneously correlated (t_0) and the second one using all lagged correlated variables (t_0+t_{-z}). Models were compared using adjusted R^2 (R^2_{adj}), which takes into account the number of independent variables used in the model (Zar 1999). A commonality analysis was conducted to partition the variance explained (R^2) in the lagged regression models (t_0+t_{-z}) into abiotic and biotic components (Seibold and McPhee 1979). This was done by first partitioning the variance into unique and common components for all variables and then adding together the components that could be solely attributed to abiotic variables, other cyanobacteria, zooplankton or the combined

variation of biotic variables (other cyanobacterial taxa and zooplankton). Commonality analysis was conducted using the package developed for R by (Nimon et al. 2008). Time-series analysis and linear regressions were conducted in SYSTAT vs. 11.

5.4 Results

Ten colonial cyanobacterial species were found in greater than 50% of the samples collected (Table 5.1, Appendix B). *Microcystis* reached higher density in 2003 compared with 2004 (Figure 5.1), due largely to the prevalence of *M. wesenbergii* which reached the highest density of any colonial cyanobacterial species in 2003 with a density of 1.9×10^6 cells L^{-1}. *M. aeruginosa* also reached its maximum density in 2003 (Figure 5.2, Table 5.1). *Anabaena* density was higher in 2003 compared with 2004 (Figure 5.1). In 2003, *A. crassa* and *A. viguieri* contributed more to overall *Anabaena* density than in 2004 but both species contributed less in both years relative to *A. flos-aquae* and *A. lemmermanii* (Figure 5.3). Other commonly found species were *Aphanizomenon flos-aquae* which reached highest densities in the fall of 2004; *Cylindrospermopsis raciborskii* which although present in a large number of samples never reached a very high density (maximum 5.8×10^4 cells L^{-1}); and *Woronichinia* sp. that had high densities in both years in the fall (Table 5.1, Figure 5.4).

Five genera of cladocerans were found in more than 30% of samples: *Bosmina* (72% of all samples), *Ceriodaphnia* (54%), *Daphnia* (48%), *Diaphanosoma* (45%) and *Chydorus* (31%). *Chydorus,* however, was not included in the analyses as it is a predatory species. Within each year, cladoceran dynamics were highly variable (Figure 5.5) with coefficients of variation reaching as high as 322% for *Daphnia* in 2003 (Table 5.1). Generally, the cladoceran genera showed defined periods of population increase and decline within each year. *Bosmina* increased only for a short period in the fall of 2003 and again in the spring of 2004; *Ceriodaphnia* reached maximum densities in late summer in 2003 and 2004; *Daphnia* reached highest densities in the spring of both years; and *Diaphanosoma* increased in late summer in both years though it reached higher maximums in 2003 (Figure 5.5).

Both calanoid and cyclopoid copepods were found in Contance Lake. Cyclopoids and nauplii were found in every sample where calanoid copepods were rarely found in 2003 (Table 5.1, Figure 5.6). Cyclopoids reached highest density in early spring of 2004 (82 individuals L^{-1}) but by June fell to below 20 indivduals L^{-1} where densities remained for the rest of the year. In 2003, cyclopoids never reached more than 31 individuals L^{-1}. In both years, nauplii dynamics were similar to the cyclopoids (Figure 5.6).

Four rotifer genera were commonly found in Constance Lake: *Asplanchna* (55% of samples), *Keratella* (98%), *Polyartha* (99%), and *Trichocera* (77%). *Polyartha* reached the highest densities of any of these genera in spring of 2003 (4,679 individuals L^{-1}; Table 5.1; Figure 5.7). Except for *Asplanchna* in 2004, each genus underwent a rapid increase in density between June and mid-July in each year and *Keratella* and *Polyartha* showed a slight increase again in late fall. *Trichocera* only peaked in the spring of each year and in 2004, *Asplanchna* only increased in the fall (Figure 5.7).

Of the fifteen cross-correlations detected among growth rates of cyanobacterial taxa, only two were negative (Table 5.2). *Woronichinia* was negatively correlated with both *A. flos-aquae* and *M. aeruginosa* at t_{-3} and t_{-6} respectively. *Woronichinia,* however, was positively correlated with *Microcystis* at t_{-3}. And *Microcystis* was positively correlated with *Woronichinia* at t_{-6}. The strongest correlation was between *Woronichinia* and *Anabaena* at t_{-6} ($r = 0.65$; $P < 0.001$), followed by an instantaneous correlation between *M. aeruginosa* and *M. wesenbergii* ($r = 0.33$; $P < 0.001$).

A number of correlations were detected between cyanobacterial species and zooplankton genera and again the majority of these relationships (15 out of 19) were positive (Table 5.3). The only negative relationships were between Cyclopoids and *Anabaena, A. flos-aquae* and *A. lemmermanii* at t_0, t_0 and t_{-6} respectfully, and between *Keratella* and *Aphanizomenon flos-aquae* at t_{-3}. Cyclopoids also had a positive relationship with *Woronichinia* at t_0. Only one relationship was detected with cladocerans: *Daphnia* were positively correlated with *Anabaena flos-aquae* at t_{-12}. Fourteen of the correlations detected were with rotifers and nine of those were with *Trichocera.*

Nutrients, turbidity and average water column temperature all showed correlations with cyanobacterial growth rates (Table 5.3). Ammonium and TP both were negatively correlated with total cyanobacterial biomass at t_{-3} and ammonium was also negatively correlated with the growth rate of *M. aeruginosa* at t_{-3}. Total nitrogen was negatively correlated with the growth rates of *Anabaena* and *A. flos-aquae* at t_{-3} and t_0 respectively. Although TP was negatively related to total cyanobacterial biomass, it was positively related to growth rates of *Microcystis, M. aeruginosa, Woronichinia sp.* and *Aphanizomenon flos-aquae* at lags up to three days. Turbidity was positively correlated with *Microcystis* and *Microcystis aeruginosa* at t_0 but was negatively correlated with *A. flos-aquae* at t_{-3}. *Microcystis, M. aeruginosa* and *M. wesenbergii* were all correlated with temperature at t_{-3}, t_{-3} and t_{-6}.

Lagged linear regression models for each cyanobacterial population growth rate against cross-correlated variables revealed highly significant models in all cases (Table 5.4). Two sets of models were run, the first using only variables that were instantaneously correlated (t_0) and the second using all variables (t_0+t_{-z}). The amount of variation (R^2) explained by the first set of models ranged from 0.174 for *M. aeruginosa* to 0.095 for *Woronichinia* and between 0.330 for *Woronichinia* to 0.106 for *Aphanizomenon flos-aquae* for the second set of models. For all cyanobacterial taxa, the addition of lagged variables increased the amount of variation explained by the model. In the cases of *A. flos-aquae* and *Woronichinia,* the amount of explained variation was double.

Biotic variables were responsible for most of the explained variation in each lagged regression model but abiotic variables explained about an equal amount of the variation for *Microcystis* and *Aphanizomenon flos-aquae* (Table 5.5). For *M. aeruginosa, M. wesenbergii* and *Woronichinia,* the associations with other cyanobacterial taxa were responsible for more variation than zooplankton. For *Anabaena, Microcystis, A. flos-aquae, A. lemmermanii,* and *Aphanizomenon flos-aquae,* the opposite was true. All variables explaining the most variation were biotic except for turbidity in the case of *A. flos-aquae.*

5.5 Discussion

A number of common bloom-forming and toxic cyanobacterial species were present in Constance Lake between 2003 and 2005, but their biomass never reached bloom levels. In this study, cyanobacterial population dynamics were analyzed against environmental factors to identify limits of cyanobacterial growth. Limits to growth were expected as positive correlations between cyanobacterial growth rates and abiotic factors or negative correlations with zooplankton and/or between cyanobacterial genera. As expected, most correlations with abiotic variables were positive with the exception of correlation with ammonium and total nitrogen. Surprisingly, most correlations with zooplankton and other cyanobacterial taxa were also positive. A time lag occurred in most cyanobacterial populations before responding to changes in abiotic conditions, zooplankton or other cyanobacterial growth rates. When lagged factors were included in regression models the amount of variation explained doubled in some cases (Table 5.4). For all cyanobacterial taxa except *A. flos-aquae*, biotic variables, either other cyanobacteria or zooplankton taxa, explained more variation in population growth rate dynamics than abiotic variables (Table 5.5). This suggests that more emphasis needs to be placed on understanding the interactions of populations within multi-species communities.

Competition among cyanobacteria within the same genus was expected as these species are the most similar and therefore have the highest degree of resource overlap (Hardin 1960). Surprisingly, there was no evidence of competition among species within the same genus and all but two of the correlations detected among cyanobacterial taxa were positive (Table 5.2). Both the two most abundant *Anabaena* species (*A. flos-aquae* and *A. lemmermanii*) and the two most abundant *Microcystis* species (*M. aeruginosa* and *M. wesenbergii)* were positively correlated. As an answer to his original "Paradox of the Plankton", Hutchinson (1961) suggests that multiple species may exist when equilibrium conditions are not met. This is likely in the shallow, polymictic lake studied and positive correlations among similar species may then be due to species responding to similar environmental conditions. The correlations detected were based on three day intervals, however, and at this short time scale, even species within the same genus have different

correlations with environmental conditions (Table 5.3). Alternatively, these taxa may be actively facilitating the growth of other cyanobacterial taxa. For example, facilitation between cyanobacteria species has been documented when light and nitrogen are limited (Agawin et al. 2007).

A number of species pairs showed correlations at two time lags. For example, positive correlations between species occurred in series when the first species (e.g. *A. flos-aquae* or *A. lemmermanii)* positively affected a second species (*A. lemmermanii* or *M. wesenbergii* respectively) at t-3, and then was also instantaneously correlated with the second species. This pattern was also present between *Anabaena* and *Woronichinia* at t-6. One explanation is that the first species facilitates the growth of a second species which then benefits from the growth of this species. This may occur to aid in the protection from grazers. For example, if *A. lemmermanii* produced a macromolecule to defend against cyclopoids, then it may be in the best interest of *A. flos-aquae* to facilitate the growth of *A. lemmermanii* to gain protection. Zooplankton can also facilitate growth by recycling nutrients (Wetzel 2001). For example, *Trichocera* was positively correlated with the growth of *A. lemmermannii* and *M. wesenbergii* (Table 5.3). If *A. lemmermanii* facilitates the growth of *M. wesenbergii, A. lemmermanii* would also gain an advantage through the presence of more *Trichocera.*

Only one correlation was detected between the growth rate of a cyanobacteria taxon and a cladoceran genus: *A. flos-aquae* and *Daphnia* at t_{-12} days. Though *Bosmina* were generally more numerous than *Daphnia,* they had no influence on cyanobacterial populations. van Gremberghe et al. (2008) found that the cladoceran community was important in structuring cyanobacteria community dynamics in a mesotrophic lake. Community composition, however, was determined by denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA gene fragments and therefore represents the genotype community rather than the morphotype community composition (van Gremberghe et al. 2008). Cladocerans may have influenced the genotype composition of cyanobacterial populations but these changes were not detected in the changes of morpho-species population growth rates. The low density of cladocerans in Constance Lake, likely due to the presence of planktivorous fish which are known to feed on larger

cladocerans (Lynch and Shapiro 1981), may not have been adequate to reduce cyanobacterial biomass (Sarnelle 2007).

In contrast to the cladocerans, there were a greater number of correlations with rotifers (Table 5.3). In particular, all cyanobacteria taxa were positively correlated with *Trichocera* and the only negative correlation was between *Anabaena* and *Polyartha* at t-3 days. Increases in colonial cyanobacterial biomass have been associated with decreases in large cladocerans and increases in smaller cladocerans, rotifers and copepods (Edmondson and Litt 1982; Orcutt and Pace 1984; Hansson et al. 2007). These smaller species are thought to be less susceptible to cyanobacteria (Gilbert 1990) compared to larger cladocerans which are considered to be more susceptible to feeding interference and/or toxicity from cyanobacteria (Porter and Orcutt 1980a; Gliwicz and Lampert 1990; Hansson et al. 2007). However, the correlations at a fine temporal scale suggests that there may be a direct relationship between rotifers and cyanobacteria rather than the proliferation of rotifers due a release from competition by larger cladocerans (Sartonov 1995). One possibility is that the relationship between rotifers and cyanobacteria was mediated by bacteria. Bacteria can influence growth rates of phytoplankton by influencing nutrient availability (Danger et al. 2007) or by allelopathically inhibiting algal growth (Fukami et al. 1997) or causing cell lysis (Manage et al. 2000). Rotifer populations may benefit from the bacteria associated with cyanobacteria (Ooms-Wilms 1997) and in doing so promote the growth of cyanobacteria by reducing the inhibitory effects of bacteria.

Very little work has been done on the interaction between copepods and cyanobacteria in part because copepods are able to select their food and therefore are thought to avoid cyanobacteria (Kerfoot and Kirk 1991). DeMott and Moxter (1991) demonstrated that the calanoid copepod, *Diaptomus birgei,* does actively select non-toxic strains of cyanobacteria. There was a low density of calanoid copepods in Constance Lake in 2003 and 2004 (maximum density was 4 individuals L^{-1}) but cyclopoid copepods reached densities of 82 individuals L^{-1} in 2004 (Table 5.1). Adult Cyclopoid copepods were negatively correlated with *Anabaena* and *A. lemmermanii* at t_{-6} days and instantaneously negatively correlated with *A. flos-aquae.* Cyclopoid copepods can be

herbivores, predators or omnivores (Kumar and Rao 1999; Wetzel 2001) but since but since this class was not identified to genus it is not possible to know whether the cyclopoids were herbivorous actively decreasing *Anabaena* biomass, or predator cyclopoids which may have been impacting *Anabaena* through a secondary path with microzooplankton.

Compared with the biotic interactions, abiotic variables were less important in explaining the variation in cyanobacterial population growth dynamics (Table 5.5). These same abiotic variables are commonly measured and have been the focus of numerous past studies on cyanobacteria (Jacoby et al. 2000; Welker et al. 2003; Downing et al. 2001). Total phosphorus and turbidity were positively instantaneously correlated with *Microcystis* and *M. aeruginosa.* TP and turbidity were in themselves highly correlated so whether the low light conditions or high turbidity were specifically favourable cannot be discerned. However, both high TP and low light, in this case caused by high turbidity, has been shown to positively influence *Microcystis* (Huisman et al. 1999a; Nalewajko and Murphy 2001). Also, temperature at lags of 3 and 6 days were correlated with *Microcystis* population growth rates. Maximum photosynthetic capacity of *Microcystis* is known to occur under high temperatures (Robarts and Zohary 1987) and as light was never limiting, the lagged correlation with temperature is likely the time needed to convert new energy into growth.

In temperate lakes, nitrogen can be as limiting as phosphorus on phytoplankton growth (Elser et al. 1990). Ammonium is the most easily assimilated form of nitrogen but the results show a negative correlation with total cyanobacterial biomass and with *M. aeruginosa* at lags of 3 days. Yoshida et al. (2007) found that the green alga, *Scenedesmus quadricauda* outgrew and almost excluded *Microcystis novacekii* under conditions of pulsed ammonium supply compared to a low continuous ammonium supply in which *M. novacekii* suppressed *S. quadricauda.* In 2003 and 2004, the variability in ammonium was higher than other abiotic variables (Chapter 4) suggesting that conditions were more analogous to a pulsed rather than continuous supply of ammonium. *Anabaena* and *A. flos-aquae* were negatively correlated with total nitrogen (at t_{-3} and t_0 respectively). *Anabaena* species have the ability to fix atmospheric nitrogen (Komárek et

al. 2003) so this negative correlation might suggest an advantage for *Anabaena* when total nitrogen was low. Nitrogen fixation requires higher light conditions than nitrogen uptake (Agawin et al. 2007), which could explain the negative correlation with turbidity at t_{-3} (Table 5.3).

Population fluctuations are thought to arise by a combination of short and long term abiotic influences (weather vs. climate forcing) and biotic processes which consist of intrinsic processes within a species and interactions among individuals of different species (Bjørnstad and Grenfell 2001). Plankton community composition is reset each spring and yearly variations within one lake will largely be due to community dynamics and changing weather. In some studies, weather conditions are concluded as the main cause of promoting or disrupting cyanobacterial blooms (e.g. Jacoby et al. 2000). Less well studied are community processes and the resultant influence of population dynamics. Biological communities are known to respond to linear changes in environmental conditions in a non-linear manner (Hsieh and Ohman 2006). Time lags in the response of a species to changing conditions have been mathematically shown to causes non-linearities in population dynamics but the specific factors that cause these lags remain to be discovered (Turchin 1990; Bjørnstad and Grenfell 2001). Understanding inter-specific interactions as they occur in natural ecosystems requires *in situ* observations as these interactions are in part a result of the history of the system (Landis et al. 2000). By analysing two years of high frequency multi-species data from Constance Lake, some highly significant inter-specific interactions were identified that occurred both with and without time lags. Biotic relationships explained the majority of variation in cyanobacterial population dynamics.

5.6 Tables

Table 5.1 Descriptive statistics for cyanobacterial taxa, zooplankton genera and chlorophyll sampled in Constance Lake over three years (2003, n=53; 2004, n=64; 2005, n=10).

	2003	2004	2005
Food web component	mean/range/%CV	mean/range/%CV	mean/range/%CV
Total cyanobacteria biomass ($\mu g\ L^{-1}$)	54/3 - 200/88	35/0 - 114/72	46/1 - 153/103
Cyanobacteria taxa (10^3 cells L^{-1})			
Anabaena	254/0 - 786/80	385/0 - 1789/90	405/1 - 1596/120
Microcystis	670/0 - 2586/91	343/0 - 1252/90	313/14 - 1353/139
Anabaena flos-aquae	90/0 - 534/148	277/0 - 1108/95	125/0 - 470/109
A. lemmermannii	66/0 - 470/129	61/0 - 823/225	90/0 - 428/158
Anabaena crassa	58/0 - 321/159	33/0 - 175/134	81/0 - 461/173
Anabaena viguieri	31/0 - 425/228	11/0 - 96/175	101/0 - 753/235
Microcystis aeruginosa	316/13 - 1211/80	266/0 - 751/84	180/10 - 630/117
Microcystis flos-aquae	32/0 - 238/174	36/0 - 621/233	47/0 - 308/210
Microcystis wesenbergii	326/0 - 1934/132	42/0 - 233/124	86/4 - 547/201
Woronichinia sp	97/0 - 619/136	63/0 - 515/191	78/0 - 272/151
Aphanizomenon flos-aquae	6/0 - 17/74	34/0 - 264/179	84/0 - 198/88
Cylindrospermopsis raciborskii	17/0 - 58/79	3/0 - 30/220	15/0 - 57/117
Zooplankton genus (individuals L^{-1})			
Bosmina	2/0 - 25/211	3/0 - 41/232	9/0 - 33/121
Ceriodaphnia	2/0 - 29/237	1/0 - 6/141	4/0 - 17/153
Daphnia	3/0 - 64/322	2/0 - 32/291	8/0 - 71/291
Diaphanosoma	1/0 - 10/168	0/0 - 3/199	2/0 - 10/191
Calanoids	1/0 - 4/116	0/0 - 3/462	0/0 - 1/172
Cyclopoids	9/2 - 31/70	10/0 - 82/138	15/1 - 60/123
Nauplii	15/2 - 55/64	18/3 - 70/73	20/4 - 40/68
Asplanchna	106/0 - 720/160	168/0 - 1225/172	129/0 - 641/201
Keratella	711/138 - 1686/60	769/0 - 3080/85	940/150 - 4000/124
Polyartha	995/143 - 4679/78	902/0 - 2881/79	884/75 - 2425/80
Trichocera	165/0 - 1133/129	112/0 - 1066/148	109/0 - 488/128

Table 5.2 Lagged and instantaneous correlations between cyanobacterial population growth rates in Constance Lake during 2003 & 2004. The taxa listed in column A is shifted the given number of days (lag) from the taxa listed in column B (A_{t-z} •B).

A - Lagged Taxa	B - Taxa	Lag	r	*p*
Anabaena	*Microcystis*	-9	0.27	0.018
Anabaena	*Woronichinia*	0	0.31	0.001
Anabaena	*Woronichinia*	-6	0.65	<0.001
Microcystis	*Woronichinia*	-6	0.25	0.007
Woronichinia	*Microcystis*	-3	0.21	0.025
Woronichinia	*M. aeruginosa*	-3	0.21	0.027
Woronichinia	*M. aeruginosa*	-6	-0.24	0.013
Woronichinia	*A. flos-aquae*	-3	-0.23	0.014
A. flos-aquae	*A. lemmermanii*	0	0.27	0.005
A. flos-aquae	*A. lemmermanii*	-3	0.23	0.015
A. flos-aquae	*M. aeruginosa*	-6	0.24	0.012
M. wesenbergii	*M. aeruginosa*	0	0.33	<0.001
A. lemmermanii	*Woronichinia*	-3	0.31	0.001
A. lemmermanii	*M. wesenbergii*	0	0.19	0.044
A. lemmermanii	*M. wesenbergii*	-3	0.31	0.001

Table 5.3 Cross correlations between selected cyanobacteria and zooplankton population growth rates and changes in environmental conditions. The number in parenthesis indicates the number of days that zooplankton or environmental factors are lagged relative to cyanobacteria.

	Total Cyanobac. Biomass	*Anabaena*	*Microcystis*	*Anabaena flos-aquae*	*Anabaena lemmermanii*	*Microcystis aeruginosa*	*Microcystis wesenbergii*	*Woronichinia sp.*	*Aphanizomenon flos-aquae*
Zooplankton									
Bosmina	ns	ns	ns	ns	ns	ns	ns	ns	ns
Daphnia	ns	ns	ns	0.21(12)*	ns	ns	ns	ns	ns
Cyclopoid	ns	-0.21(6)*	ns	-0.22(0)*	-0.29(6) †	ns	ns	0.29(0) †	ns
Asplanchna	ns	0.40(9)†	ns	ns	0.20(9)*	ns	ns	ns	ns
Keratella	ns	ns	ns	0.23(12)*	ns	ns	ns	ns	-0.24(3)*
Polyartha	ns	0.35(9)‡	ns	ns	ns	ns	ns	ns	ns
Trichocera	0.26(0)†	0.26(0)†	0.28(0) ‡	0.22(12)*	0.46(0) ‡	0.26(0) †	0.30(0) † 0.33(3) ‡	0.23(6)*	ns
Environmental Factors									
NH_4	-0.23(3)*	ns	ns	ns	ns	-0.22(3)*	ns	ns	ns
RP	ns	ns	ns	ns	ns	ns	ns	ns	ns
TN	ns	-0.27(3)†	ns	-0.20(0)*	ns	ns	ns	ns	ns
TP	-0.22(3)*	ns	0.29(0)†	ns	ns	0.19(0)*	ns	0.27(6)†	0.23(3)*
Turbidity	ns	ns	0.28(0) †	-0.21(3) *	ns	0.28(0) †	ns	ns	ns
Light Ext.	ns	ns	ns	ns	ns	0.23(0)*	ns	ns	ns
Temp	ns	ns	0.22(3)*	ns	ns	0.29(3) †	0.20(6) *	ns	ns
pH	ns	ns	ns	ns	ns	ns	ns	ns	ns

*$P<0.05$; †$P<0.01$; ‡$P<0.001$

Table 5.4 Cyanobacteria population growth rates regressed against biotic and abiotic variables without time lags (t_0) and both instantaneous and lagged (t_0+t_{-z}) variables. If no subscript is present, variables were not lagged. ANA – *Anabaena,* MIC – *Microcystis,* AFLO – *A. flos-aquae,* ALEM – *A. lemmermanii,* MAER – *M. aeruginosa,* MWES – *M. wesenbergii,* WOR – *Woronichinia,* ASP – *Asplanchna,* DAPH – *Daphnia,* CYC – cyclopoids, KER – *Keratella,* POLY – *Polyartha,* TRI – *Trichocera,* TURB – turbididty, TEMP – average water column temperature, RP - reactive phosphorus, TP – total phosphorus, TN – total nitrogen, EXT – light extinction coefficient.

Taxa	Model	Independent Variables	R^2	R^2_{adj}	df	F	*p*
Cyanobacteria	t_0	TRI	0.106	0.098	1,113	13.40	<0.001
Biomass	t_0+t_{-z}	TRI -NH_{4t-3}-TP_{t-3}	0.139	0.124	2,110	8.90	<0.001
Anabaena	t_0	WOR+TRI	0.127	0.111	2,108	7.89	0.001
	t_0+t_{-z}	WOR+TRI+ASP_{t-9} +$POLY_{t-9}$	0.211	0.179	4,100	6.89	<0.001
Microcystis	t_0	TRI+TP+TURB	0.168	0.145	3,111	7.46	<0.001
	t_0+t_{-z}	TRI+TP+ANA_{t-9}+$TEMP_{t-3}$	0.236	0.207	4,104	8.03	<0.001
A. flos-aquae	t_0	ALEM-CYC -TN	0.159	0.136	3,111	7.00	<0.001
	t_0+t_{-z}	ALEM-CYC -TN+$DAPH_{t-12}$ +KER_{t-12}+TRI_{t-12} -$TURB_{t-3}$	0.325	0.277	7,99	6.81	<0.001
A. lemmermanii	t_0	AFLO+TRI	0.152	0.136	2,110	9.84	<0.001
	t_0+t_{-z}	AFLO+TRI+$AFLO_{t-3}$ -CYC_{t-6} +ASP_{t-9}	0.225	0.187	5,101	5.87	<0.001
M. aeruginosa	t_0	MWES+TRI +TURB	0.174	0.151	3,111	7.77	<0.001
	t_0+t_{-z}	MWES+EXT+$AFLO_{t-6}$+WOR_{t-3} -WOR_{t-6}+$TEMP_{t-3}$	0.322	0.283	6,104	8.23	<0.001
M. wesenbergii	t_0	MAER+TRI	0.163	0.145	2,112	10.67	<0.001
	t_0+t_{-z}	MAER+TRI+$ALEM_{t-3}$+$TEMP_{t-6}$	0.283	0.256	4,106	10.45	<0.001
Woronichinia	t_0	CYC	0.095	0.087	1,112	11.76	0.001
sp.	t_0+t_{-z}	CYC+ANA_{t-6}+TP_{t-6}	0.330	0.331	3,106	17.37	<0.001
Aphanizomenon	t_0[1]						
flos-aquae	t_0+t_{-z}	-KER_{t-3}+TP_{t-3}	0.106	0.090	2,110	6.52	0.002

[1] No variables were instantaneously correlated with *Aphanizomenon flos-aquae.*

Table 5.5 Percentage of total R^2 from complete regression models that is due to abiotic factors (a), other cyanobacteria taxa (c), and zooplankton (z), as well as both cyanobacteria and zooplankton together (c∩z). Also, listed are the two variables and their time lags, that explain the largest amount of variation within each model.

	Total	% R^2							
Taxa	R^2	a	c	z	c∩z	variable	% R^2	variable	% R^2
Cyanobacterial Biomass	0.139	29	0	54	54	TRI	54	NH_{4t-3}	38
Anabaena	0.247	0	41	50	100	WOR	41	POL_{t-9}	17
Microcystis	0.188	43	18	29	49	TRI	29	TP	23
A. flos aquae	0.308	30	10	46	68	$TURB_{t-3}$	18	$DAPH_{t-12}$	16
A. lemmermanii	0.339	0	18	70	100	ASP_{t-9}	24	TRI	21
M. aeruginosa	0.386	19	68	0	68	MWES	31	WOR_{t-6}	20
M.wesenbergii	0.322	11	45	26	85	$ALEM_{t-3}$	30	TRI	26
Woronichinia sp.	0.190	7	52	13	83	AN_{t-6}	52	CYCLO	13
Aphanizomenon flos-aquae	0.057	46	0	50	50	KER_{t-3}	50	TP_{t-3}	46

5.7 Figures

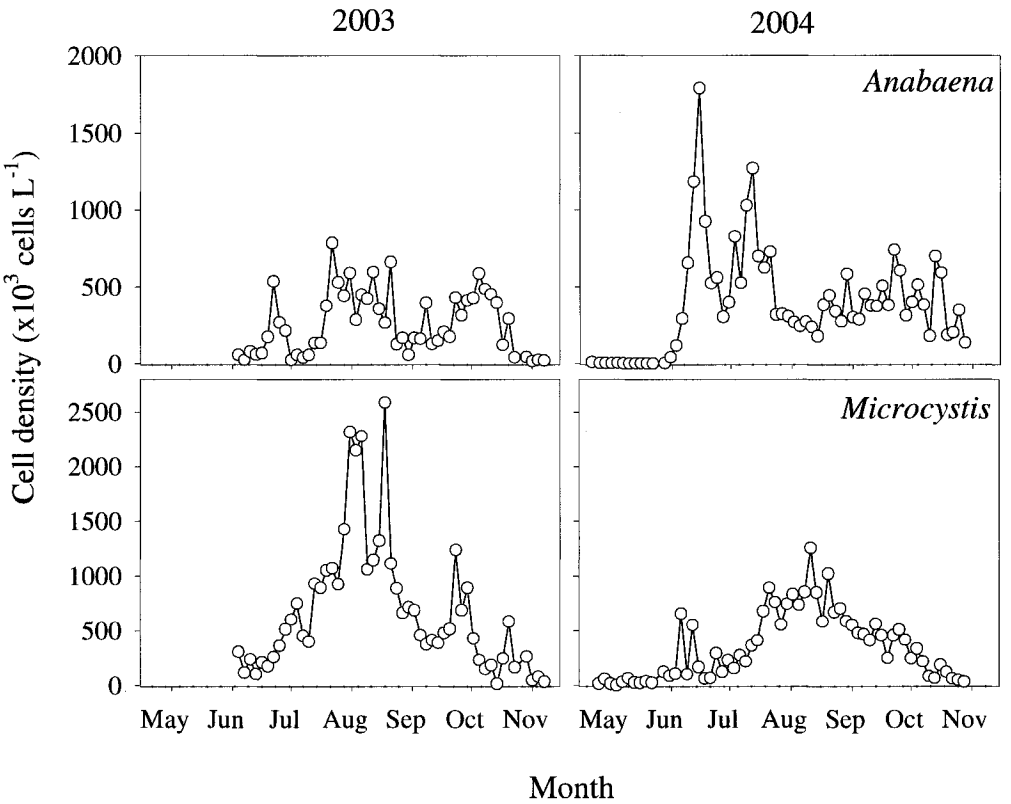


Figure 5.1 Population dynamics of the two most common genera of colonial cyanobacteria in Constance Lake, Canada. Note the different scales.

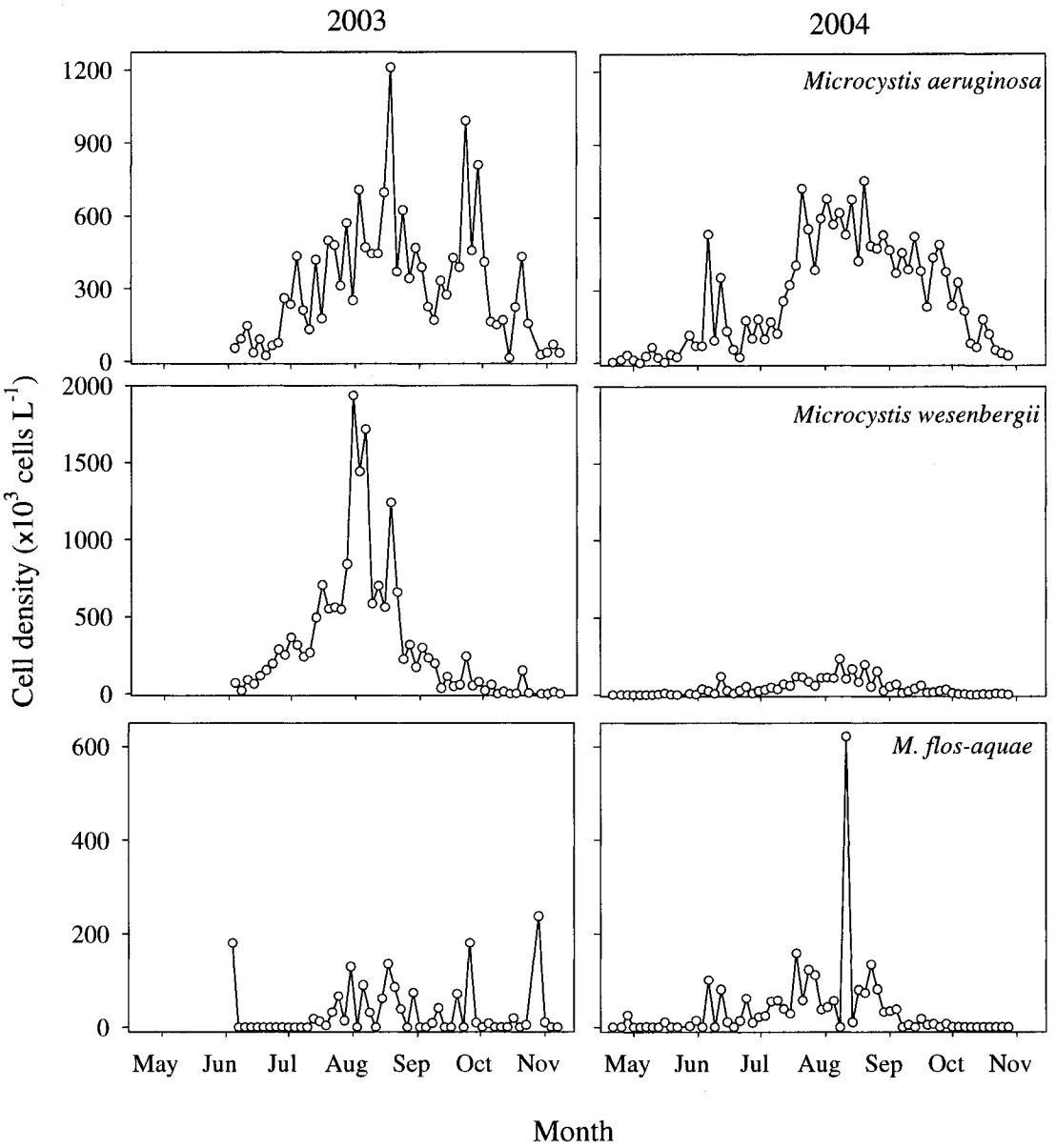


Figure 5.2 Population dynamics of the three most common species of *Microcystis* sampled in Constance Lake over two years. Note the different scales.

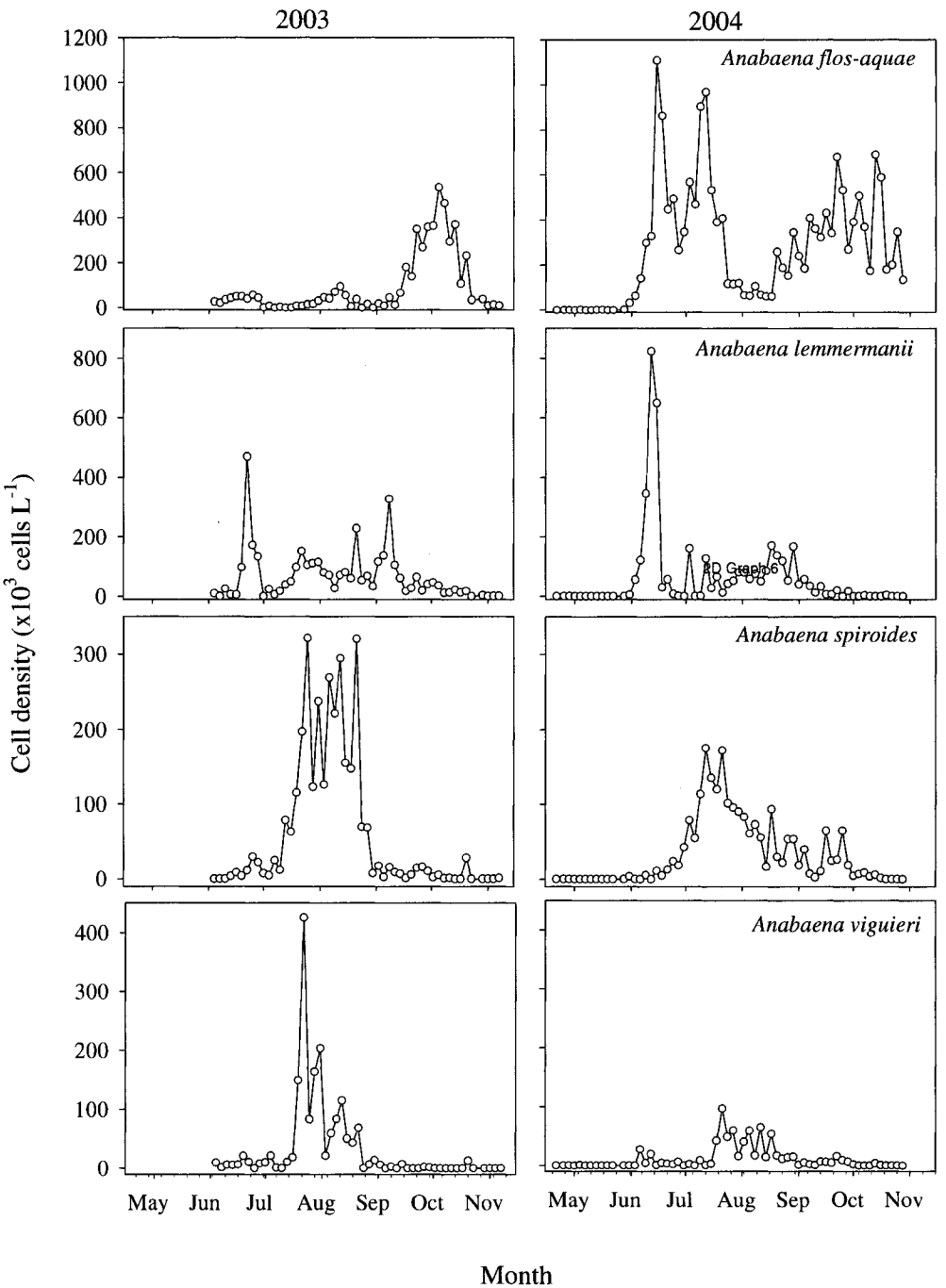


Figure 5.3 Population dynamics of the four most common *Anabaena* species sampled in Constance Lake over two years. Note the different scales.

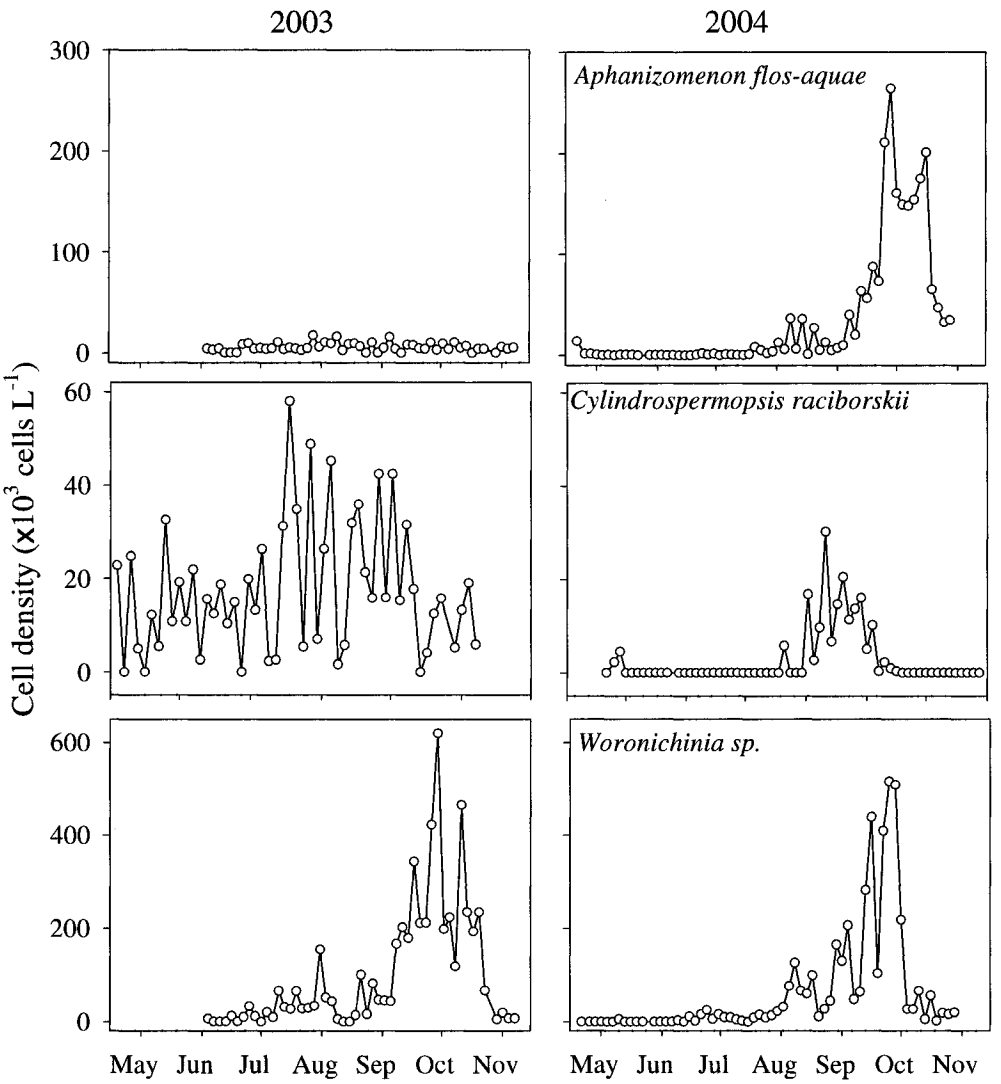


Figure 5.4 Population dynamics of *Aphanizomenon flos-aquae, Cylindrospermopsis raciborskii,* and *Woronichinia sp.* in Constance Lake over two years. Note the different scales.

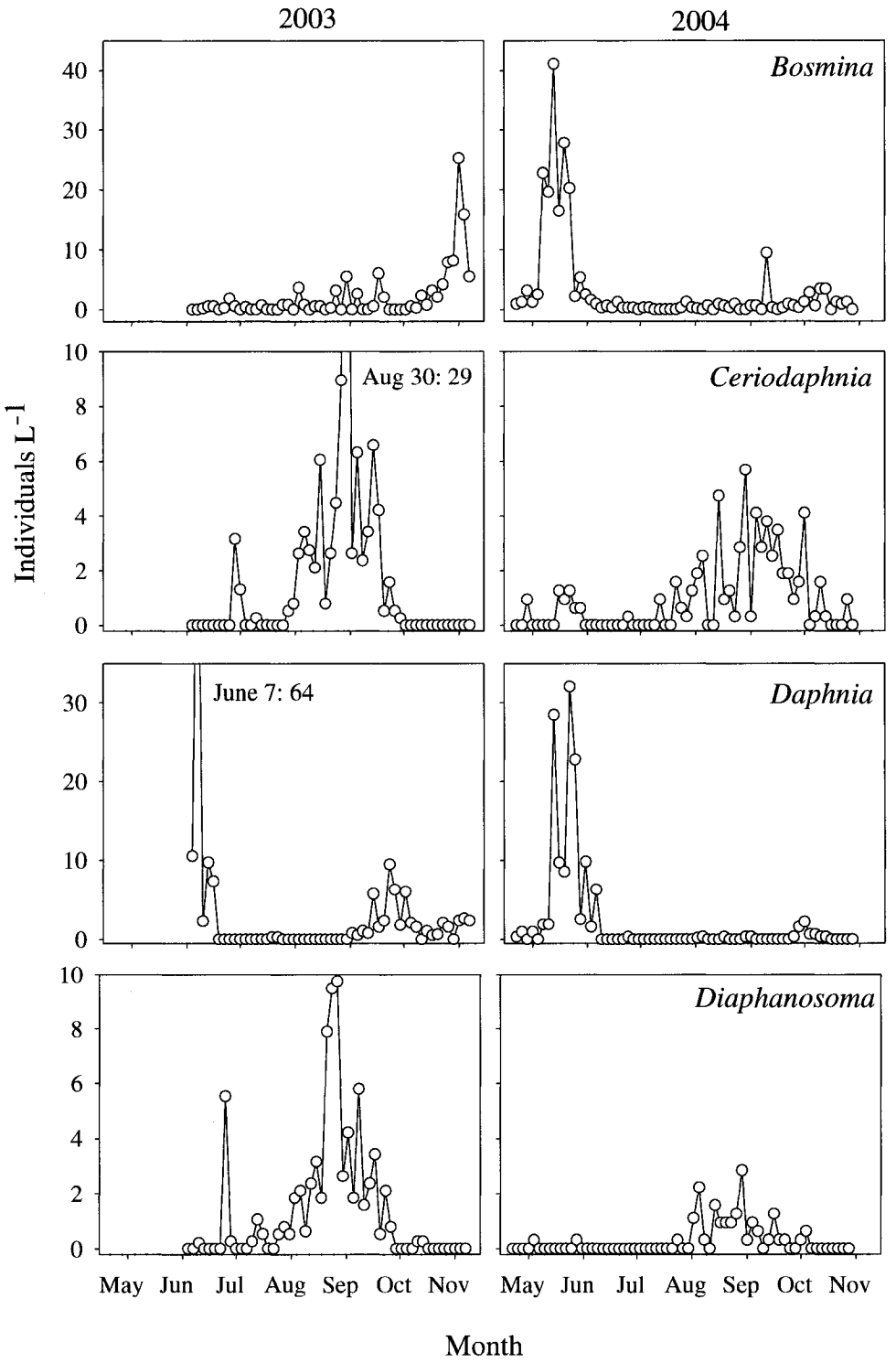


Figure 5.5 Population dynamics of the most common genera of cladocerans in Constance Lake, Canada. Note the different scales.

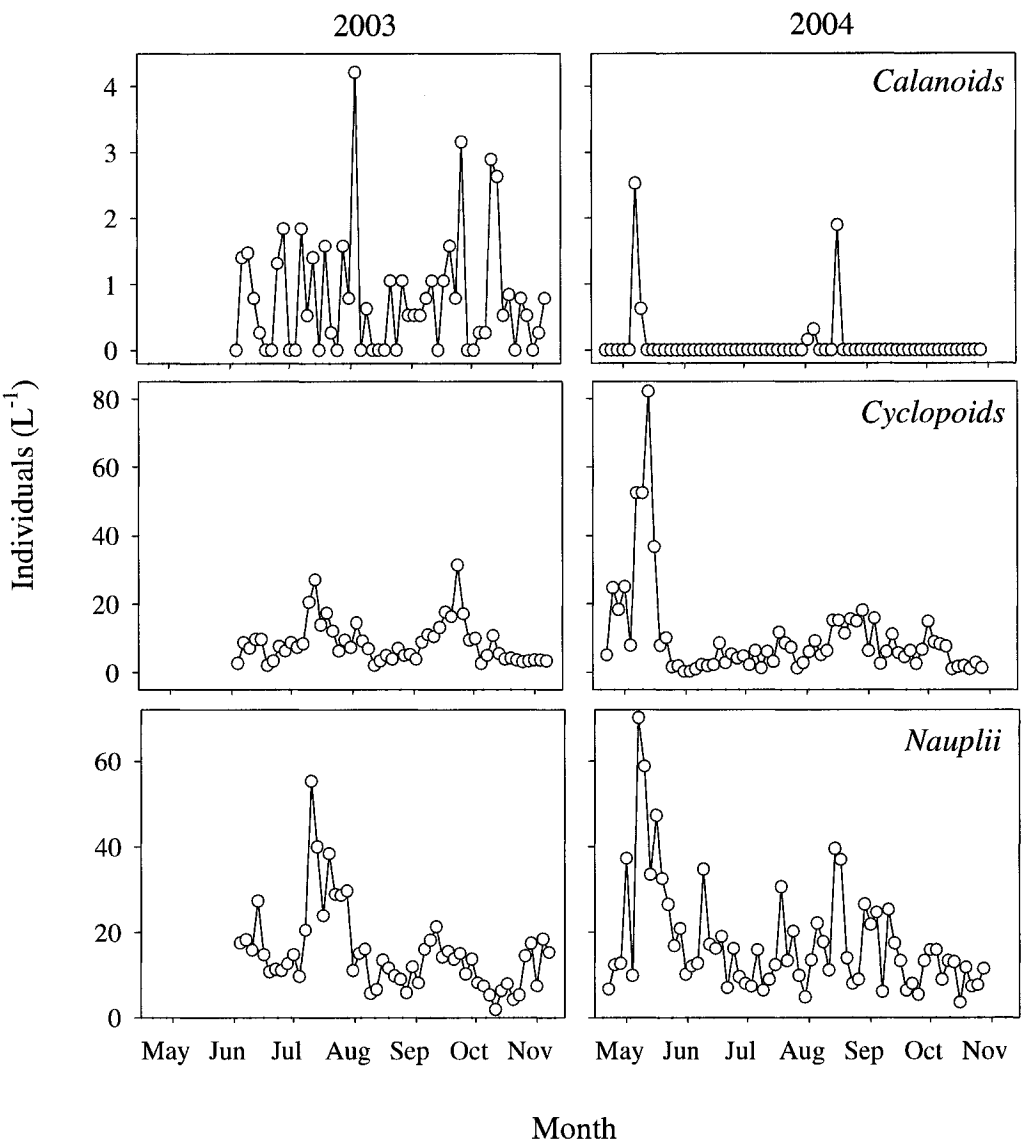


Figure 5.6 Population dynamics of the most common genera of copepods in Constance Lake, Canada. Note the different scales.

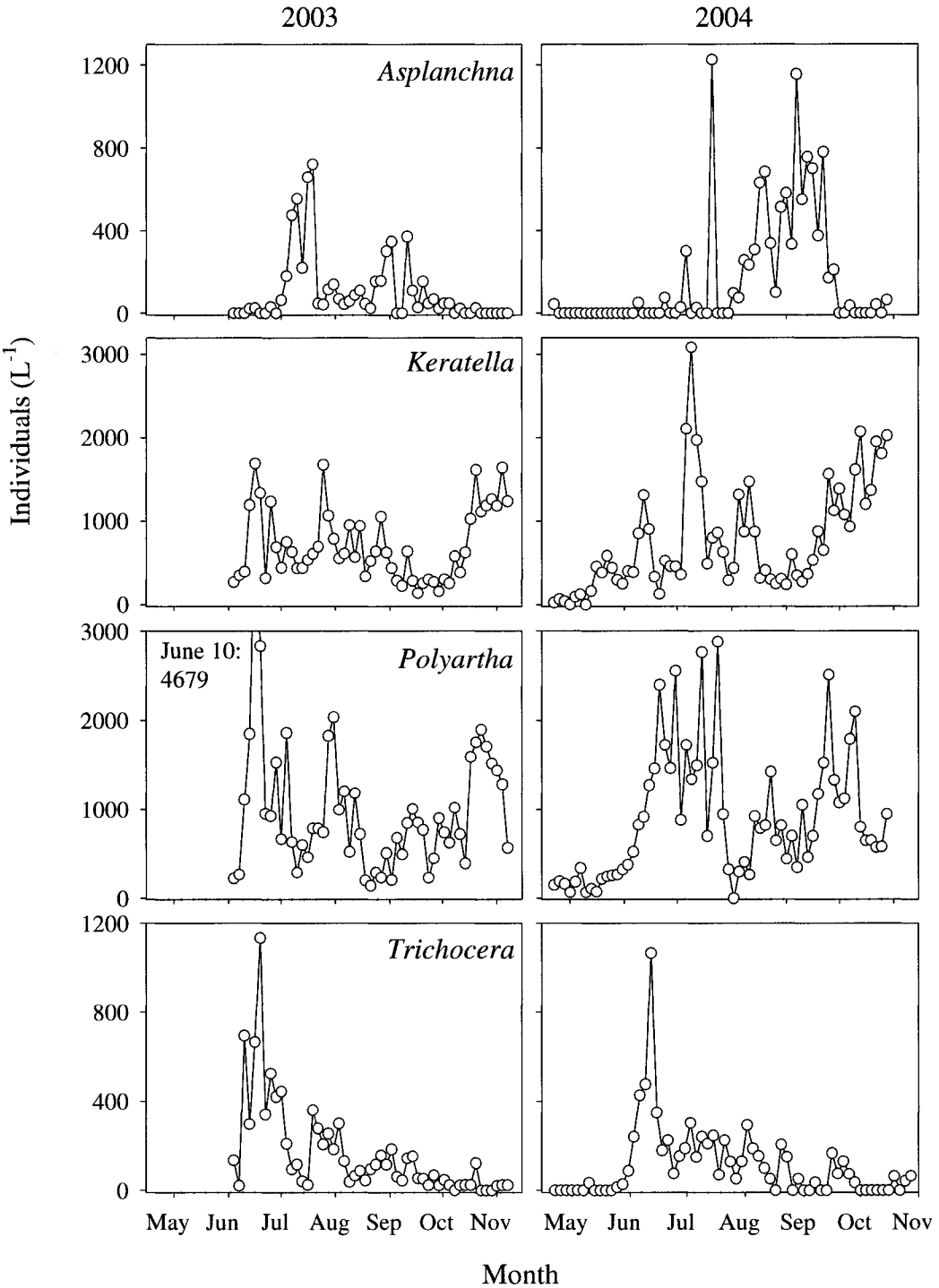


Figure 5.7 Population dynamics of the most common genera of rotifers in Constance Lake, Canada. Note the different scales.

6 Conclusions

Since Reynolds' (1975) first description of cyanobacterial blooms, a great deal of research has been conducted using the methods of field observations, mesocosms, and laboratory experiments. Laboratory studies have investigated the response of different zooplankton species to cyanobacteria, studied the physiological adaptations of cyanobacteria to abiotic conditions and uncovered the genetic basis of microcystin production. Mesocosm studies have sought to unravel the food web interactions between nutrients, cyanobacteria, zooplankton and fish and field observations have uncovered regional scale relationships such as those between nutrient enrichment and the likelihood of a cyanobacterial blooms, as well as relationships with abiotic conditions that may be regulating cyanobacteria and cyanotoxins *in situ* (e.g., Kotak et al. 1995; Kotak et al. 1996).

Though much has been learned over the last three decades many questions remain. The large number of laboratory studies and mesocosm studies of cyanobacteria-zooplankton interaction has made it difficult to draw any conclusions. Chapter one showed the high degree of variability in population growth rates both within and between zooplankton species fed diets containing cyanobacteria. It also showed that most zooplankton could maintain positive population growth rates when fed diets containing cyanobacteria, though those growth rates are below optimal levels.

Over the same period that this meta-analysis was conducted, studies were published which showed the potential for *Daphnia* to adapt to the presence of microcystin and cyanobacteria in general (Gustafsson and Hansson 2004; Gustafsson et al. 2005). As both the length of time necessary for adaptation to occur and the mechanism involved will influence species dynamics and interactions (Holt 2005; Stomp et al. 2008), identifying both the time required and the adaptive trait was the logical next step. The results of chapter two show that, rather than the decades of time previously thought necessary for *Daphnia* to adapt to toxic cyanobacteria (Hairston et al. 2001), *Daphnia* can adapt in periods as short as 8 weeks. A model of the experimental results

suggests that the adaptive trait could be either differential gut passage or feeding selectivity.

The results of both chapters one and two suggest that zooplankton play a role in controlling cyanobacterial biomass and may shift cyanobacterial strains towards toxic genotypes. In contrast, field observations showed no correlations between zooplankton and microcystins in Constance Lake, even with high frequency sampling. This could have been due to the low abundance of cladocerans throughout much of the years studied (Figure 5.5). However, there were negative correlations between *Anabaena* taxa and *Daphnia* and cyclopoid copepods. *Daphnia* and copepods have been previously shown to graze on *Anabaena* (DeMott and Moxter 1991) and this negative correlation may in part explain why cyanobacterial taxa in Constance Lake never reached bloom levels. The positive correlations between cyanobacterial and rotifer growth rates and also among cyanobacterial taxa were more unexpected. Such facilitative relationships are rarely observed or studied though they may play a large part in maintaining phytoplankton diversity (Agawin et al. 2007).

In the last chapter of this thesis, the dynamics of all potentially bloom-forming cyanobacteria that occurred in Constance Lake were investigated in the light of limiting abiotic conditions, competition and grazing pressure. The high frequency sampling allowed for the simultaneous examination of each of these interactions *in situ,* an objective that has thus far been limited to experimental mesocosms (e.g.Ghadouani et al. 2003; Sarnelle 2007; Gobler et al. 2007). The list of abiotic factors that may limit cyanobacterial population growth is well documented (Hyenstrand et al. 1998). Some of these factors, such as temperature and nutrients, were important in Constance Lake. However, biotic interactions with zooplankton, especially rotifers, and other cyanobacterial taxa were more important in explaining the variability of each species.

Applying laboratory results to whole ecosystems remains a challenge for ecologists. Where laboratory studies are designed to look at simple interactions, ecosystems contain all possible interactions. One of the first hurdles to overcome in moving from laboratory studies to predictive ecology in ecosystems is the selection of an

appropriate scale (time and space) that best explains the main sources of variability without distortion from small scale stochastic events. Regional studies have better predictive potential for cyanobacterial biomass in relation to abiotic and biotic variables most likely due to the greater range of these variables (Reynolds 1998) but cyanobacterial blooms and the management of these blooms occur at the within lake scale. Sampling at six day intervals in the case of microcystins and three day intervals for cyanobacterial taxa only explained a maximum of 35 % of the variance. Further variation may be explained by the internal dynamics of each species (Turchin 1990; Turchin 1999) or possibly allelopathic interactions with species in other phyla (e.g. Keating 1977; Vardi et al. 2002).

Community relations, especially predator-prey interactions have the capacity to shape species composition and alter species dynamics (Sarnelle 1993). Species interactions will also depend upon both the genetic diversity and adaptation potential of the species involved (Chapters 1 & 2; Yoshida et al. 2003). The finding that not only is there a wide variation in the response of zooplankton to cyanobacteria but also that zooplankton can adapt to the presence of cyanobacteria has changed the direction of future research from laboratory studies using single clones to investigating interactions of populations (Sarnelle 2007). Although much of the emphasis in explaining cyanobacterial blooms has been focussed on bottom-up influence of abiotic variables (e.g. Hyenstrand et al. 1998) when considering blooms within a single lake, community processes could be more important (Chapter 5). Given the potential for adaptation of both zooplankton and cyanobacteria to changing conditions, ecosystem models which extend beyond single genotype interactions to consider the adaptive potential of each population may better explain multi-species dynamics in semi-closed ecosystems such as lakes.

7 Appendix A

Table A1. The intra-class correlation coefficient and associated significance values for zooplankton taxa. Significant values <0.05 indicate effect sizes are more similar within a study than across all studies.

Taxa	N	# of studies	ICC	*P*
Bosmina longirostris	3	2	-0.32	0.562
Ceriodaphnia	34	6	-0.02	0.499
Ceriodaphnia cornuta	28	4	-0.06	0.606
Ceriodaphnia dubia	6	2	0.64	0.065
Daphnia	214	33	0.40	<0.001
Daphnia ambigua	5	2	0.52	0.155
Daphnia carinata	8	2	0.62	0.034
Daphnia cucullata	16	2	-0.18	0.749
Daphnia galaeta	22	6	0.53	0.007
Daphnia longispina	12	5	0.93	<0.001
Daphnia magna	46	8	0.72	<0.001
Daphnia pulex	95	12	0.12	0.034
Moina	27	7	0.72	<0.001
Moina macrocopa	7	2	-0.41	0.948
Moina micrura	20	5	0.78	<0.001
Brachionus	56	11	0.48	<0.001
Brachionus calyciflorus	45	10	0.62	<0.001
Brachionus rubens	11	2	-0.08	0.43
Keratella	19	5	0.55	0.009
Keratella cochlearis	14	5	0.54	0.041
Keratella crassa	4	2	-1.49	0.786

Table A2. Results of a random sign test to determine the influence of multiple experiments per study on the effect size of herbivorous zooplankton fed treatment diets containing cyanobacteria. Genus entries are bolded and combine effect sizes from all studies with species in the specified genus.

Zooplankton taxa	no. experiments	n	mean effect size	s^2	P
Daphnia	1	33	-0.58	0.07	0.001
	3	87	-0.61	0.05	0.001
	6	139	-0.61	0.04	0.001
	10	177	-0.62	0.04	0.001
	15	200	-0.62	0.03	0.001
	ALL	214	-0.62	0.03	0.001
Daphnia carinata	1	2	-0.74	0.27	0.123
	3	6	-0.75	0.16	0.023
	4	8	-0.75	0.13	0.013
Daphnia galeata	1	6	-0.29	0.08	0.028
	3	16	-0.31	0.05	0.02
	4	22	-0.39	0.04	0.001
Daphnia longispina	1	5	-0.52	0.06	0.023
	3	11	-0.49	0.04	0.03
	4	12	-0.47	0.04	0.02
Daphnia magna	1	8	-0.931	0.107	0.005
	3	24	-0.934	0.064	<0.001
	6	34	-1.005	0.057	<0.001
	10	40	-1.016	0.056	<0.001
	15	46	-1.009	0.057	<0.001
Daphnia pulex	1	12	-0.48	0.19	0.035
	3	32	-0.52	0.12	0.02
	6	54	-0.58	0.10	0.001
	10	68	-0.59	0.08	0.001
	15	82	-0.61	0.07	0.001

Zooplankton taxa	no. experiments	n	mean effect size	s^2	P
Moina	1	7	-1.21	0.21	0.022
	3	18	-1.27	0.14	0.001
	6	25	-1.10	0.12	0.001
	8	27	-1.03	0.11	0.001
Moina micrua	1	5	-0.68	0.09	0.014
	3	12	-0.63	0.06	0.02
	6	18	-0.54	0.05	0.01
	8	20	-0.50	0.05	0.01
Brachionus	1	12	-1.10	0.22	0.017
	3	34	-1.14	0.14	0.001
	6	47	-1.12	0.13	0.001
	10	56	-1.04	0.12	0.001
Brachionus calyciflorus	1	10	-0.98	0.19	0.027
	3	28	-1.01	0.12	0.01
	6	38	-1.02	0.12	0.01
	10	45	-0.92	0.13	0.01
Keratella	1	5	-0.22	0.07	0.105
	3	12	-0.22	0.05	0.023
	6	16	-0.21	0.05	0.05
	10	19	-0.22	0.04	0.08
Keratella cochlearis	1	5	-0.20	0.07	0.117
	3	11	-0.22	0.06	0.3
	6	14	-0.22	0.05	0.1

Table A3. The results of re-sampling with replacement for t-tests comparing effect sizes of *Daphnia magna* fed toxic and non-toxic treatment diets (putative toxins); mean zooplankton effect sizes for treatment diets containing *Microcystis aeruginosa* with or without microcystins; and also for mean zooplankton effect sizes for treatment diets containing *Microcystis aeruginosa* in colonial or single celled form (morphology).

Taxa	Comparison	No. experiments selected	N	Mean Effect Size	Standard deviation	p-value
Daphnia	putative	1	10	2.40	0.72	0.029
magna	toxins	3	25	3.73	0.71	0.001
		6	32	3.79	0.70	0.002
		ALL	38	3.40	0.66	0.001
Microcystis	microcystins	1	22	0.53	0.53	0.343
aeruginosa		3	59	0.64	0.54	0.307
		6	92	0.88	0.50	0.166
		10	119	1.15	0.49	0.113
		15	138	1.24	0.45	0.107
		ALL	151	1.18	0.47	0.118
Microcystis	morphology	1	34	-0.36	0.52	0.355
aeruginosa		3	87	-0.30	0.64	0.364
		6	138	-0.19	0.73	0.338
		10	180	0.03	0.80	0.416
		15	210	-0.30	0.94	0.314
		ALL	229	-0.41	0.97	0.261

Table A4. List of studies used in the meta-analysis comparing *r* for grazers fed control foods comprised of chlorophytes and/or flagellates and treatment foods containing cyanobacteria. Grazer types: cl = cladoceran [Genera = [1]*Bosmina*, [2]*Ceriodaphnia*, [3]*Chydorus*, [4]*Daphnia*, [5]*Moina*, [6]*Moinadaphnia*, [7]*Scapholeberis*, [8]*Simocephalus*] and r = rotifer [Genera = [9]*Asplanchna*, [10]*Brachionus*, [11]*Hexarthra*, [12]*Keratella*, [13]*Synchaeta*]. Toxins analyzed and found to be present: [14]anatoxin, [15]deazaadenosine, [16]glucopyranoside, [17]microcystin. Morphology types: colonial = colonial [Genera = [18]*Microcystis*], filament = filamentous [Genera = [19]*Anabaena*, [20]*Aphanizomenon*, [21]*Cylindrospermopsis*, [22]*Oscillatoria*/*Planktothrix*], and single = single-celled [Genera = [19]*Anabaena*, [23]*Anacystis*, [24]*Gleocapsa*, [25]*Merismopedia*, [18]*Microcystis*, [26]*Synechococcus*, [27]*Synechocystis*]. ND = toxin presence not-determined by chemical analysis.

Studies	Grazer types	Cyanobacterial toxicity	Cyanobacterial morphology	Effect size estimates
Ahlgren et al. 1990	cl[3,4]	ND	single[18], filamentous[22]	4
Alva-Martínez et al. 2001	cl[2,4,5]	ND	single[18]	9
Alva-Martínez et al. 2004	cl[4]	ND	single[18]	8
Arnold 1971	cl[4]	non-toxic, ND	single[19,23,24,25,26,27]	14
Brett 1993	cl[4]	ND	single[18]	2
Cecchine G.A III. 1997	r[10]	toxic[17]	single[18]	9
Chen and Xie 2003	cl[5]	ND	colonial[18]	3
Chen and Xie 2004	cl[4]	toxic[17]	single[18]	4
Claska and Gilbert 1998	cl[4]	toxic[14,15,16]	filament[19]	25
de Bernardi et al. 1981	cl[4]	ND	single[18]	6
de Figueiredo et al. 2004	cl[4]	ND	filament[20]	6
Ferrão-Filho and Azevedo 2003	cl[2,5]	toxic[17]	single[18], colonial[18]	16
Ferrão-Filho et al. 2000	cl[2,4,5,6]	toxic[17]	single[18]	25
Ferrão-Filho et al. 2002	cl[2,5]	toxic[17]	single[18]	2
Fulton and Paerl 1987	r[10]	ND	single[18], colonial[18]	4
Fulton and Paerl 1988	cl[4], r[10]	ND	colonial[18]	4
Geng et al. 2006	r[10]	toxic[17]	single[18]	6
Gilbert 1990	cl[1,2,4], r[12,13]	toxic[15,16]	filament[19]	22
Gilbert 1994	r[12]	toxic[14]	filament[19]	1
Gilbert 1996b	r[10]	toxic[14]	filament[19]	5
Gilbert 1996a	r[9,10]	toxic[14]	filament[19]	6
Gilbert and Durand 1990	cl[4], r[12]	non-toxic	filament[19]	4
Hanazato and Yasuno 1987	cl[5]	ND	single[18], colonial[18]	2
Henning et al. 1991	cl[4]	toxic[17], non-toxic, ND	single[18]	4
Hietala et al. 1995	cl[4]	toxic[17]	single[18]	10
Hietala et al. 1997a	cl[4]	toxic[17]	single[18]	18
Hietala et al. 1997b	cl[4]	toxic[17]	single[18]	4
Kurmayer 2001	cl[1,4]	non-toxic	filament[20]	4
Lundstedt and Brett 1991	cl[4]	ND	single[18]	1
Lürling 2003a	cl[4]	toxic[17], non-toxic	single[18]	8
Lürling 2003b	cl[4]	toxic[17], non-toxic	single[18]	16
Lürling and van der Grinten 2003	cl[4]	toxic[17], non-toxic	single[18]	5
Martin-Creuzburg et al. 2005	cl[4]	ND	single[26]	6
Nandini and Rao 1998	cl[2,4,5,7,8], r[10,11]	ND	single[18], colonial[18]	32
Nandini et al. 2000	cl[4]	ND	colonial[18]	2
Porter and Orcutt 1980b	cl[4]	toxic[14]	single[19], filament[19]	4
Repka 1996	cl[4]	toxic[17]	filament[22]	6
Repka 1997	cl[4]	toxic[17]	filament[22]	9
Repka 1998	cl[4]	toxic[17]	filament[22]	12
Rothhaupt 1991	r[10]	ND	single[18], filament[19,21]	8
Sartonov 1995	cl[4], r[12]	non-toxic	single[18]	4
Shurin and Dodson 1997	cl[4]	toxic[17]	single[18]	3
Smith and Gilbert 1995	cl[4], r[12]	toxic[17], non-toxic	single[18]	18
Starkweather 1981	r[10]	non-toxic	filament[19]	2
Starkweather and Kellar 1983	r[10]	toxic[14]	filament[19]	3
Weithoff and Walz 1995	r[10]	toxic[17]	filament[22]	10
Forbes and Calow 1999	cl[4]	toxic[17]	single[18]	2
			Total	378

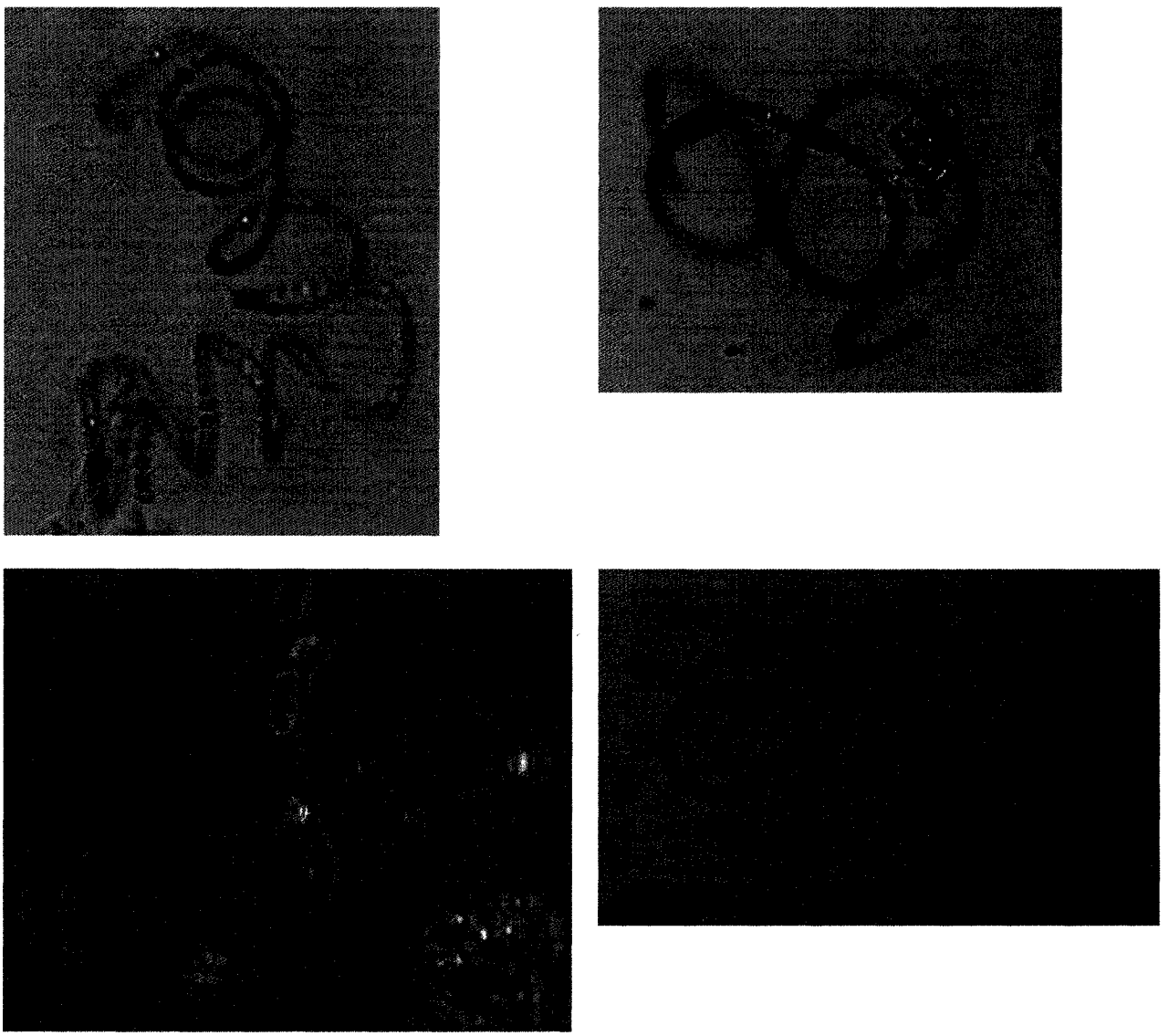
8 Appendix B – Common Cyanobacteria Species found in Constance Lake

8.1 Methods

Samples were collected from Constance Lake, a shallow mesotrophic lake in west Ottawa, between May 2003 and October 2005. Water samples were collected with an integrated tube sampler to a depth of 2 m. In the laboratory, 2 L of water was screened through a 35μm Nitex mesh and preserved in formalin. Sub-samples were pipetted directly to an Utermöhl counting chamber and a couple of drops of diluted (10%) Lugol's iodine solution was added to help identify the cyanobacteria. Pictures were taken at 300X magnification using a Sony Cyber-shot 2.1 mega pixels digital camera with an eye piece adaptor for a Wild M40 inverted microscope. Cell and colony dimensions are based on mean measurements made over all years (n = 230). Five measurements per species were made on three samples each month except for in 2005 when measurements were made on all samples.

8.2 Anabaena flos-aquae

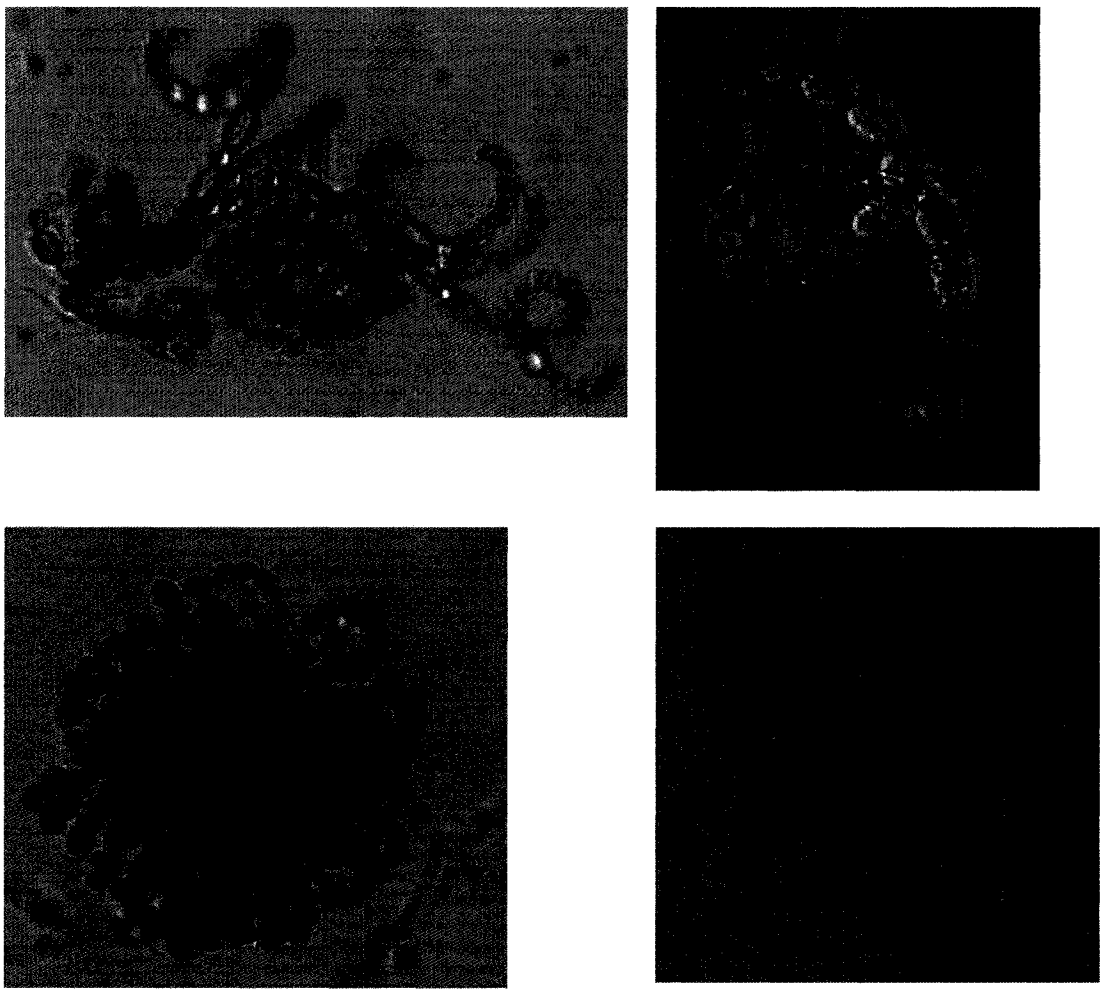
Trichome	flexulose, some thin mucilage, barely visible
Vegetative Cells	~5 μm diameter, spherical, well defined margin, cells well separated
Akinete	10-12 x 5 -7 μmj straight edged, cylindrical, scattered throughout trichome
Heterocyte	slightly smaller than cells and spherical



Digital photographs of *Anabaena flos-aquae.* From top left and moving clockwise, pictures were taken June 15, 2004; Sept 22, 2004; Sept 22, 2004; Oct 22, 2004

8.3 Anabaena lemmermannii

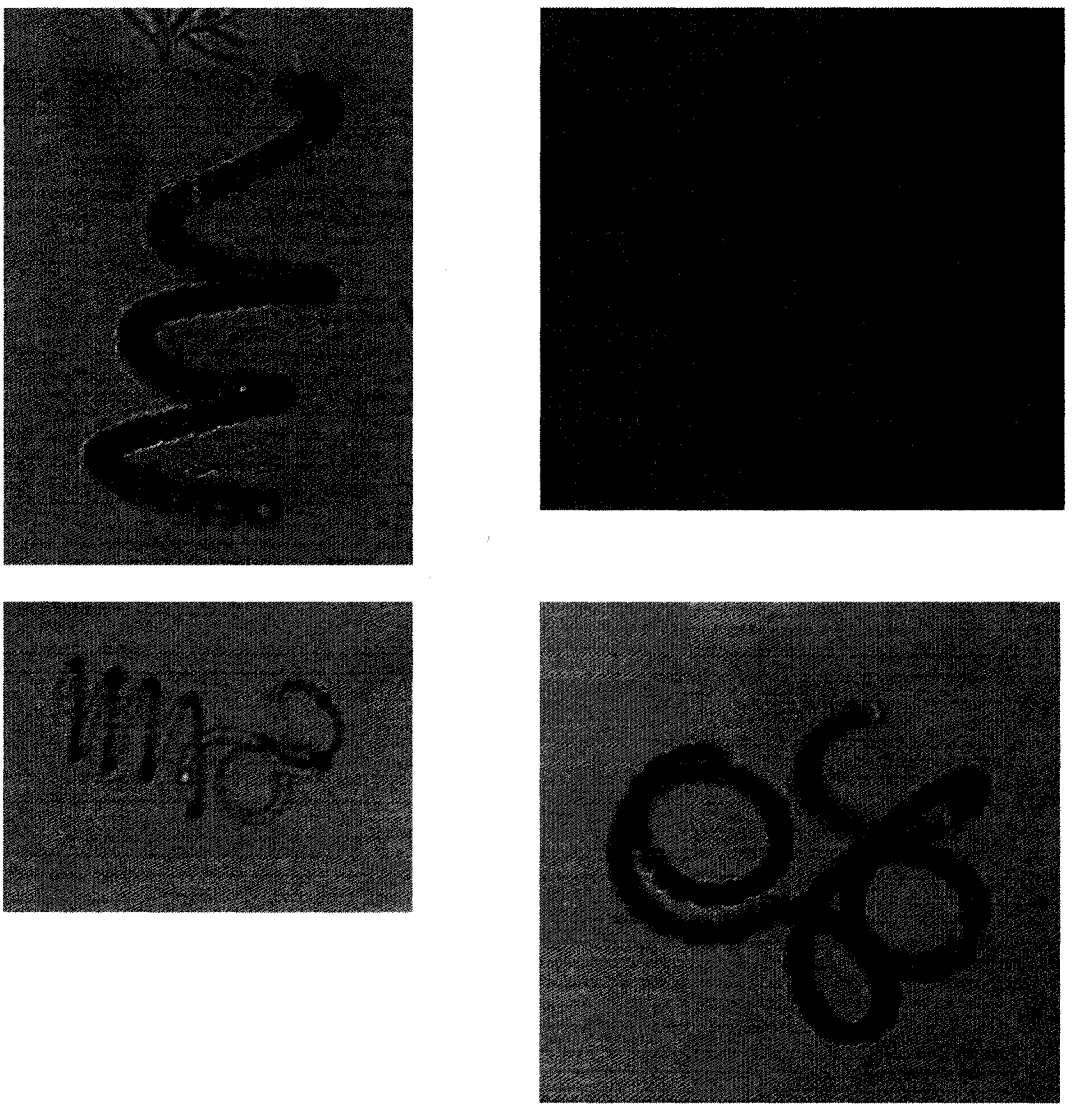
Trichome	Flexulose, can produce tangled colonies
Vegetative Cells	~7-8 x 6-7 μm, slightly longer than wide
Akinete	~10 x 8 μm, found on either side of heterocyte
Heterocyte	~6 x 6 μm, round



Digital photographs of *Anabaena.* All pictures were taken June15, 2004.

8.4 Anabaena crassa

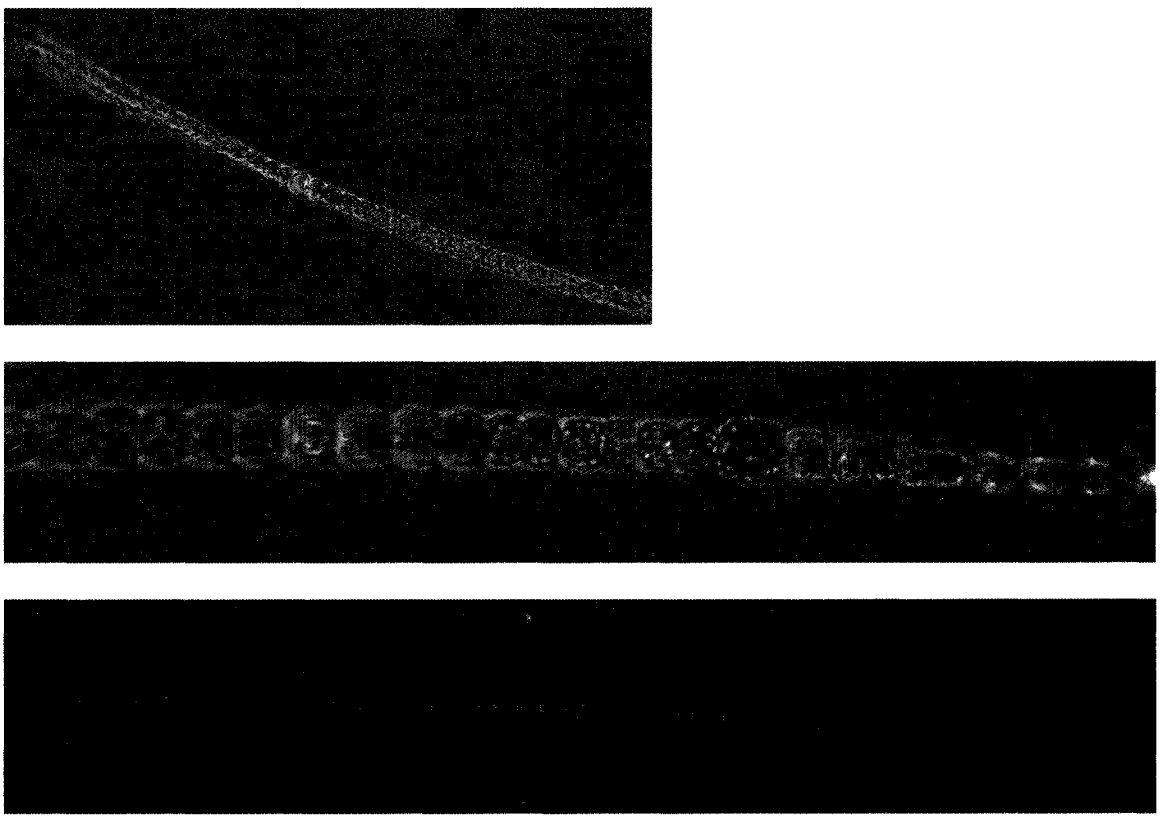
Trichrome	Spiraled or sometimes flexulose, 60 μm long and 16 μm between spirals
Vegetative Cells	~8 x 8 μm, roundish but squished together
Akinete	~16 x 12; dispersed throughout trichrome, can be two in a row
Heterocyte	~8 x 8 μm; round, same size as cells



Digital photographs of *Anabaena spiroides.* From top left and moving clockwise, pictures were taken June 15, 2004; June 24, 2004; Aug 13, 2004; June 24, 2004.

8.5 Anabaena viguieri

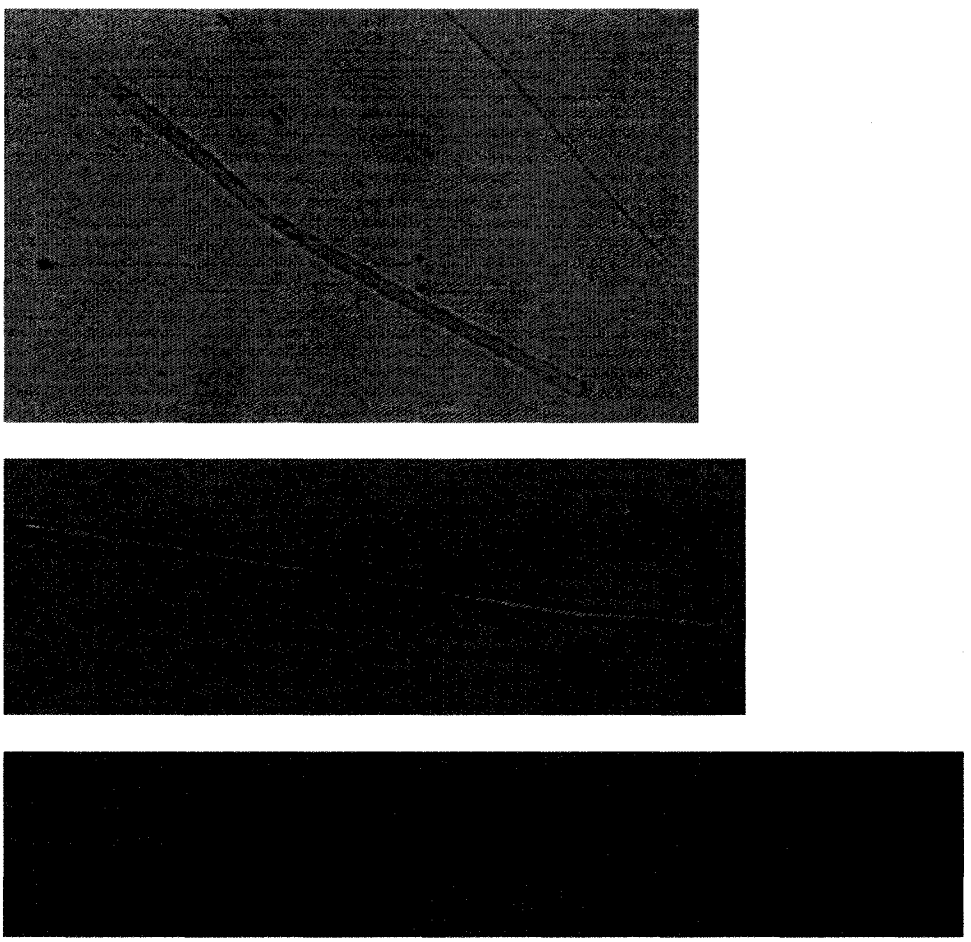
Trichome	Straight, thin
Vegetative Cells	6-7 x 8; cells spherical to barrel shaped
Akinete	20 x 12 μm
Heterocyte	8-9 x 8, spherical



Digital photographs of *Anabaena viguieri.* From top, samples were taken June 15, 2004; Aug 17, 04; Aug 17, 04. The arrow in the middle picture points to an akinete in early stages of development.

8.6 Aphanizomenon flos-aquae

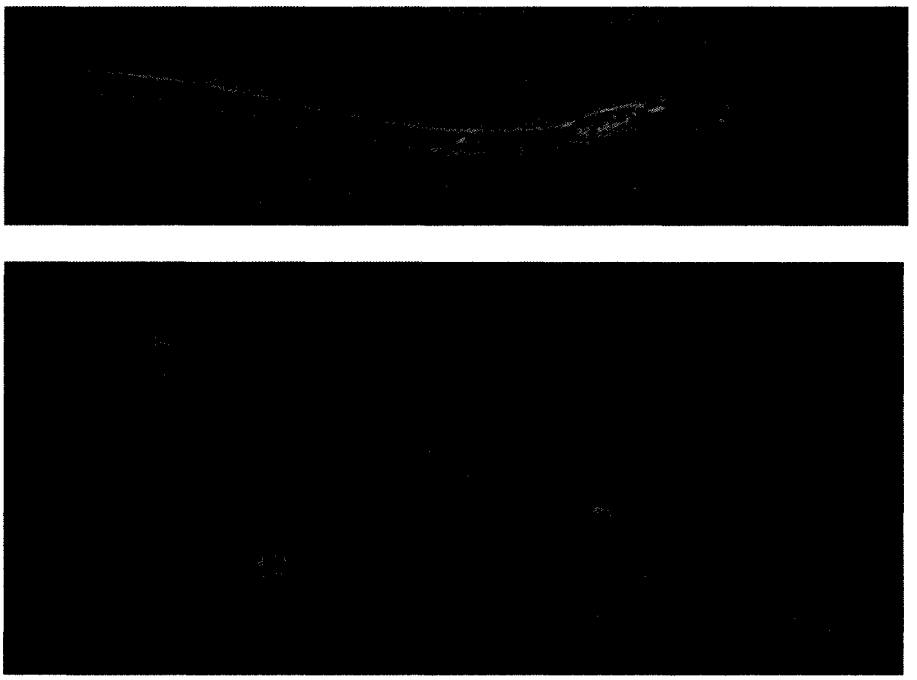
Trichome	Straight, long
Vegetative Cells	~ 8 x 4.5; barrel shaped; no gas vesicles, just aerotopes
Akinete	~ 72 x 8; long and slender, found in the middle of the trichome
Heterocyte	~ 1 2x 8, often terminal



Digital photographs of *Aphanizomemon flos-aquae.* From top, pictures were taken Aug 17, 2004; Sept 22, 2004; Sept 28, 2004.

8.7 Cylindrospermopsis raciborskii

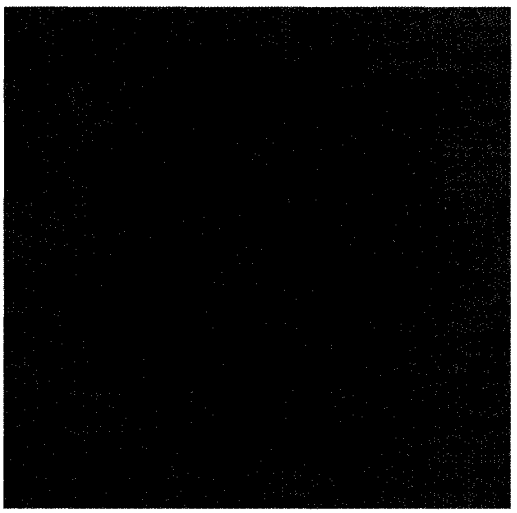
Trichome	Straight
Vegetative Cells	~ 3 x 8 μm but difficult to see cell divisions
Akinete	~ 5 x 16 μm, always right before heterocyte on the end or close to the end, sometimes two in a row
Heterocyte	~ 3 x 7 μm, always terminal

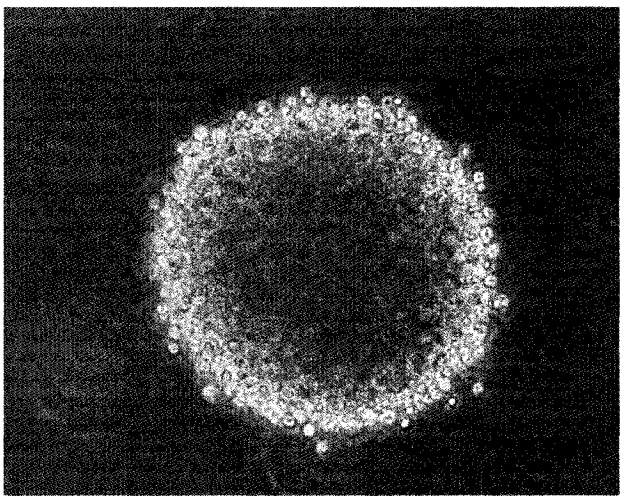


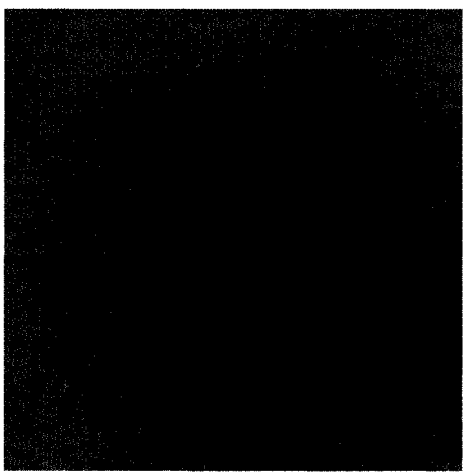
Digital photographs of *C. raciborskii* taken Sept 28, 2004 (top) and Aug 18, 2004 (bottom).

8.8 Microcystis flos-aquae

Colony Shape	More or less spherical, compact with irregular margin
Vegetative Cells	~ 3.5 μm, spherical
Mucilage	Not easily visable



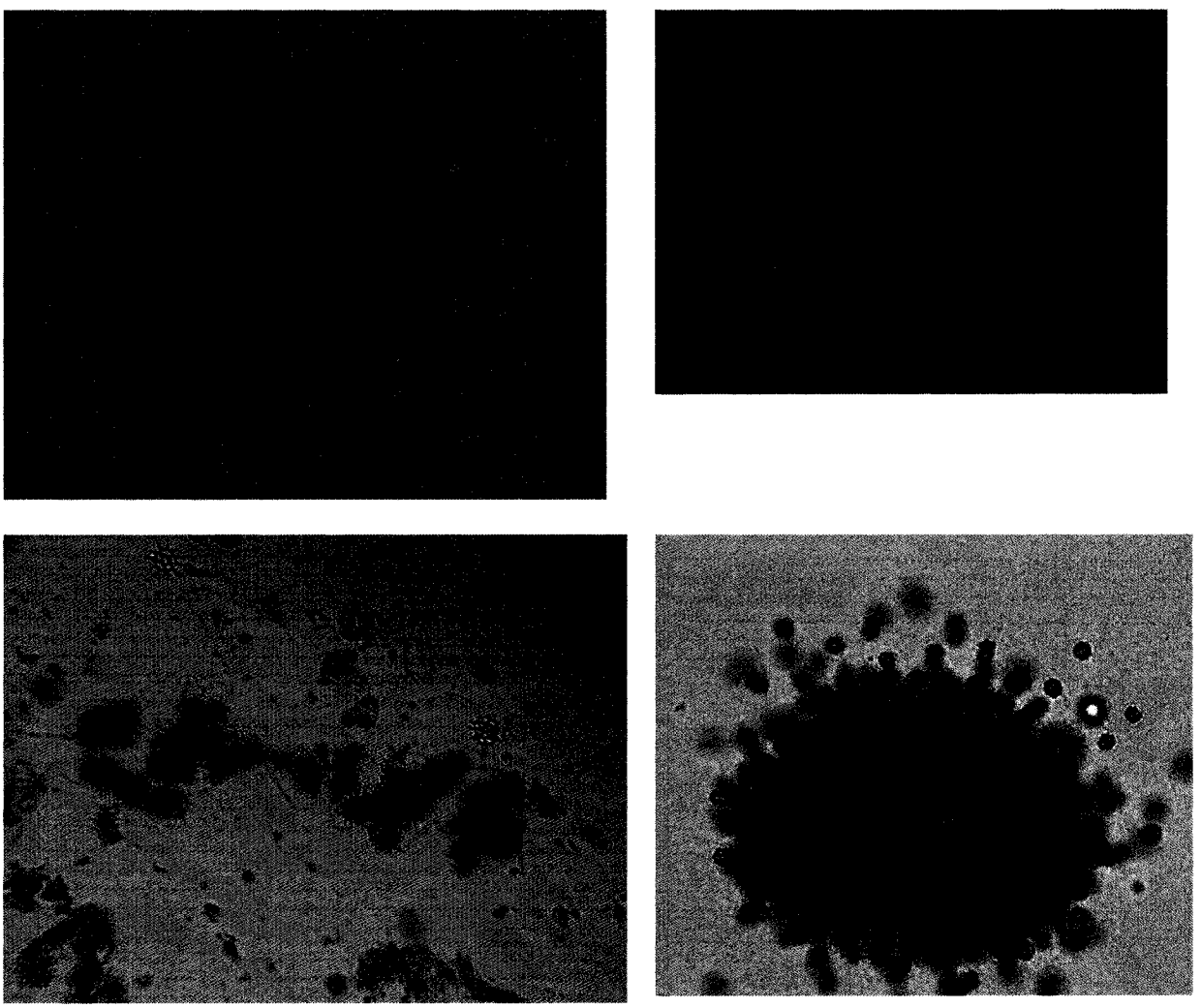




Digital photographs of *M. flos-aquae* from sample taken July 6, 2004.

8.9 Microcystis aeruginoa

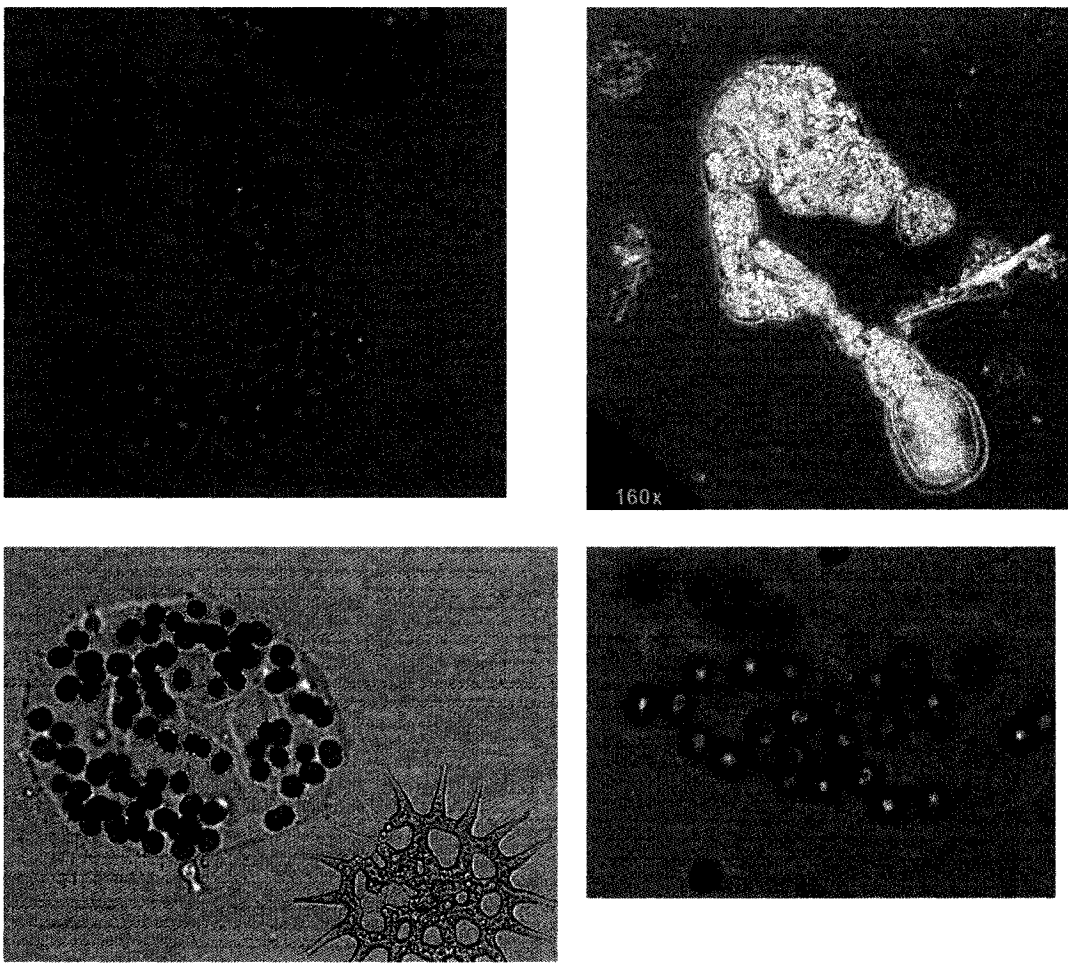
Colony Shape	spherical when small but becoming lobed and clathrate when larger
Vegetative Cells	spherical, ~5 μm
Mucilage	thin, no easily seen



Digital photographs of *M. aeruginosa.* From top left and moving clockwise, pictures were taken pictures taken Aug 18, 03; July 21, 04; Aug 18, 2003; and August 9, 2006.

8.10 Microcystis wesenbergii

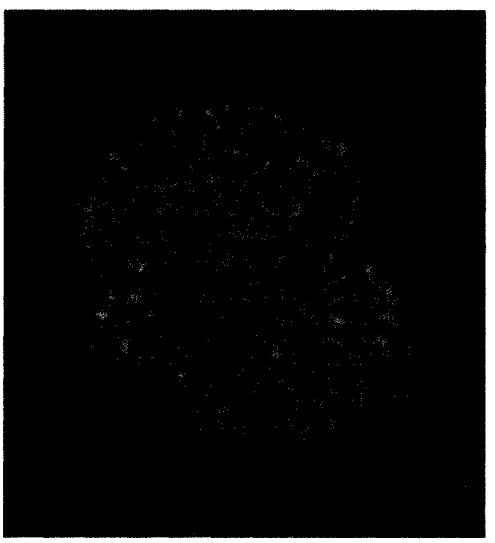
Colony Shape	Lobed and clathrate as colonies get larger
Vegetative Cells	~5-6 μm, round
Mucilage	Thick with refractive edge



Digital photographs of *M. wesenbergii.* From top left and moving clockwise, first three pictures were taken July 21, 2004 and the fourth picture was taken July 5, 2006.

8.11 Woronichinia sp.

Colony Shape	spherical, cells radially arranged
Vegetative Cells	~5.5 x 4, cells slightly oblong, especially along margin
Mucilage	Sometimes not visible, sometimes visible around margin



Digital photographs of *Woronichinia sp.* taken Aug 18, 2003.

9 References

ADAMS,D.C., J.GUREVITCH, AND M.S.ROSENBERG 1997. Resampling tests for meta-analysis of ecological data. Ecology **78**: 1277-1283.

AGAWIN,N.S.R., S.RABOUILLE, M.J.W.VELDHUIS, L.SERVATIUS, S.HOL, H.M.J.VAN OVERZEE, AND J.HUISMAN 2007. Competition and facilitation between unicellular nitrogen-fixing cyanobacteria and non-nitrogen fixing phytoplankton species. Limnol.Oceanogr. **52**: 2233-2248.

AHLGREN,G., I.B.GUSTAFSSON, AND M.BOBERG 1992. Fatty acid composition and chemical composition of freshwater microalgae. J Phycol **28**: 37-50.

AHLGREN,G., L.LUNDSTEDT, M.T.BRETT, AND C.FORSBERG 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. J.Plankton Res. **12**: 809-818.

ALBAY,M., R.AKCAALAN, H.TUFEKCI, J.S.METCALF, K.A.BEATTIE, AND G.A.CODD 2003. Depth profiles of cyanobacterial hepatotoxins (microcystins) in three Turkish freshwater lakes. Hydrobiologia **505**: 89-95.

ALVA-MARTÍNEZ,A.F., S.S.S.SARMA, AND S.NANDINI 2001. Comparative population dynamics of three species of cladocera in relation to different levels of *Chlorella vulgaris* and *Microcystis aeruginosa.* Crustaceana **74**: 749-764.

ALVA-MARTÍNEZ,A.F., S.S.S.SARMA, AND S.NANDINI 2004. Population growth of *Daphnia pulex* (Cladocera) on a mixed diet (*Microcystis aeruginosa* with *Chlorella* or *Scenedesmus*). Crustaceana **77**: 973-988.

AMÉ,M.V., M.DEL PILAR DÍAZ, AND D.A.WUNDERLIN 2003. Occurrence of toxic cyanobacterial blooms in San Roque Reservoir (Córdoba, Argentina): A field and chemometric study. Environ Toxicol **18**: 192-201.

AN,J. AND 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.

ANDERSEN,T. AND D.O.HESSEN 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnol.Oceanogr. **36**: 807-814.

ARANDA-RODRIGUEZ,R., A.TILLMANNS, F.BENOIT, F.R.PICK, J.HARVIE, AND L.SOLENAIA 2005. Pressurized liquid extraction of toxins from cyanobacterial cells. Environ Toxicol **20**: 390-396.

ARNOLD,D.E. 1971. Ingestion, assimilation, survival, and reproduction by daphnia puplex fed seven species of blue-green algae. Limnol.Oceanogr. **16**: 906-920.

ARNQVIST,G. AND D.WOOSTER 1995. Meta-analysis: synthesizing research findings in ecology and evolution. TREE **10**: 236-240.

BALCER,M.D., N.L.KORDA, and S.I.DODSON. 1984. Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. University of Wisconsin Press.

BALDIA,S.F., M.C.G.CONACO, T.NISHIJIMA, S.IMANISHI, AND K.HARADA 2003. Microcystin production during algal bloom occurrence in Laguna de Bay, the Philippines. Fisheries Science **69**: 110-116.

BALLOT,A., L.KRIENITZ, K.KOTUT, C.WIEGAND, J.S.METCALF, G.A.CODD, AND S.PFLUGMACHER 2004. Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya - Lakes Bogoria Nakura and Elmenteita. J.Plankton Res. **26**: 925-935.

BALLOT,A., L.KRIENITZ, K.KOTUT, C.WIEGAND, AND S.PFLUGMACHER 2005. Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. Harmful Algae **4**: 139-150.

BALLOT,A., S.PFLUGMACHER, C.WIEGAND, K.KOTUT, AND L.KRIENITZ 2003. Cyanobacterial toxins in Lake Baringo, Kenya. Limnologica **33**: 2-9.

BEDNARSKA,A. AND P.DAWIDOWICZ 2007. Change in filter-screen morphology and depth selection: Uncoupled responses of *Daphnia* to the presence of filamentous cyanobacteria. Limnol.Oceanogr. **52**: 2358-2363.

BERTHON,J.L. AND S.BROUSSE 1995. Modification of migratory behaviour of planktonic Crustacea in the presence of a bloom of *Microcystis aeruginosa* (Cyanobacteria). Hydrobiologia **300/301**: 185-193.

BILLAM,M., L.TANG, Q.CAI, S.MUKHI, H.GUAN, P.WANG, Z.WANG, C.W.THEODORAKIS, R.J.KENDALL, AND J.WANG 2006. Seasonal variations in the concentration of microcystin-LR in two lakes in western Texas, USA. Environ Toxicol Chem **25**: 349-355.

BJØRNSTAD,O.N. AND B.T.GRENFELL 2001. Noisy clockwork: Time-series analysis of population fluctuations in animals. sc **293**: 638-643.

BOGIALLI,S., M.BRUNO, R.CURINI, A.DI CORCIA, C.FANALI, AND A.LAGANA 2006. Monitoring algal toxins in lake water by liquid chromotography tandem mass spectrometry. Environ.Sci.Technol. **40**: 2917-2923.

BOLCH,C.J.S., S.I.BLACKBURN, G.J.JONES, P.T.ORR, AND P.M.GREWE 1997. Plasmid content and distributrion in the toxic cyanobacterial genus *Microcystis* Kutzing ex Lemmermann (Cyanobacteria: Chroococcales). Phycologia **36**: 6-11.

BRETT,M.T. 1993. Resource quality effects on *Daphnia longispina* offspring fitness. J.Plankton Res. **15**: 403-412.

BRIAND,J.F., S.JACQUET, C.FLINOIS, C.AVOIS-JACQUET, C.MAISONNETTE, B.LEBERRE, AND J.F.HUMBERT 2005. Variations in the microcystin production of *Planktothrix rubescens* (Cyanobacteria) assessed from a four-year survey of Lac du Bourget (France) and from Laboratory Experiments. Microb Ecol **50**: 418-428.

BRIAND,J.F., C.ROBILLOT, C.QUIBLIER-LLOBERAS, AND C.BERNARD 2002. A perennial bloom of *Planktothrix agardhii* (Cyanobacteria) in a shallow eutrophic French lake: Limnological and microcystin production studies. Arch.Hydrobiol. **153**: 605-622.

BURNISON,B.K. 1980. Modified dimethysulfoxide (DMSO) extraction from chlorophyll analysis of phytoplankton. Can J Fish Aquat Sci **37**: 729-733.

BURNS,C.W. 1968. The relationship between body size of filter-feeding cladocera and the maximum size of particle ingested. Limnol.Oceanogr. **13**: 675-678.

CARMICHAEL,W.W. 1994. The toxins of cyanobacteria. Sci Am **270**: 78-86.

CARMICHAEL,W.W. 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs". Hum Ecol Risk Assess **7**: 1393-1407.

CARMICHAEL,W.W., V.R.BEASLEY, D.L.BUNNER, J.N.ELOFF, I.R.FALCONER, P.GORHAM, K.I.HARADA, T.KRISHNAMURTHY, M.J.YU, R.E.MOORE, K.L.RINEHART, M.RUNNEGAR, O.M.SKULBERG, AND M.WATANABE 1988. Naming of the cyclic heptapeptide toxins of cyanobacteria (blue-green algae). Toxicon **26**: 971-973.

CECCHINE G.A III. Combinations of natural and anthropogenic stressors affect populations of freshwater rotifers. 28-63. 1997. Georgia Institute of Technology.

CHATFIELD,C. 2004. The analysis of time-series - An introduction, 6th ed. CRC Press.

CHEN,F.Z. AND P.XIE 2003. The effects of fresh and decomposed *Microcystis aeruginosa* on cladocerans from a subtropic Chinese Lake. Journal of Freshwater Ecology **18**: 97-104.

CHEN,F.Z. AND P.XIE 2004. The toxicities of single-celled *Microcystis aeruginosa* PCC7820 and liberated *M. aeruginosa* to *Daphnia carinata* in the absence and presence of the green alga *Scenedesmus obliquus*. Journal of Freshwater Ecology **19**: 539-545.

CHEN,W., L.SONG, D.OU, AND N.GAN 2005. Chronic toxicity and responses of several important enzymes in *Daphnia magna* on exposure to sublethal microcystin-LR. Environ Toxicol **20**: 330.

CHRISTOFFERSEN,K., B.RIEMANN, A.KLYSNER, AND M.SØNDERGAARD 1993. Potential role of fish predation and natural populations of zooplankton in structuring a plankton community in eutrophic lake water. Limnol.Oceanogr. **38**: 561-573.

CLASKA,M.E. AND J.J.GILBERT 1998. The effect of temperature on the response of *Daphnia* to toxic cyanobacteria. Freshwater Biol. **39**: 221-232.

CODD,G.A. 2000. Cyanobacterial toxins, the perception of water quality, and the prioritization of eutrophication control. Ecol Eng **16**: 51-60.

CONTI,A.L.R., J.M.GUERRERO, AND J.M.REGUEIRA 2006. Levels of microcystins in two Argentinean reservoirs used for water supply and recreation: differences in implementation of safe levels. Environ Toxicol **20**: 263-269.

CUVIN-ARALAR,M.L., J.FASTNER, U.FOCKEN, K.BECKER, AND E.V.ARALAR 2002. Microcystins in natural blooms and laboratory cultured *Microcystis aeruginosa* from Laguna de Bay, Philippines. Syst Appl Microbiol **25**: 179-182.

DANGER,M., C.OUMAROU, D.BENEST, AND G.LACROIX 2007. Bacteria can control stoichiometry and nutrient limitation of phytoplankton. Functional Ecology **21**: 202-210.

DE BERNARDI,R. AND G.GIUSSANI 1990. Are blue-green algae a suitable food for zooplankton: An overview. Hydrobiologia **200/201**: 29-41.

DE BERNARDI,R., G.GIUSSANI, AND E.L.PEDRETTI 1981. The significance of blue-green algae as food for filterfeeding zooplankton: experimental studies on *Daphnia* spp. fed by *Microcystis aeruginosa*. Verh.Internat.Verein.Limnol. **21**: 477-483.

DE FIGUEIREDO,D.R., S.C.ANTUNES, M.J.PEREIRA, AND F.GONCALVES 2004. Chronic effects of *Aphanizomenon flos-aquae* on the survival and reproduction of daphnids. Fresen Environ Bull **13**: 665-670.

DE NOBEL,W.T., H.C.P.MATTHIJS, E.VON ELERT, AND L.R.MUR 1998. Comparison of the light-limited growth of the nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon*. New Phytol. **138**: 579-587.

DEMOTT,W.R. 1982. Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina*. Limnol.Oceanogr. **27**: 518-527.

DEMOTT,W.R. 1986. The role of taste in food selection by freshwater zooplankton. Oecologia **69**: 334-340.

DEMOTT,W.R. 1999. Foraging strategies and growth inhibition in five daphnids feeding on mixtures of toxic cyanobacterium and green algae. Freshwater Biol. **42**: 263-274.

DEMOTT,W.R. AND S.DHAWALE 1995. Inhibition of in vitro protein phosphatase activity in three zooplankton species by microcystin-LR, a toxin from cyanbacteria. Arch.Hydrobiol. **134**: 417-424.

DEMOTT,W.R., R.D.GULATI, AND E.VAN DONK 2001. *Daphnia* food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. Limnol.Oceanogr. **46**: 2054-2060.

DEMOTT,W.R. AND F.MOXTER 1991. Foraging cyanobacteria by copepods: Responses to chemical defense and resource abundance. Ecology **72**: 1820-1834.

DEMOTT,W.R. AND D.C.MÜLLER-NAVARRA 1997. The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia,* a cyanobacterium and lipid emulsions. Freshwater Biol. **38**: 649-664.

DI STEFANO,J., F.FIDLER, AND G.CUMMING 2005. Effect size estimates and confidence intervals: an alternative focus for the presentation and interpretation of ecological data, p. 71-102. *In* A.R.Burk [ed.], New Trends in Ecological Research. Nova Science Publishers,Inc.

DILLON,P.J. AND F.H.RIGLER 1974. The phosphorus-chlorphyll relationship in lakes. Limnol.Oceanogr. **19**: 767-773.

DIXIT,A.S., R.I.HALL, P.R.LEAVITT, R.QUINLAN, AND J.P.SMOL 2000. Effects of sequential depositional basins on lake response to urban and agriculture pollution: a palaeoecological analysis of the Qu'Appelle Valley, Saskatchewan, Canada. Freshwater Biol. **43**: 319-337.

DOMINGOS,P., T.K.RUBIM, R.J.R.MOLICA, S.M.F.O.AZEVEDO, AND W.W.CARMICHAEL 1999. First report of microcystin production by picoplanktonic cyanobacteria isolated from a northeast Brazilian drinking water supply. Environ Toxicol **14**: 31-35.

DOW,C.S. AND U.K.SWOBODA 2000. Cyanotoxins, p. 613-632. *In* B.A.Whitton and M.Potts [eds.], The ecology of cyanobacteria - Their diversity in time and space. Kluwer Academic Publishers.

DOWNING,J.A., S.B.WATSON, AND E.MCCAULEY 2001. Predicting cyanobacteria dominance in lakes. Can J Fish Aquat Sci **58**: 1905-1908.

DOWNING,T.G., C.MEYER, M.M.GEHRINGER, AND M.VAN DE VENTER 2005. Microcystin content of *Microcystis aeruginosa* is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. Environ Toxicol **20**: 257-262.

EDDY,S. and A.C.HODSON. 1961. Taxonomic keys to the common animals of the northern central states. Burgess Publishing Company.

EDMONDSON,W.T. AND A.H.LITT 1982. *Daphnia* in Lake Washington. Limnol.Oceanogr. **27**: 272-293.

ELSER,J.J. 1999. The pathway to noxious cyanobacteria blooms in lakes: the food web as the final turn. Freshwater Biol. **42**: 537-543.

ELSER,J.J., E.R.MARZOLF, AND C.R.GOLDMAN 1990. Phosphorus and nitrogen limitation of a phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. Can J Fish Aquat Sci **47**: 1468-1477.

EPP,G.T. 1996. Grazing on filamentous cyanobacteria by *Daphnia pulicaria*. Limnol.Oceanogr. **41**: 560-567.

ESPIE,G.S., A.G.MILLER, R.A.KANDASAMY, AND D.T.CANVIN 1991. Active HCO^{3-} transport in cyanobacteria. Can.J.Bot. **69**: 936-944.

FALCONER,I.R. 1996. Potential impact on human health of toxic cyanobacteria. Phycologia **35**: 6-11.

FASTNER,J., G.A.CODD, J.S.METCALF, P.WOITKE, C.WIEDNER, AND H.UTKILEN 2002. An international intercomparison exercise for the determination of purified microcystin-LR and microcystins in cyanobacterial field material. Anal Bioanal Chem **374**: 437-444.

FASTNER,J., U.NEUMANN, B.WIRSING, J.WECKESSER, C.WIEDNER, B.NIXDORF, AND I.CHORUS 1999. Microcystins (hepatoxic heptapeptides) in German fresh water bodies. Environ Toxicol **14**: 13-22.

FERGUSON,R. Saving Canada's Sickest Lake. The Globe and Mail December 11, 2004, F6. 2004.

FERRÃO-FILHO,A.S., S.AZEVEDO, AND W.R.DEMOTT 2000. Effects of toxic and non-toxic cyanobacteria on the life history of tropical and temperate cladocerans. Freshwater Biol. **45**: 1-19.

FERRÃO-FILHO,A.S. AND S.M.AZEVEDO 2003. Effects of unicellular and colonial forms of toxic *Microcystis aeruginosa* from laboratory cultures and natural populations on tropical cladocerans. Aquatic Ecology **37**: 23-35.

FERRÃO-FILHO,A.S., P.DOMINGOS, AND S.AZEVEDO 2002. Influences of a *Microcystis aeruginosa* Kutzing bloom on zooplankton populations in Jacarepagua Lagoon (Rio de Janeiro, Brazil). Limnologica **32**: 295-308.

FEUILLADE,M., G.JANN-PARA, AND J.FEUILLADE 1996. Toxic compounds to *Artemia* from blooms and isolates of the cyanobacterium *Planktothrix rubescens*. Arch.Hydrobiol. **138**: 175-186.

FISCHER,W.J., I.GARTHWAITE, C.O.MILES, K.M.ROSS, J.B.AGGEN, A.R.CHAMBERLIN, N.R.TOWERS, AND D.R.DIETRICH 2001. Congener-independent immunoassay for microcystins and nodularins. Environ.Sci.Technol. **35**: 4849-4856.

FOGG,G.E. and B.THAKE. 1975. Algal Cultures and Phytoplankton Ecology, 3 ed. University of Wisconsin Press.

FORBES,V.E. AND P.CALOW 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? Environ Toxicol Chem **18**: 1544-1556.

FRANK,C.A.P. 2002. Microcystin-producing cyanobacteria in recreational waters in southwestern Germany. Environ Toxicol **17**: 361-366.

FRYER,G. 1987. The feeding mechanisms of the Daphniidae (Crustacea: Cladocera) : recent suggestions and neglected considerations. J.Plankton Res. **9**: 419-432.

FRYER,G. 1991. Functional morphology and the adaptive radiation of the Daphniidae (Branchiopoda: Anomopoda). Philiosophical Transactions: Biological Sciences **331**: 1-99.

FUKAMI,K., T.NISHIJIMA, AND Y.ISHIDA 1997. Stimulative and inhibitory effects of bacteria on the growth of microalgae. Hydrobiologia **358**: 185-191.

FULTON,R.S. AND H.W.PAERL 1987. Toxic and inhibitory effects of the blue-green alga *Microcystis aeruginosa* herbivorous zooplankton. J.Plankton Res. **9**: 837-855.

FULTON,R.S. AND H.W.PAERL 1988. Effects of the blue-green alga *Microcystis aeruginosa* on zooplankton competitive relations. Oecologia **76**: 383-389.

GATES,S. 2002. Review of methodology of quantitative reviews using meta-analysis in ecology. Journal of Animal Ecology **71**: 547-557.

GENG,H., P.XIE, AND J.XU 2006. Effect of toxic *Microcystis aeruginosa* PCC7820 in combination with a green alga on the experimental population of *Brachionus calyciflorus* and *B. rubens*. Bull.Environ.Contam.Toxicol. **76**: 963-969.

GERRISTEN,J. AND K.G.PORTER 1982. The role of surface chemistry in filter feeding by zooplankton. sc **216**: 1225-1227.

GERRISTEN,J., K.G.PORTER, AND J.R.STRICKLER 1988. Not by sieving alone: observations of suspension feeding in *Daphnia*. Bulletin of Marine Science **43**: 336-376.

GHADOUANI,A. AND B.PINEL-ALLOUL 2002. Phenotypic plasticity in *Daphnia pulicaria* as an adaptation to high biomass of colonial and filamentous cyanobacteria: experimental evidence. J.Plankton Res. **24**: 1047-1056.

GHADOUANI,A., B.PINEL-ALLOUL, AND E.E.PREPAS 2003. Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. Freshwater Biol. **48**: 363-381.

GIANI,A., D.F.BIRD, Y.T.PRAIRIE, AND J.F.LAWRENCE 2005. Empirical study of cyanobacterial toxicity along a trophic gradient of lakes. Can J Fish Aquat Sci **62**: 2100-2109.

GILBERT,J.J. 1990. Differential effect of *Anabaena affins* on cladocerans and rotifers: mechanisms and implications. Ecology **71**: 1727-1740.

GILBERT,J.J. 1994. Susceptibility of planktonic rotifers to a toxic strain of *Anabaena flos-aquae*. Limnol.Oceanogr. **39**: 1286-1297.

GILBERT,J.J. 1996a. Effect of temperature on the response of planktonic rotifers to a toxic cyanobacterium. Ecology **77**: 1174-1180.

GILBERT,J.J. 1996b. Effects of food availability on the response of planktonic rotifers to a toxic strain of the cyanobaterium *Anabaena flos-aquae*. Limnol.Oceanogr. **41**: 1565-1572.

GILBERT,J.J. AND M.W.DURAND 1990. Effect of *Anabaena flos-aquae* on the abilities of *Daphnia* and *Keratella* to feed and reproduce on unicellular algae. Freshwater Biol. **24**: 577-596.

GILLOOLY,J.F. 2000. Effect of body size and temperature on generation time in zooplankton. J.Plankton Res. **22**: 241-251.

GLIWICZ,Z.M. 1990. Why do cladocerans fail to control algal blooms? Hydrobiologia **200/201**: 83-97.

GLIWICZ,Z.M. 2002. On the different nature of top-down and bottom-up effects in pelagic food web. Freshwater Biol. **47**: 2296-2312.

GLIWICZ,Z.M. AND W.LAMPERT 1990. Food thresholds in *Daphnia* species in the absence and presence of blue-green filaments. Ecology **71**: 691-702.

GLIWICZ,Z.M. AND E.SIEDLAR 1980. Food size limitation and algae interfering with food collection in *Daphnia*. Arch.Hydrobiol. **88**: 155-177.

GOBLER,C.J., T.W.DAVIS, K.J.COYNE, AND G.L.BOYER 2007. Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake. Harmful Algae **6**: 119-133.

GOTELLI,N.J. 1998. A Primer of Ecology, 2 ed. Sinauer Associates, Inc.

GRAHAM,J.L., J.R.JONES, S.B.JONES, AND T.E.CLEVENGER 2006. Spatial and temporal dynamics of microcystin in a Missouri Reservoir. Lake Reservior Manage **22**: 59-68.

GRAHAM,J.L., J.R.JONES, S.B.JONES, J.A.DOWNING, AND T.E.CLEVENGER 2004. Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. Water Res **38**: 4395-4404.

GROSS,E.M. 2003. Allelopathy of aquatic autotrophs. Critical Reviews in Plant Sciences **22(3&4)**: 313-319.

GUO,N. AND P.XIE 2006. Development of tolerance against toxic *Microcystis aeruginosa* in three cladocerans and the ecological implications. Environ.Pollut. **143**: 513-518.

GUREVITCH,J. AND L.V.HEDGES 1993. Meta-analysis: Combining the results of independent experiments, p. 378-398. *In* S.M.Scheiner and J.Gurevitch [eds.], Design and Analysis of Ecological Experiments. Chapman & Hall.

GUREVITCH,J. AND L.V.HEDGES 1999. Statistical issues in ecological meta-analyses. Ecology **80**: 1142-1149.

GUSTAFSSON,S. AND L.-A.HANSSON 2004. Development of tolerance against toxic cyanobacteria in *Daphnia*. Aquatic Ecology **38**: 37-44.

GUSTAFSSON,S., K.RENGEFORS, AND L.-A.HANSSON 2005. Increased consumer fitness following transfer of toxin tolerance to offspring via maternal effects. Ecology **86**: 2561-2567.

HAIDER,S., V.NAITHANI, P.N.VISWANATHAN, AND P.KAKKAR 2003. Cyanobacterial toxins: a growing environmental concern. Chemosphere **52**: 1-21.

HAIRSTON,N.G., C.L.HOLTMEIER, W.LAMPERT, L.J.WEIDER, D.M.POST, J.M.FISCHER, C.E.CÁCERES, J.A.FOX, AND U.GAEDKE 2001. Natural selection for grazer resistance to toxic cyanobacteria: evolution of phenotypic plasticity. Evolution **55**: 2203-2214.

HAMILTON,P.B., L.M.LEY, S.DEAN, AND F.R.PICK 2005. The occurrence of the cyanobacterium *Cylindrospermopsis raciborskii* in Constance Lake: an exotic cyanoprokaryote new to Canada. Phycologia **44**: 17-25.

HANAZATO,T. AND M.YASUNO 1987. Evaluation of *Microcystis* as food for zooplanktonin a europhic lake. Hydrobiologia **144**: 251-259.

HANEY,J.F. AND D.J.HALL 1973. Sugar-coated *Daphnia*: A preservation technique for Cladocera. Limnol.Oceanogr. **18**: 331-333.

HANSSON,L.-A., S.GUSTAFSSON, K.RENGEFORS, AND L.BOMARK 2007. Cyanobacterial chemical warfare affects zooplankton community composition. Freshwater Biol. **52**: 1290-1301.

HARADA,K., F.KONDO, AND L.A.LAWTON 1999. Laboratory analysis of cyanotoxins, p. 370-399. *In* I.Chorus and J.Bartram [eds.], Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. WHO.

HARADA,K., H.MURATA, Z.QIANG, M.SUZUKI, AND F.KONDO 1996. Mass spectrometric screening method for microcystins in cyanobacteria. Toxicon **34**: 701-710.

HARDIN,G. The competitive exclusion principle. Sience 131, 1292-1297. 1960.

HARRIS,D.C. 2003. Quantitative Chemical Analysis, 6 ed. Freeman, Basingstoke and Macmillan.

HARRIS,G.P. 1986. Phytoplankton Ecology. Chapman and Hall Ltd.

HARRIS,G.P. 1994. Pattern, process and prediction in aquatic ecology. A limnological view of some general ecological problems. Freshwater Biol. **32**: 143-160.

HARTMANN,H.J. AND D.D.KUNKEL 1991. Mechanisms of food selection in *Daphnia*. Hydrobiologia **225**: 129-154.

HAWKINS,P. AND W.LAMPERT 1989. The effect of *Daphnia* body size on filtering rate inhibition in the presence of a filamentous cyanobacterium. Limnol.Oceanogr. **34**: 1084-1089.

HAXTON,T.J. AND C.S.FINDLAY 2006. Meta-analysis of the ecological impacts of freshwater power management. Can J Fish Aquat Sci **submitted**.

HEALTH CANADA. Guidelines for Canadian drinking water quality: supporting documentation — Cyanobacterial toxins - microcystin-LR. 1-15. 2002. Ottawa, ON, Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada.

HENNING,M., H.HERTEL, J.G.WALL, AND J.G.KOHL 1991. Strain-specific influence of *Microcystis aeruginosa* on food ingestion and assimilation of some cladocerans and copepods. Int Rev Gesamten Hydrobiol **76**: 37-45.

HIETALA,J., C.LAURÉN-MÄÄTTÄ, AND M.WALLS 1997a. Life history responses of *Daphnia* clones to toxic *Microcystis* at different food levels. J.Plankton Res. **19**: 917-926.

HIETALA,J., C.LAURÉN-MÄÄTTÄ, AND M.WALLS 1997b. Sensitivity of *Daphnia* to toxic cyanobacteria: effects of genotype and temperature. Freshwater Biol. **37**: 299-306.

HIETALA,J., M.REINIKAINEN, AND M.WALLS 1995. Variation in life history responses of *Daphnia* to toxic *Microcystis aeruginosa*. J.Plankton Res. **17**: 2307-2318.

HISBERGUES,M., G.CHRISTIANSEN, L.ROUHIAINEN, K.SIVONEN, AND T.BORNER 2003. PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. Arch.Microbiol. **180**: 402-410.

HOLT,R.D. 2005. On the integration of community ecology and evolutionary biology: historical perspectives and current perspectives, p. 235-272. *In* B.E.Beisner and K.Cuddington [eds.], Ecological paradigms lost: Routes of theory change. Elsevier Academic Press.

HOTTO,A., M.SATCHWELL, AND G.BOYER 2005. Seasonal production and molecular characterization of microcystins in Oneida Lake, New York, USA. Environ Toxicol **20**: 243-248.

HSIEH,C. AND M.D.OHMAN 2006. Biological responses to environmental forcing: The linear tracking window hypothesis. Ecology **87**: 1932-1938.

HUISMAN,J., R.R.JONKER, C.ZONNEVELD, AND F.J.WEISSING 1999a. Competition for light between phytoplankton species: experimental tests of mechanistic theory. Ecology **80**: 211-222.

HUISMAN,J., P.VAN OOSTVEEN, AND F.J.WEISSING 1999b. Critical depth and critical turbulence: Two different mechanisms for the development of phytoplankton blooms. Limnol.Oceanogr. **44**: 1781-1787.

HUISMAN,J., P.VAN OOSTVEEN, AND F.J.WEISSING 1999c. Species dynamics in phytoplankton blooms: Incomplete mixing and competition for light. American Naturalist **154**: 46-68.

HUMMERT,CH., J.DAHLMANN, K.REINHARDT, H.PH.H.DANG, D.K.DANG, AND B.LUCKAS 2001. Liquid chromatography - mass spectrometry identification of microcystins in *Microcystis aeruginosa* strain from Lake Thanh Cong, Hanoi, Vietnam. Chromatographia **54**: 569-575.

HUTCHINSON,G.E. 1961. The paradox of the plankton. The American Naturalist **95**: 137-145.

HYENSTRAND,P., P.BLOMQVIST, AND A.PETTERSSON 1998. Factors determiing cyanobacterial success in aquatic systems- a literature review. Arch.Hydrobiol.Spec.Is.Adv.Limnol. **15**: 41-62.

INFANTE,A. AND S.E.B.ABELLA 1985. Inhibition of *Daphnia* by *Oscillatoria* in Lake Washington. Limnol.Oceanogr. **30**: 1046-1052.

ISO. Water quality - Determination of microcystins - Method using solid phase extraction (SPE) and high performance liquid chromotography (HPLC) with ultraviolet (UV) detection. 1, -17. 2005. International Organization for Standardization.

JACOBY,J.M., D.C.COLLIER, E.B.WELCH, F.J.HARDY, AND M.CRAYTON 2000. Environmental factors associated with a toxic bloom of Microcystis aeruginosa. Can.J.Fish.Aquat.Sci. **57**: 231-240.

JANG,M.H., J.M.JUNG, AND N.TAKAMURA 2007. Changes in microcystin production in cyanobacteria exposed to zooplankton at different population densities and infochemical concentrations. Limnol.Oceanogr. **52**: 1454-1466.

JANN-PARA,G., I.SCHWOB, AND J.FEUILLADE 2004. Occurrence of toxic *Planktothrix rubescens* blooms in lake Nantua, France. Toxicon **43**: 279-285.

JANSE,I., W.E.A.KARDINAAL, M.K.AGTERVELD, M.MEIMA, P.M.VISSER, AND G.ZWART 2005. Contrasting microcystin production and cyanobacterial population dynamics in two *Planktothrix* dominated freshwater lakes. Environ Microb **7**: 1514-1524.

JAYATISSA,L.P., E.I.L.SILVA, J.MCELHINEY, AND L.A.LAWTON 2006. Occurrence of toxigenic cyanobacterial blooms in freshwaters of Sri Lanka. Syst Appl Microbiol **29**: 156-164.

JEFFREY,S.W. AND G.F.HUMPHREY 1975. New spectrophotometric equations for determining chlorophyll a, b, c1 and c2 in higher plants, algae and natural phytoplankton . Biochem Physiol Pflanzen **167**: 191-194.

JOHNSON,C.N. 1998. Species extinction and the relationship between distribution and abundance. Nature **394**: 272-274.

JOHNSTON,B.R. AND J.M.JACOBY 2003. Cyanobacterial toxicity and migration in a mesotrophic lake in western Washington, USA. Hydrobiologia **495**: 79-91.

JONES,G.J. AND 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 Res **28**: 871-876.

JONES,R.I. 1978. Adaptations to fluctuating irradiance by natural phytoplankton communities. Limnol.Oceanogr. **23**: 920-926.

JONES,S.B. AND J.R.JONES 2002. Seasonal variation in cyanobacterial toxin production in two Nepalese lakes. Verh.Internat.Verein.Limnol. **28**: 1017-1022.

JUNGMANN,D. 1992. Toxic compounds isolated from *Microcystis* PCC7806 that are more active against *Daphnia* than two microcystins. Limnol.Oceanogr. **37**: 1777-1783.

JURCZAK,T., M.TARCZYNSKA, K.KARLSSON, AND J.MERILUOTO 2004. Characterization and diversity of cyanobacterial hepatotoxins (microcystins) in blooms from Polish freshwater identified by liquid chromatography-electrospray ionization mass spectrometry. Chromatographia **59**: 571-578.

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

KARDINAAL,W.E.A., I.JANSE, M.KAMSTVAN AGTERVELD, M.MEIMA, J.SNOEK, L.R.MUR, J.HUISMAN, G.ZWART, AND P.M.VISSER 2007a. Microcystis genotype succession in relation to microcystin concentrations in freshwater lakes. Aquat Microb Ecol **48**: 1-12.

KARDINAAL,W.E.A., L.TONK, I.JANSE, S.HOL, P.SLOT, J.HUISMAN, AND P.M.VISSER 2007b. Competition for light between toxic and nontoxic strains of the harmful cyanobacterium *Microcystis*. Appl.Environ.Microbiol. **73**: 2939-2946.

KEATING,K.I. 1977. Allelopathic influence on blue-green bloom sequence in a eutrophic lake. sc **196**: 885-887.

KEATING,K.I. 1978. Blue-green algal inhibition of diatom growth: Transition from mesotrophic to eutrophic community structure. sc **199**: 971-973.

KEMP,A. AND J.JOHN 2006. Microcystins associated with *Microcystis* dominated blooms in the southwest wetlands, western Australia. Environ Toxicol **21**: 125-130.

KENEFICK,S.L., S.E.HRUDEY, H.G.PETERSON, AND E.E.PREPAS 1993. Toxin release from *Microcystis aeruginosa* after chemical treatment. Water Sci.Technol. **27**: 433-440.

KERFOOT,W.C. AND K.L.KIRK 1991. Degree of taste discrimination among suspension-feeding cladocerans and copepods: Implications for detritivory and herbivory. Limnol.Oceanogr. **36**: 1107-1123.

KILHAM,P. AND R.E.HECKY 1988. Comparative ecology of marine and freshwater phytoplankton. Limnol.Oceanogr. **33**: 776-795.

KIRK,K.L. AND J.J.GILBERT 1992. Variation in herbivore response to chemical defenses: zooplankton foraging on toxic cyanobacteria. Ecology **73**: 2208-2217.

KOMÁREK,J. 1991. A review of water-bloom-forming *Microcystis* species, with regard to populations from Japan. Algological Studies **64**: 115-127.

KOMÁREK,J. 1999. Survey of investigated species of *Aphanizomenon* and *Anabaena*. Unpublished

KOMÁREK,J. 2003. Coccoid and colonial cyanobacteria, p. 59-116. *In* J.D.Wehr, R.G.Sheath, and J.H.Thorp [eds.], Freshwater Algae of North America: Ecology and Classification. Academic Press.

KOMÁREK,J. AND K.ANAGNOSTIDIS 1989. Modern approaches to the classification system of cyanophytes. 4. Nostocales. Archiv für Hydrobiologie **56**: 247-345.

------. Cyanoprokaryota 1. Teil: Chroococcales. 1, -548. 1998. Stuttgart, Fischer Verlag. Süsswasserflora von Mitteleuropa 19/1.

KOMÁREK,J., H.KLING, AND J.KOMARKOVA 2003. Filamentous cyanobacteria, p. 117-196. *In* J.D.Wehr, R.G.Sheath, and J.H.Thorp [eds.], Freshwater Algae of North America: Ecology and Classification. Academic Press.

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. Can J Fish Aquat Sci **57**: 1584-1593.

KOTAK,B.G., A.K.Y.LAM, E.E.PREPAS, S.L.KENEFICK, AND S.E.HRUDEY 1995. Variability of the hepatoxin microcystin-LR in hypereutrophic drinking water lakes. J Phycol **31**: 248-262.

KOTAK,B.G., R.W.ZURAWELL, E.E.PREPAS, AND C.F.B.HOLMES 1996. Microcystin-LR concentrations in aquatic food web compartments from lakes of varying trophic status. Can J Fish Aquat Sci **53**: 1974-1985.

KRIVTSOV,V., E.G.BELLINGER, AND D.C.SIGEE 2005. Elemental composition of *Microcystis aeruginosa* under conditions of lake nutrient depletion. Aquatic Ecology **39**: 123-134.

KUBWABO,C., N.VAIS, AND F.M.BENOIT 2004. Identification of microcystin-RR and [Dha7] microcystin-RR in commercial standards by electrospray ionization mass spectrometry. Journal of AOAC International **87**: 1028-1031.

KUIPER-GOODMAN,T., I.R.FALCONER, AND J.FITZGERALD 1999. Human health aspects, p. 126-160. *In* I.Chorus and J.Bartram [eds.], Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. World Health Organisation.

KUMAR,R. AND T.R.RAO 1999. Demographic responses of adult *Mesocyclops thermocyclopoides* (Copepoda, Cyclopoida) to different plant and animal diets. Freshwater Biol. **42**: 487-501.

KURMAYER,R. 2001. Competitive ability of *Daphnia* under dominance of non-toxic filamentous cyanobacteria. Hydrobiologia **442**: 289.

KURMAYER,R., G.CHRISTIANSEN, AND I.CHORUS 2003. The abundance of microcystin-producing genotypes correlates positively with colony size in *Microcystin* sp. and determines its microcystin net production in Lake Wansee. Appl.Environ.Microbiol. **69**: 787-795.

KURMAYER,R., E.DITTMAN, J.FASTNER, AND I.CHORUS 2002. Diversity of microcystin genes within a population of the toxic cyanobacterium *Microcystis spp.* in Lake Wannsee (Berlin, Germany). Microb Ecol **43**: 107-118.

LAM,A.K.Y., E.E.PREPAS, D.SPINK, AND S.E.HRUDEY 1995. Chemical control of hepatotoxic phytoplankton blooms: Implications for human health. Water Res **29**: 1845-1854.

LAMPERT,W. 1974. A method for determining food selection by zooplankton. Limnol.Oceanogr. **19**: 995-998.

LAMPERT,W. 1981. Inhibitory and toxic effects of blue-green algae on *Daphnia*. Int Rev Gesamten Hydrobiol **66**: 285-298.

LAMPERT,W. 1987. Laboratory studies on zooplankton-cyanobacteria interactions. New Zealand Journal of Marine and Freshwater Research **21**: 483-490.

LANDIS,W.G., J.MANKIEWICZ, R.A.MATTHEWS, AND G.B.MATTHEWS 2000. A test of the community conditioning hypothesis: Persistence of effects in model ecological structures dosed with the jet fuel JP-8. Environ Toxicol Chem **19**: 327-336.

LASLETT,G.M., R.M.CLARK, AND G.J.JONES 1997. Estimating the precision of filamentous blue-green algae cell concentration from a single sample. Environmetrics **8**: 313-339.

LATHROP,R.C. 1999. Summer water clarity responses to phosphorus, *Daphnia* grazing, and internal mixing in Lake Mendota. Limnol.Oceanogr. **44**: 137-146.

LAWTON,L.A., C.EDWARDS, AND G.A.CODD 1994. Extraction and High-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. Analyst **119**: 1525-1530.

LI,R., M.WATANABE, AND M.M.WATANABE 2000. Taxonomic studies of planktic species of *Anabaena* based on morphological characteristics in cultured strains. Hydrobiologia **438**: 117-138.

LINDENSCHMIDT,K.E. AND I.CHORUS 1998. The effect of water column mixing on phytoplankton succession, diversity and similarity. J.Plankton Res. **20**: 1927-1951.

LINDHOLM,T., P.VESTERKVIST, L.SPOOF, C.LUNDBERG-NIINISTO, AND J.MERILUOTO 2003. Microcystin occurrence in lakes in Aland, SW Finland. Hydrobiologia **505**: 129-138.

LONG,B.M., G.J.JONES, AND P.T.ORR 2001. Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate. Appl.Environ.Microbiol. **67**: 278-283.

LUNDSTEDT,L. AND M.T.BRETT 1991. Differential growth rates of three cladoceran species in response to mono- and mixed- algal cultures. Limnol.Oceanogr. **36**: 159-165.

LÜRLING,M. 2003a. Daphnia growth on microcystin-producing and microcystin-free *Microcystis aeruginosa* in different mixtures with the green alga *Scenedesmus obliquus*. Limnol.Oceanogr. **48**: 2214-2220.

LÜRLING,M. 2003b. Effects of microcystin-free and microcystin-containing strains of the cyanobacterium *Microcystis aeruginosa* on growth of the grazer *Daphnia magna*. Environ Toxicol **18**: 202-210.

LÜRLING,M. AND E.VAN DER GRINTEN 2003. Life-history characteristics of *Daphnia* exposed to dissolved microcystin-LR and to the cyanobacterium *Microcystis aeruginosa* with and without microcystins. Environ Toxicol Chem **22**: 1281-1287.

LYNCH,M. 1980. The evolution of cladoceran life histories. The Quarterly Review of biology **55**: 23-42.

LYNCH,M. AND J.SHAPIRO 1981. Predation, enrichment, and phytoplankton community structure. Limnol.Oceanogr. **26**: 86-102.

MAATOUK,I., N.BOUAICHA, D.FONTAN, AND Y.LEVI 2002. Seasonal variation of microcystin concentrations in the Saint-Caprais reservoir (France) and their removal in a small full-scale treatment plant. Water Res **36**: 2891-2897.

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 Lett. **264**: 187-192.

MANAGE,P.M., Z.KAWABATA, AND S.NAKANO 2000. Algicidal effect of the bacterium *Alcaligenes denitrificans* on *Microcystis* spp. Aquat Microb Ecol **22**: 111-117.

MANKIEWICZ,J., J.KOMARKOVA, K.IZYDORCZYK, T.JURCZAK, M.TARCZYNSKA, AND M.ZALEWSKI 2005. Hepatotoxic cyanobacterial blooms in the lakes of Nothern Poland. Environ Toxicol **20**: 499-506.

MANLY,B.F.J. 2007. Randomization, bottstrap and monte carlo methods in biology, 3 ed. Chapman & Hall/CRC.

MARSHALL,C.T. AND R.H.PETERS 1989. General patterns in the seasonal development of chlorophyll *a* for temperate lakes. Limnol.Oceanogr. **34**: 856-867.

MARTIN-CREUZBURG,D., A.WACHER, AND E.VON ELERT 2005. Life history consequences of sterol availability in the aquatic keystone species *Daphnia*. Oecologia **144**: 362-372.

MATHYS,W. AND B.SURHOLD 2004. Analysis of microcystins in freshwater samples using high performance liquid chromotography and en enzyme-linked immunosorbent assay. Int.J.Hyg.Environ.Health **207**: 601-605.

MAY,R.M. 1976. Simple mathematical models with very complicated dynamics. Nature **261**: 459-470.

MCELHINEY,J. AND L.A.LAWTON 2005. Detection of the cyanobacterial hepatotoxins microcystins. Toxicol.Appl.Pharmacol. **203**: 219-230.

MCQUEEN,D.J. AND D.R.S.LEAN 1987. Influence of water temperature and nitrogen to phosphorus ratios on the dominance of blue-green algae in Lake St. George, Ontario. Can J Fish Aquat Sci -598.

MEHNER,T., J.BENNDORF, P.KASPRZAK, AND R.KOSCHEL 2002. Biomanipulation of lake ecosystems: successful applications and expanding complexity in the underlying science. Freshwater Biol. **47**: 2453-2465.

METCALF,J.S., K.A.BEATTIE, J.RESSLER, S.GERBERSDORF, S.PFLUGMACHER, AND G.A.CODD 2002. Cross-reactivity and performance assessment of four microcystin immunoassays with detoxication products of the cyanobacterial toxin, microcystin-LR. Journal of Water Supply: Research and Technology - AQUA **51**: 145-151.

METCALF,J.S. AND G.A.CODD 2003. Analysis of cyanobacterial toxins by immunological methods. Chem.Res.Toxicol. **16**: 1-8.

METCALF,J.S., P.HYENSTRAND, K.A.BEATTIE, AND G.A.CODD 2000. Effects of physiochemical variables and cyanobacterial extracts on the immunoassay of microcystin-LR by two ELISA kits. Journal of Applied Microbiology **89**: 532-538.

MILLER,A.G., G.S.ESPIE, AND D.T.CANVIN 1991. Active CO_2 transport in cyanobacteria. Can.J.Bot. **69**: 925-935.

MORENO,I., G.REPETTO, E.CARBALLAL, A.GAGO, AND A.M.CAMEAN 2005. Cyanobacteria and microcystins occurrence in the Guadiana River (SW Spain). Int.J.Environ.Anal.Chem. **85**: 461-474.

MORENO,I.M., P.PEREIRA, S.FRANCA, AND A.CAMEAN 2004. Toxic cyanobacteria strains isolated from blooms in the Guadiana River (southwestern Spain). Biol Res **37**: 405-471.

MOUNTFORT,D.O., P.HOLLAND, AND J.SPROSEN 2005. Method of detecting classes of microcystins by combination of protein phosphatase inhibition assay and ELISA: comparison with LC-MS. Toxicon **45**: 199-206.

MOURELATOS,S. AND G.LACROIX 1990. In situ filtering rates of Cladoceran: Effect of body lenght, temperature, and food concentration. Limnol.Oceanogr. **35**: 1101-1111.

MSAGATI,T.A.M., B.A.SIAME, AND D.D.SHUSHU 2006. Evaluation of methods for the isolation, detection and quantification of cyanobacterial hepatotoxins. Aquatic Toxicology **78**: 382-397.

MULLER-NAVARRA,D.C., M.T.BRETT, A.M.LISTON, AND C.R.GOLDMAN 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature **406**: 74-76.

MUR,L.R., O.M.SKULBERG, AND H.UTKILEN 1999. Cyanobacteria in the environment, p. 21-40. *In* I.Chorus and J.Bartram [eds.], Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. World Health Organization.

MURPHY,T.P., K.IRVINE, J.GUO, J.DAVIES, H.MURKIN, M.CHARLTON, AND S.B.WATSON 2003. New microcystin concerns in the lower Great Lakes. Water Qual Res J Can **38**: 127-140.

NAKAGAWA,S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. Behavioral Ecology **15**: 1044-1045.

NALEWAJKO,C. AND T.P.MURPHY 2001. Effects of temperature, and availability of nitrogen and phosphorus on the abundance of *Anabaena* and *Microcystis* in Lake Biwa, Japan: an experimental approach. Limnol **2**: 45-48.

NANDINI,S. AND T.R.RAO 1998. Somatic and population growth in selected cladoceran and rotifer species offered the cyanobacterium *Microcystis aeruginosa* as food. Aquatic Ecology **31**: 283-298.

NANDINI,S., S.S.S.SARMA, AND P.RAMIREZ-GARCIA 2000. Life table demography and population growth of *Daphnia laevis* (Cladocera, Anomopoda) under different densities of *Chlorella vulgaris* and *Microcystis aeruginosa*. Crustaceana **73**: 1273-1286.

NASRI,A., N.BOUAICHA, AND J.FASTNER 2004. First report of a microcystin-containing bloom of cyanobacteria *Microcystis* spp. in Lake Oubeira, Eastern Algeria. Arch.Environ.Contam.Toxicol. **46**: 197-202.

NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL. Australian Drinking Water Guidelines. 1-376. 1996. Agriculture and Resource Management Council of Australia and New Zealand.

NIMON,K., M.LEWIS, R.KANE, AND R.M.HAYNES 2008. An R package to compute commonality coefficients in the multiple regression case: An introduction to the package and practical examples. Behavior Research Methods **40**: 457-466.

NIZAN,S., D.DIMENTMAN, AND M.SHILO 1986. Acute toxic effects of the cyanobacterium *Microcystis aeruginosa* on *Daphnia magna*. Limnol.Oceanogr. **31**: 497-502.

ONTARIO MINISTRY OF THE ENVIRONMENT. Handbook of analytical methods for environmental samples. Vols. 1 and 2. 1983. Toronto, Ontario, Canada.

OOMS-WILMS,A.L. 1997. Are bacteria an important food source for rotifers in eutrophic lakes? J.Plankton Res. **19**: 1125-1141.

ORCUTT,J.D. AND M.L.PACE 1984. Seasonal dynamics of rotifer and crustacean zooplankton populations in a eutrophic monomictic lake with a note on rotifer sampling techniques. Hydrobiologia **119**: 73-80.

ORR,P.T. AND G.J.JONES 1998. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. Limnol.Oceanogr. **43**: 1604-1614.

ORTEA,P.M., O.ALLIS, B.M.HEALY, M.LEHANE, A.N.SHUILLEABHÁIN, A.FUREY, AND K.J.JAMES 2004. Determination of toxic cyclic heptapeptides by liquid chromatography with detection using ultra-violet, protein phosphatase assay and tandem mass spectrometry. Chemosphere **55**: 1395-1402.

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. Environ Toxicol **20**: 270-276.

PACE,M.L., J.J.COLE, AND S.R.CARPENTER 1998. Trophic cascades and compensation: differential responses of microzooplankton in whole-lake experiments. Ecology **79**: 138-152.

PADILLA,C., S.SANZ-ALFEREZ, AND F.F.DEL CAMPO 2006. Toxin characterization and identification of a *Microcystis flos-aquae* strain from a Spanish drinking-water reservoir. Arch.Hydrobiol. **165**: 383-399.

PARK,H., C.IWAMI, M.F.WATANABE, K.HARADA, T.OKINO, AND H.HAYASHI 1998. Temporal variabilities of the concentrations of intra- and extracellular microcystin and toxic *Microcystis* species in a hypertrophic lake, Lake Suwa, Japan (1991-1994). Environ Toxicol Water Qual **13**: 61-72.

PETERS,R.H. 1984. Methods for the study of feeding, grazing and assimilation by zooplankton, p. 336-412. *In* J.A.Downing and F.H.Rigler [eds.], A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific Publications.

PFLUGMACHER,S., C.WIEGAND, A.OBEREMM, K.A.BEATTIE, E.KRAUSE, G.A.CODD, AND C.E.W.STEINBERG 1998. Identification of an enzymatically formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR:the first step of detoxication. Biochim.Biophys.Acta **1425**: 527-533.

PICK,F.R. 1991. The abundance and composition of freshwater picocyanobacteria in relation to light penetration. Limnol.Oceanogr. **36**: 1457-1462.

PICK,F.R. AND D.R.S.LEAN 1987. The role of macronutrients (C,N,P) in controlling cyanobacterial dominance in temperate lakes. New Zealand Journal of Marine and Freshwater Research **21**: 425-434.

PIETSCH,C., C.WIEGAND, M.V.AMÉ, A.NICKLISCH, D.WUNDERLIN, AND S.PFLUGMACHER 2001. The effects of a cyanobacterial crude extract on different aquatic organisms: evidence for cyanobacterial toxin modulating factors. Environ Toxicol **16**: 535-542.

PLATH,K. 1998. Adaptive feeding behavior of *Daphnia magna* in response to short-term starvation. Limnol.Oceanogr. **43**: 593-599.

PORTER,K.G. 1973. Selective grazing and differential digestion of algae by zooplankton. Nature **244**: 179-180.

PORTER,K.G. AND R.MCDONOUGH 1984. The energetic cost of response to blue-green algal filaments by cladocerans. Limnol.Oceanogr. **29**: 365-369.

PORTER,K.G. AND J.D.ORCUTT 1980a. Nutritional adequacy, manageability, and toxicity as factors that determine the food quality of green and blue-green algae for *Daphnia*. American Society of Limnology and Oceanography Special Symposium **3**: 268-280.

PORTER,K.G. AND J.D.ORCUTT 1980b. Nutritional adequacy, manageability, and toxicity as factors that determine the food quality of green and blue-green algae for *Daphnia*, p. 268-281. *In* W.C.Kerfoot [ed.], Evolution and ecology of zooplankton communities. University Press of New England.

POWER,M.E. 1992. Top-down and bottom-up forces in food webs: Do plants have primacy? Ecology **73**: 733-746.

RANTALA,A., D.P.FEWER, M.HISBERGUES, L.ROUHIAINEN, J.VAITOMAA, T.BORNER, AND K.SIVONEN 2004. Phylogenetic evidence for the early evolution of microcystin synthesis. PNAS **101**: 568-573.

REINIKAINEN,M. 1994. Effects of Microcystis spp. and Selected Cyanotoxins. Algological Studies **75**: 229-237.

REINIKAINEN,M., M.KETOLA, AND M.WALLS 1994. Effects of the concentration of toxic *Microcystis aeruginosa* and an alternative food on the survival of *Daphnia pulex*. Limnol.Oceanogr. **39**: 424-432.

REPKA,S. 1996. Inter and intraspecific differences in *Daphnia* life histories response to two food sources: the green alga *Scenedesmus* and the filamentous cyanobacterium *Oscillatoria*. J.Plankton Res. **18**: 1213-1233.

REPKA,S. 1997. Effects of food type on the life history of *Daphnia* clones from lakes differing in trophic state. I. *Daphnia galeata* feeding on *Scenedesmus* and *Oscillatoria*. Freshwater Biol. **37**: 675-683.

REPKA,S. 1998. Effects of food type on the life history of *Daphnia* clones from lakes differing in trophic state. II *Daphnia cucullata* feeding on mixed diets. Freshwater Biol. **38**: 685-692.

REYNOLDS,C.S. 1984a. Phytoplankton periodicity: the interactions of form, function and environmental variabillity. Freshwater Biol. **14**: 111-142.

REYNOLDS,C.S. 1984b. The ecology of freshwater phytoplankton. Cambridge University Press.

REYNOLDS,C.S. 1990. Temporal scales of variability in pelagic environments and the response of phytoplankton. Freshwater Biol. **23**: 25-53.

REYNOLDS,C.S. 1998. What factors influence the species composition of phytoplankton in lakes of different trophic status? Hydrobiologia **369/370**: 11-26.

REYNOLDS,C.S., M.DOKULIL, AND J.PADISAK 2000. Understanding the assembly of phytoplankton in relation to the trophic spectrum: where are I now? Hydrobiologia **424**: 147-152.

REYNOLDS,C.S., V.L.HUSZAR, C.KRUK, L.NASELLI-FLORES, AND S.MELO 2002. Towards a functional classification of the freshwater phytoplankton. J.Plankton Res. **24**: 417-428.

REYNOLDS,C.S., R.L.OLIVER, AND A.E.WALSBY 1987. Cyanobacterial dominance: the role of buoyancy regulation in dynamic lake environments. New Zealand Journal of Marine and Freshwater Research **21**: 379-390.

REYNOLDS,C.S. AND A.C.PETERSEN 2000. The distribution of planktonic Cyanobacteria in Irish lakes in relation to their trophic status. Hydrobiologia **424**: 91-99.

REYNOLDS,C.S. AND A.E.WALSBY 1975. Water-blooms. Biological Reviews of the Cambridge Philosophical Society **50**: 437-481.

REYNOLDS,C.S., S.W.WISEMAN, AND M.J.O.CLARKE 1984. Growth and loss rate responses of phytoplankton to intermittent artificial mixing and their potential application to the control of planktonic algal biomass. Journal of Applied Ecology **21**: 11-39.

RINEHART,K.L., M.NAMIKOSHI, AND B.W.CHOI 1994. Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). J Appl Phycol **6**: 159-176.

ROBARTS,R.D. AND 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.

ROHRLACK,T., K.CHRISTOFFERSEN, E.DITTMAN, I.NOGUEIRA, V.VASCONCELOS, AND T.BORNER 2005. Ingestion of microcystins by *Daphnia*: Intestinal uptake and toxic effects. Limnol.Oceanogr. **50**: 440-448.

ROHRLACK,T., K.CHRISTOFFERSEN, M.KAEBERNICK, AND B.A.NEILAN 2004. Cyanobacterial protease inhibitor microviridin J causes a lethal molting disruption in *Daphnia pulicaria.* Appl.Environ.Microbiol. **70**: 5047-5050.

ROHRLACK,T., E.DITTMAN, T.BÖRNER, AND K.CHRISTOFFERSEN 2001. Effects of cell-bound microcystins on survival and feeding of *Daphnia* spp. Appl.Environ.Microbiol. **67**: 3523-3529.

ROJO,C. AND M.ALVAREZ-COBELAS 2000. A plea for more ecology in phytoplankton ecology. Hydrobiologia **424**: 141-146.

ROLLAND,A., D.F.BIRD, AND A.GIANI 2005. Seasonal changes in composition of the cyanobacterial community and the occurrence of hepatotoxic blooms in the eastern townships, Quebec, Canada. J.Plankton Res. **27**: 683-694.

ROSENBERG,M.S. 2006. The file drawer problem revisted: A general weighted method for calculating fail-safe numbers in meta-analysis. Evolution **59**: 464-468.

ROTHHAUPT,K.O. 1991. The influence of toxic and filamentous blue-green algae on feeding and population growth of the rotifer *Brachionus rubens*. Int.Revues ges.Hydrobiol. **76**: 67-72.

SABOUR,B., M.LOUDIKI, B.OUDRA, V.VASCONCELOS, R.MARTINS, S.OUBRAIM, AND B.FAWZI 2002. Toxicology of a *Microcystis ichthyoblabe* waterbloom from Lake Oued Mellah (Morocco). Environ Toxicol **17**: 24-31.

SARNELLE,O. 1993. Herbivore effects on phytoplankton succession in a eutrophic lake. Ecological Monographs **63**: 129-149.

SARNELLE,O. 2007. Initial conditions mediate the interaction between *Daphnia* and bloom-forming cyanobacteria. Limnol.Oceanogr. **52**: 2120-2127.

SARNELLE,O. AND A.E.WILSON 2005. Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria. Limnol.Oceanogr. **50**: 1565-1570.

SARTONOV,A. 1995. Effects of *Microcystis aeruginosa* on interference competition between *Daphnia pulex* and *Keratella cochlearis*. Hydrobiologia **307**: 117-126.

SCHEFFER,M. 1998. Ecology of Shallow Lakes, 1 ed. Kluwer Academic Publishers.

SCHINDLER,D.W. and J.R.VALLENTYNE. 2008. The algal bowl: overfertilization of the world's freshwaters and estuaries. University of Alberta Press.

SCHOPF,J.W. 1993. Microfossils of the early archean apex chert: New evidence of the antiquity of life. sc **260**: 640-646.

SCOTT,W.E. 1991. Occurrence and significance of toxic cyanobacteria in Southern Africa. Water Sci.Technol. **23**: 175-180.

SEIBOLD,D.R. AND R.D.MCPHEE 1979. Commonality analysis: a method for decomposing explained variance in multiple regression analysis. Human Communication Research **5**: 355-366.

SEKADENDE,B.C., T.J.LYIMO, AND R.KURMAYER 2005. Microcystin production by cyanobacteria in the Mwanza Gulf (Lake Victoria, Tanzania). Hydrobiologia **543**: 299-304.

SHAPIRO,J. Current beliefs regarding dominance by blue-greens: the case for the importance of CO2 and pH. 1989. 24th Congress of the International Association of Theoretical and Applied Limnology. 1989.

SHAPIRO,J. 1997. The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes. Freshwater Biol. **37**: 307-323.

SHEN,P.P., Q.SHI, Z.C.HUA, F.X.KONG, Z.G.WANG, S.X.ZHUANG, AND D.C.CHEN 2003. Analysis of microcystins in cyanobacteria blooms and surface water samples from Meiliang Bay, Taihu Lake, China. Environ Int **29**: 641-647.

SHURIN,J.B. AND S.I.DODSON 1997. Sublethal toxic effects of cyanobacteria and nonylphenol on environmental sex determination and development in *Daphnia*. Environ Toxicol Chem **16**: 1269-1276.

SIVONEN,K. 1996. Cyanobacteria toxins and toxin production. Phycologia **35**: 12-24.

SIVONEN,K. AND G.J.JONES 1999. Cyanobacterial Toxins, p. 41-111. *In* I.Chorus and J.Bartram [eds.], Toxic cyanobacteria in water - A guide to their public health consequences, monitoring and management. E & FN Spoon.

SMITH,A.D. AND J.J.GILBERT 1995. Relative susceptibilities of rotifers and cladocerans to *Microcystis aeruginosa*. Arch.Hydrobiol. **132**: 309-336.

SMITH,V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. sc **221**: 669-671.

SOMMER,U., Z.M.GLIWICZ, W.LAMPERT, AND A.DUNCAN 1986. The PEG model of seasonal succession of planktonic events in fresh waters. Arch.Hydrobiol. **106**: 433-471.

SONG,L., T.SANO, R.LI, M.WATANABE, Y.LIU, AND K.KAYA 1998. Microcystin production of *Microcystis virdis* (cyanobacteria) under different culture conditions. Phycological Research **46**: 19-23.

STARKWEATHER,P.L. 1981. Trophic relationships between the rotifer *Brachionus calyciflorus* and the blue-green alga *Anabaena flos-aquae*. Verh Int Verein Limnol **21**: 1514.

STARKWEATHER,P.L. AND P.E.KELLAR 1983. Utilization of cyanobacteria by *Brachionus calyciflorus: Anabaena flos-aquae* (NRC-44-1) as a sole or complementary food source. Hydrobiologia **104**: 373-377.

STOMP,M., M.A.VAN DIJK, H.M.J.VAN OVERZEE, M.T.WORTEL, C.A.M.SIGON, M.EGAS, H.HOOGVELD, H.J.GONS, AND J.HUISMAN 2008. The timescale of phenotypic plasticity and its impact on competition in fluctuating environments. The American Naturalist **172**: E169-E185.

STÜCKEN,A. AND.C.WIEDNER. 2008.A pictoral key to *Nostocales*.
Unpublished Work

SUNDA,W.G., E.GRANELI, AND C.J.GOBLER 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. J Phycol **42**: 963-974.

THORP,J.H. and A.P.COVICH. 2001. Ecology and classification of North American freshwater invertebrates. Academic Press.

THOSTRUP,L. AND K.CHRISTOFFERSEN 1999. Accumulation of microcystin in *Daphnia magma* feeding on toxic *Microcystis*. Arch.Hydrobiol. **145**: 447-467.

TILLET,D., E.DITTMAN, M.ERHARD, H.VON DOHREN, T.BORNER, AND B.A.NEILAN 2000. Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide-polyketide synthetase system. Chemistry and Biology **7**: 753-764.

TILLMANNS,A.R., F.R.PICK, AND R.ARANDA-RODRIGUEZ 2007. Sampling and analysis of microcystins: Implications for the development of standardized methods. Environ Toxicol **22**: 132-143.

TILLMANNS,A.R., A.E.WILSON, F.R.PICK, AND O.SARNELLE 2008. Meta-analysis of cyanobacteria effects on zooplankton population growth rate: species-specific responses. Fundamental and Applied Limnology **171**: 285-295.

TILMAN,D. AND J.A.DOWNING 1994. Biodiversity and stability in grasslands. Nature **367**: 363-365.

TILMAN,D., M.MATTSON, AND S.LANGER 1981. Competition and nutrient kinetics along a temperature gradient: An experimental test of a mechanistic approach to niche theory. Limnol.Oceanogr. **26**: 1020-1033.

TURCHIN,P. 1990. Rarity of density dependence or population regulation with lags? Nature **344**: 660-663.

TURCHIN,P. 1999. Population regulation: A synthetic view. Oikos **84**: 153-159.

UTKILEN,H., J.FASTNER, AND J.BARTRAM 1999. Fieldwork: site inspection and sampling, p. 1-17. *In* I.Chorus and J.Bartram [eds.], Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. World Health Organization.

VAN DONK,E. AND D.O.HESSEN 1993. Grazing resistance in nutrient-stressed phytoplankton. Oecologia **93**: 508-511.

VAN GREMBERGHE,I., J.VAN WICHELEN, K.VAN DER GUCHT, P.VANORMELINGEN, S.D'HONDT, C.BOUTTE, A.WILMOTTE, AND W.VYVERMAN 2008. Covariation between zooplankton composition and cyanobacterial community dynamics in Lake Blaarmeersen (Belgium). FEMS Microbial Ecology **63**: 222-237.

VARDI,A., D.SCHATZ, K.BEERI, U.MOTRO, A.SUKENIK, A.LEVINE, AND A.KAPLAN 2002. Dinoflagellate-cyanobacterium communication may determine the composition of phytoplankton assemblage in a mesotrophic lake. Current Biology **12**: 1767-1772.

VERHAGEN,J.H.G. 1994. Modeling phytoplankton patchiness under the influence of wind-driven currents in lakes. Limnol.Oceanogr. **39**: 1551-1565.

VEZIE,C., J.RAPALA, J.VAITOMAA, J.SEITSONEN, AND K.SIVONEN 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis* strains and on intracellular microcystin concentrations. Microb Ecol **43**: 443-454.

VIEIRA,J.M.S., M.T.P.AZEVEDO, S.M.F.O.AZEVEDO, R.Y.HONDA, AND B.CORREA 2005. Toxic cyanobacteria and microcystin concentrations in a public water supply reservoir in the Brazilian Amazonian region. Toxicon **45**: 901-909.

VIVIBERG,J. 1989. Culture techniques for studies on the growth, development and reproduction of copepods and cladocerans under laboratory and *in situ* conditions: a review. Freshwater Biol. **21**: 317-373.

VON ELERT,E., D.MARTIN-CREUZBURG, AND J.R.LE COZ 2003. Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (*Daphnia galeata)*. Proc.R.Soc.London, B **270**: 1209-1214.

VON ELERT,E. AND T.WOLFFROM 2001. Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*. Limnol.Oceanogr. **46**: 1552-1558.

WANG,X., P.PARKPIAN, N.FUJIMOTO, K.M.RUCHIRAWAT, R.D.DELAUNE, AND A.JUGSUJINDA 2002. Environmental conditions associating microcystins production to *Microcystis aeruginosa* in a reservoir of Thailand. J Environ Sci Health **A37**: 1181-1207.

WATANABE,M.M., K.KAYA, AND N.TAKAMURA 1992. Fate of the toxic cyclic heptapeptides, the microcystins, from blooms of *Microcystis* (Cyanobacteria) in a hypertrophic lake. J Phycol **28**: 761-767.

WEBSTER,K.E. AND R.H.PETERS 1978. Some size-dependent inhibitions of larger cladoceran filterers in filamentous suspensions. Limnol.Oceanogr. **23**: 1238-1245.

WEITHOFF,G. AND N.WALZ 1995. Influence of filamentous cyanobacterium *Planktothrix agardhii* on population growth and reproductive pattern of rotifer *Brachionus calyciflorus*. Hydrobiologia **313/314**: 381-386.

WELKER,M., H.VON DOHREN, H.TAUSCHER, C.E.W.STEINBERG, AND M.ERHARD 2003. Toxic *Microcystis* in shallow lake Muggelsee (Germany) - dynamics, distribution, diversity. Arch.Hydrobiol. **157**: 227-248.

WETZEL,R.G. 2001. Limnology, 3 ed. Academic Press.

WETZEL,R.G. and G.E.LIKENS. 1990. Limnological Analysis, 2 ed. Springer-Verlag Inc.

WHITE,S.A., L.D.FABBRO, AND L.J.DUIVENVOORDEN 2003. Changes in cyanoprokaryote populations, *Microcystis* morphology, and microcystin concentrations in Lake Elphinstone (Central Queensland, Australia). Environ Toxicol **18**: 403-412.

WHITTON,B.A. AND M.POTTS 2000. Introduction to the cyanobacteria, p. 1-11. *In* B.A.Whitton and M.Potts [eds.], The Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer Academic Publishers.

WIEDNER,C., P.M.VISSER, J.FASTNER, J.S.METCALF, G.A.CODD, AND L.R.MUR 2003. Effects of light on the microcystin content of *Microcystis* strain PCC 7806. Appl.Environ.Microbiol. **69**: 1475-1481.

WILLAME,R., T.JURCZAK, J.IFFLY, T.KULL, J.MERILUOTO, AND L.HOFFMANN 2005. Distribution of hepatotoxic cyanobacterial blooms in Belgium and Luxembourg. Hydrobiologia **551**: 99-117.

WILLIAMS,D.A., A.M.RAE, AND R.R.WOLF. Paleozoic geology of the Ottawa area, southern Ontario. (Map P2716). 1984. Ontario Geological Survey.

WILSON,A.E. AND M.E.HAY 2007. A direct test of cyanobacterial chemical defense: Variable effects of microcystin-treated food on two *Daphnia pulicaria* clones. Limnol.Oceanogr. **In press**.

WILSON,A.E., O.SARNELLE, B.A.NEILAN, T.P.SALMON, M.M.GEHRINGER, AND M.E.HAY 2005. Genetic variation of the bloom-forming cyanobacterium *Microcystis aeruginosa* within and among lakes: implications for harmful algal blooms. Appl.Environ.Microbiol. **71**: 6126-6133.

WILSON,A.E., O.SARNELLE, AND A.R.TILLMANNS 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. Limnol.Oceanogr. **51**: 1915-1924.

WIRSING,B., T.FLURY, C.WIEDNER, U.NEUMANN, AND J.WECKESSER 1999. Estimation of the microcystin content in cyanobacterial field samples from German Lakes using the colorimetric protein-phosphatase inhibition assay and RP-HPLC. Environ Toxicol **14**: 23-29.

WORLD HEALTH ORGANIZATION. Guidelines for drinking water quality, Third edition. Volume I, 1-540. 2004. Geneva.

YOSHIDA,M., T.TOGASHI, K.TAKEYA, J.YOSHIMURA, AND T.MIYAZAKI 2007. Ammonium supply mode and the competitive interaction between cyanobacterium *Microcystis novacekii* and the green alga *Scenedesmus quadricauda*. Fundamental and Applied Limnology **170**: 133-140.

YOSHIDA,T., L.E.JONES, S.P.ELLNER, G.F.FUSSMANN, AND N.G.HAIRSTON 2003. Rapid evolution drives ecological dynamics in a predator-prey system. Nature **424**: 303-305.

ZAR,J.H. 1999. Biostatistical Analysis, 4 ed. Prentice-Hall Inc.

ZNACHOR,P., T.JURCZAK, J.KOMARKOVA, J.JEZBEROVA, J.MANKIEWICZ, K.KASTOVSKA, AND E.ZAPOMELOVA 2006. Summer changes in cyanobacterial bloom composition and microcystin concentration in eutrophic Czech reservoirs. Environ Toxicol **21**: 236-243.

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.