Cyanobacterial blooms: causes, innovative monitoring and human health impact

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree
Doctor of Philosophy in the Graduate School of The Ohio State University

By
Feng Zhang
Graduate Program in Environmental Science

The Ohio State University
2014

Dissertation Committee:
Jiyoung Lee, Advisor
Jay F. Martin
Ozeas S. Costa
C.K. Shum
Song Liang

Abstract

Cyanobacterial blooms are expected to increase in occurrence and intensity as a consequence of future climate change, as well as human-induced eutrophication, leading to adverse effects on public health and local economics. In order to efficiently mitigate cyanobacterial blooms towards protecting public health, it is very important to understand the factors that promote cyanobacterial blooms, as well as their potential health impact. This study explores the effect of two potential promoting factors, land use and zebra mussel invasion, on cyanobacterial bloom intensity using data from the 2007 National Lake Assessment (NLA). First of all, the effect of invasive zebra mussels on cyanobacterial community structure and microcystins levels was investigated, using large-scale data from the USEPA National Lake Assessment and the U.S. Geological Survey Aquatic Nuisance Species database. ANOVA Based on Dissimilarities tests (Adonis) showed that there was a significant difference in cyanobacterial communities between lakes located in areas with and without established zebra mussel populations. Lakes located in areas with zebra mussels also had significantly higher microcystins levels and cyanobacteria abundance, but lower concentrations of phosphorous. Structural equation modeling suggested that the total effect of zebra mussel establishment resulted in an overall 1.40-fold increase in microcystins levels, which presumably resulted from three contributing factors: 1) a 0.86-fold decrease in microcystins levels through total

phosphorus decrease, 2) a 1.06-fold increase through an increased cyanobacteria abundance, and 3) a 1.53-fold increase through other ways, such as an increase in cyanobacteria toxicity. Secondly, another promoting factor, land use of the lake basin, was investigated. A spatial modeling approach was used to explore relationships among watershed land-use, nutrient concentrations, cyanobacterial abundance, and microcystins production with the NLA dataset. As expected, nitrogen and phosphorus concentrations were found to be lower ($p<0.05$) in lakes surrounded by undeveloped watersheds (e.g., forested) as opposed to those dominated by agriculture. Logistic regression analysis revealed that the presence of both cyanobacteria and *Microcystis* was unrelated to in-lake total nitrogen and phosphorus concentrations. However, for lakes where cyanobacteria or *Microcystis* were present, their abundances were positively related to both in-lake total nitrogen and phosphorus concentrations. This study suggests that efforts to minimize land-use activities, which seemingly lead to nutrient runoff, could help reduce in-lake nutrient concentrations, as well as the prevalence of toxic cyanobacteria.

Then, we investigated the potential effect of cyanobacterial blooms on nonalcoholic liver disease mortality using satellite remote sensing data. A Bayesian spatial regression, implementing a negative binomial model, was used to analyze the relationship between cyanobacterial bloom coverage and death from nonalcoholic liver disease. Risk of death from nonalcoholic liver disease increased by 0.3% (95% Bayesian confidence interval 0.1% to 0.5%) with each 1 % increase in bloom coverage in the affected county after adjusting for age, gender, educational level, and race. The

significant association between cyanobacterial blooms and nonalcoholic liver disease in this study suggested a population level health impact from cyanobacterial blooms.

In the last part of the study, two MODIS-based indicators were investigated for their performance in monitoring cyanobacteria abundance and toxin levels at two drinking water plant intakes located in Lake Erie during 2013. Good correlations were observed between toxic cyanobacteria, microcystins, and MODIS-retrieved bloom indicators for the intake in the western lake (where blooms were much more serious), but not for the central lake intake where blooms were milder. The microcystins levels showed a Spearman's correlation of 0.815 ($p<0.05$) with MODIS- retrieved chlorophyll-*a* at the Toledo intake point in western Lake Erie. Both *Microcystis* abundance and toxic *Microcystis* abundance also showed a significant positive correlation with MODIS-retrieve chlorophyll-*a* for the Toledo plant, as well as the two locations combined. These results demonstrate the potential for satellite remote sensing, at a regional scale, to contribute to monitoring of cyanobacterial blooms, as a preliminary warning, to protect human health in bloom-impacted water bodies.

Dedication

This document is dedicated to my parents Quoqin Hou and Chunlin Zhang

Acknowledgments

I am extremely grateful to the support and guidance I received from my adviser Dr. Jiyoung Lee. I deeply appreciate all my dissertation committee members, Dr. Jay Martin, Dr. Ozeas Costa, Dr. C.K. Shum and Dr. Song Liang for their helpful suggestions and comments for the study. I thank Drs. Chenlin Hu and Kuo-Hsin Tseng for helping me on the data collection for the studies contained in this dissertation. Finally, I also thank my family, colleagues and friends for giving me endless support during my doctoral years. This work would not have been possible without all their encouragement and assistance.

Vita

2007.....Chengdu No.7 High School, China

2011.....B.S. Environmental Science,
Peking University

2011.....B.A. Economics, Peking University

2012-2013.....Graduate Teaching Associate,
Environmental Science Graduate
Program, The Ohio State University

2013-present.....Graduate Research Associate,
College of Public Health,
The Ohio State University

Fields of Study

Major Field: Environmental Science

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 Occurrence of toxic cyanobacterial blooms

Cyanobacteria are microorganisms that have characteristics of both bacteria and true algae and are found in a range of water bodies throughout the world (Fristachi et al., 2008). Like algae, cyanobacteria can perform photosynthesis and under favorable conditions can grow extremely rapidly in a water body, resulting in high biomass events known as blooms. Although only certain species of cyanobacteria possess toxin-producing capabilities, recent studies reported that increasing water temperature and climate-induced eutrophication, as well as other changes in the future aquatic environment, will be more favorable for bloom-forming cyanobacteria than current conditions (Paerl et al., 2011a; Ye et al., 2011). Toxic cyanobacteria are found throughout the world in inland, as well as coastal, water environments; all continents have reported toxic blooms, including Antarctica (Carmichael, 1992; Hitzfeld et al., 2000). Cyanobacterial blooms can occur in habitats ranging from small ponds to large lakes, such as the Great Lakes. The states of Iowa, Minnesota, Nebraska, Wisconsin, California, Oregon and Ohio have established monitoring programs and routinely issue alerts for harmful cyanobacterial blooms (Pelaez et al., 2010). While toxic cyanobacteria are generally associated with blooms in freshwater, the health impact of toxic marine cyanobacterial blooms has also been recognized, such as those in the Baltic Sea and

Moreton Bay, Australia (Albert et al., 2005; Kanoshinaa et al., 2003).

In recent decades, the incidence and intensity of toxic cyanobacterial blooms, as well as the associated economic impact, have increased in the United States and worldwide (O'Neil et al., 2012). This increasing trend is reflected by the increasing number of published reports and studies on toxic cyanobacterial blooms over time as shown in the graph in Figure 1.1(Cheung et al., 2013). In addition, the geographic distribution of documented toxic cyanobacteria blooms seems to have dramatically increased in recent decades in the United States and globally (Lopez et al., 2008). For example, some cyanobacterial groups show remarkable recent expansion in their geographical ranges, such as the planktonic *Cylindrospermopsis raciborskii* and the benthic filamentous genus *Lyngbya* (Paerl and Huisman, 2009). Originally, *Cylindrospermopsis raciborskii* was described as a tropical and subtropical species, but in recent years, it has expanded to Europe, the USA Midwest, and New Zealand (Bonilla et al., 2012; Paerl and Huisman, 2009). *Lyngbya* spp. have also exhibited remarkable invasive abilities in a range of aquatic ecosystems, including streams, rivers, lakes, estuaries, and coastal waters (Paerl and Huisman, 2009).

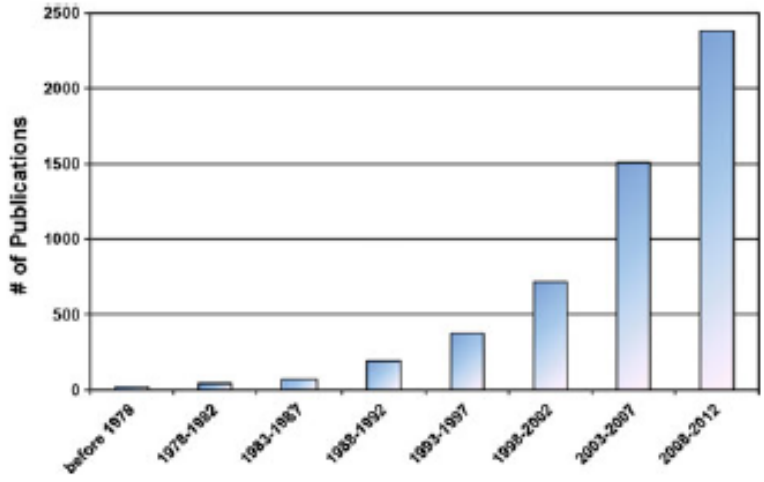


Figure 1.1. An increasing trend of published scientific studies demonstrating the worldwide spread of toxic cyanobacteria (Cheung, et al., 2013).

1.2 Cyanotoxins

There are a variety of toxin-producing cyanobacteria species that can produce toxic compounds known as cyanotoxins. Cyanobacterial blooms can compromise water quality by the production of cyanotoxins and compounds that are offensive to both taste and smell, leading to economic and public health concerns. At least 46 species have been shown to have toxic effects in vertebrates (Sivonen and Jones, 1999). The most common freshwater toxic cyanobacteria are: *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix rubescens*, *Synechococcus* spp., *Planktothrix agardhii*, *Gloeotrichia* spp., *Anabaena* spp., *Lyngbya* spp., *Aphanizomenon* spp., *Nostoc* spp., *Oscillatoria* spp., *Schizothrix* spp. and *Synechocystis* spp. (World Health Organization, 2003). Mechanisms of action of cyanotoxins range from hepatotoxic, neurotoxic, and dermatotoxic to general

inhibition of protein synthesis. Cyanotoxins fall into three broad groups of chemical structure: cyclic peptides, alkaloids, and lipopolysaccharides (LPS). Table 1.1 (below) includes a list of cyanobacteria genera, their associated toxins, and affected organs.

Table 1.1. Cyanobacteria genera and their associated toxins (modified from Lopez et al., 2008; Sivonen and Jones, 1999)

Group	Toxin	Primary target organs	Cyanobacteria Genera
Hepatotoxic cyclic peptides	Microcystins	Liver	*Anabaena, Aphanocapsa, Hapalosphon, Microcystis, Nostoc, Planktothrix (Oscillatoria)*
	Nodularins	Liver	*Nodularia spumigena*
Neurotoxic alkaloids	Saxitoxins	Nerve axons	*Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya*
	Anatoxins	Nerve synapse	*Anabaena, Aphanizomenon, Planktothrix (Oscillatoria)*
Cytotoxic alkaloids	Cylindrospermopsin	Liver	*Aphanizomenon, Cylindrospermopsin, Umezakia*
Dermatotoxic alkaloids	Lyngbyatoxins	Skin, gastro-intestinal tract	*Lyngbya*
	aplysiatoxins	Skin, gastro-intestinal tract	*Lyngbya, Schizothrix, Oscillatoria*
Irritant toxins	Lipopolysaccharide (LPS)	Potential irritant; affects any exposed tissue	All cyanobacteria
Other bioactive compounds	β-methylamino-L-alanine (BMAA)	Nerve cells	*Anabaena, Cylindrospermopsin, Microcystis, etc.*(most cyanobacteria)

Microcystins are the most well-known and studied group of cyanotoxins which there are over 80 known structural variants (Hoeger et al., 2007). Microcystins are also the most commonly occurring cyanotoxins reported in North American water bodies, while anatoxins, saxitoxins, and cylindrospermopsins has also been reported, on a limited basis (Pelaez et al., 2010). A cyanobacterial bloom survey, undertaken in Wisconsin waters in the summer of 1987, revealed that approximately 25% of the sampled sites contained cyanobacterial toxins (Repavich et al., 1990). A nationwide assessment of lakes in 2007 revealed that about 30% of the lakes in the U.S. contained detectable microcystins (USEPA, 2009). Once a cyanobacteria bloom occurs, it is very common to find detectable cyanobacteria toxins. Overall, it has been estimated that about 60% of cyanobacterial bloom samples are toxic (World Health Organization, 2003). A study, in twenty-three eutrophic lakes in the Midwest, revealed that microcystins occurred in all the blooms (Graham et al., 2010).

Nodularins are more commonly isolated from the filamentous, planktonic cyanobacterium, *Nodularia spumigena*. This species generally lives and forms toxic blooms in brackish and estuarine environments with blooms of toxic *Nodularia* occurring annually during summer months in the Baltic Sea (Sivonen et al., 1989). Nodularins are structurally similar to microcystins and can induce similar toxic effects. Mass poisoning of ducks, cattle, and dogs have been reported, which was associated with *Nodularia* blooms in northern European waters (Algermissen et al., 2011).

Saxitoxin (collectively called paralytic shellfish poisons) and its analogs are highly potent neurotoxins; Produced by several freshwater species of cyanobacteria and marine dinoflagellates. Blooms of these toxic species have led to mass kills of fish, native animals, and livestock (Pearson et al., 2010). Saxitoxin and its analogs cause globally an annual estimated 2,000 cases of paralytic shellfish poisoning (Pearson et al., 2010).

Cylindrospermopsin poisoning of humans was first identified in 1979 when over 100 children were hospitalized with symptoms of gastroenteritis on Palm Island, Australia (Griffiths and Saker, 2003). Cylindrospermopsin is highly biologically active and can interfere with several metabolic pathways (Pearson et al., 2010).The wide distribution of cylindrospermopsin-producing species presents a major problem for water management, on a global scale (Pearson et al., 2010).

1.3 Factors promoting cyanobacterial bloom formation

Factors responsible for cyanobacterial bloom formation and toxin production are very diverse. Cyanobacterial blooms are usually caused by a combination of chemical, physical, and biological factors such as the presence of good nutrients, warm temperatures, and plenty of light that all encourage an increase in numbers of cyanobacteria. Cyanobacterial blooms can occur under natural conditions, however, human activities such as the use of fertilizers, improper use of septic systems, and increased urban and agricultural activities are thought to contribute to their increasing occurrence and intensity (Anderson et al., 2002). In addition, human-induced climate change is another important contributing factor for increasing cyanobacterial blooms

(Paerl and Huisman, 2009). Seasons are another important contributing factor; in temperate zones, cyanobacterial blooms usually occur during the late summer and early autumn and may last 2-4 months, while perennial blooms are also seen in some shallow lakes (Sivonen and Jones, 1999). Over the past several centuries, increasing nutrient input, that is associated with urban, agricultural and industrial development, has promoted the dominance of harmful blooms of cyanobacteria (Paerl, 2008).

1.3.1 Nutrient loading

Elevated nutrient loading has also been proposed as the primary reason for increases in cyanobacterial blooms in a number of water bodies (Paerl, 2008; Paerl et al., 2011a). Human activities, including population growth, urbanization, and agricultural expansion, are increasing the levels of nutrients in the receiving water bodies worldwide. Land-use changes, especially increasing agricultural land and developed land in the drainage area of a water body can greatly increase the nutrient concentrations of the water body (Foley et al., 2005). Eutrophicated water bodies are vulnerable to cyanobacterial blooms, because nutrients such as nitrogen and phosphorus can stimulate the growth of cyanobacteria. Additionally, nitrogen and phosphorus have been shown to favor the growth of toxic over nontoxic cyanobacteria (Vezie et al., 2002).

In freshwater ecosystems, phosphorus availability has often been implicated as the key factor controlling phytoplankton growth and cyanobacterial bloom formation (Paerl, 2008; Schindler et al., 2008). Accordingly, controlling phosphorus inputs has been the primary strategy for control of cyanobacteria blooms by water resource managers (Strickland et

al., 2010). More attention has been given to phosphorus control, due to the assumption that nitrogen-fixing cyanobacteria can satisfy the nitrogen requirement. However, based on a study on a phosphorus fertilized lake, nitrogen fixation did not meet ecosystem nitrogen demands (Scott and McCarthy, 2010). In some cases, nitrogen fixation rates are very low, even when nitrogen fixing cyanobacteria are dominant (Ferber et al., 2004). Eutrophic lakes that are nitrogen-limited may even be dominated by cyanobacterial taxa that cannot fix nitrogen (Paerl et al., 2011b). Experimental evidence shows that nitrogen is equally likely to limit growth of algae in inland waters, and that addition of both nitrogen and phosphorus cause substantially more algal growth than either added alone (Lewis et al., 2011). There is also evidence that nitrogen availability increases the microcystins quota of *Microcystis aeruginosa* (Horst et al., 2014). In recent years, phosphorus control practices, such as bans on phosphate-containing detergents and no till agriculture, have been effective at reducing freshwater phosphorus loads but less so for nitrogen (Galloway and Cowling, 2002; Paerl et al., 2011a). In Lake Erie, *Microcystis* blooms have proliferated since the mid-1990s, even though total phosphorus inputs have remained fairly stable (Paerl and Scott, 2010). Therefore, a dual nutrient (nitrogen and phosphorus) management strategy has been proposed for controlling cyanobacterial blooms (Paerl et al., 2011a; Xu et al., 2010); there is a need to reduce both nitrogen and phosphorus inputs for long-term eutrophication and cyanobacterial bloom control.

1.3.2 Climate change

To explain the worldwide increase of harmful cyanobacterial blooms, in addition to the influence of nutrient loadings, environmental forces driven by climate change have been proposed to promote the formation of toxic cyanobacterial blooms. Our earth is warming, which is causing changes in regional climates. The patterns of warming, which influence trends in temperature and precipitation, are highly variable on a regional scale (IPCC, 2007). Rising global temperatures and changing precipitation patterns can have a promoting effect on cyanobacterial blooms. Global warming and associated hydrologic changes strongly affect the formation and toxin level of cyanobacteria blooms (Paerl and Paul, 2012).

Increasing temperature has a positive effect on the growth of cyanobacteria, as rising temperatures favor cyanobacteria in several ways. First, cyanobacteria generally grow better at higher temperatures (often above 25°C) as opposed to other phytoplankton species such as diatoms and green algae (Paerl and Paul, 2012); giving cyanobacteria a competitive advantage. Increasing water temperatures can also extend the ice-free growing season at higher latitudes and high elevations, leading to an increase of the seasonal duration of cyanobacterial bloom outbreaks in those areas (Paerl and Paul, 2012). Temperature has also been shown to affect the competition between toxic and non-toxic strains of cyanobacteria, giving toxic strains an advantage at elevated temperature (Davis et al., 2009). In addition to the direct effect of increasing temperature, increasing temperature also favors cyanobacteria growth through an intensified vertical stratification in water systems (Paerl and Paul, 2012) because many cyanobacteria species are able to

exploit stratified condition due to the gas vesicles they contained. Global surface temperature has increased and will be increasing over the 21st century, driven mainly by anthropogenic greenhouse gas concentrations(IPCC, 2007), causing an increase in toxic cyanobacterial blooms throughout the world, leading to huge challenges in water quality, water supply, and fisheries managers.

Global climate changes also result in changed patterns of precipitation and drought, which could further enhance cyanobacterial dominance; summer droughts appear to be increasing in intensity and duration, possibly due to global warming (IPCC, 2007). Larger and more intense precipitation events can increase run-off of nutrients and increase nutrient concentration in receiving waters; in contrast, increasing precipitation may prevent blooms, in short term, by flushing out dense cyanobacteria. However, over time, as the discharge subsides, the increasing nutrients eventually promote cyanobacterial blooms. This scenario will most likely occur if elevated winter–spring rainfall events are followed by periods of summer drought (Paerl and Paul, 2012). It has also been reported that prevailing dry periods favor cyanobacterial growth through increasing conductivity, longer water residence times, non-turbulent and stratified conditions and increased release of nutrients (Reichwaldt and Ghadouani, 2012).

1.3.3 Invasive zebra mussel

Zebra mussel, *Dreissena polymorpha*, is among the world's 100 worst biological invaders (IUCN, 2005). The freshwater bivalve zebra mussel have spread rapidly throughout the Laurentian watershed after they first became established in Lake St. Clair in 1988

(Griffiths et al., 1991). Zebra mussels are active grazers of phytoplankton including cyanobacteria (Pires and Van Donk, 2002) and can also filter suspended particles from the water column and excrete available nutrients back into the water. Zebra mussels have been suspected to be one of the causes of cyanobacterial blooms (Knoll et al., 2008; Orlova et al., 2004) by promoting growth through removal of competitors such as green algae and diatoms. Zebra mussels are capable of consuming large amounts of green algae and diatoms, while selectively rejecting *Microcystis aeruginosa* (Vanderploeg et al., 2001). A recent study on 61 inland lakes in Michigan revealed a positive association between zebra mussel invasion and the dominance of the potentially harmful cyanobacterium, *Microcystis* (Raikow et al., 2004). In Saginaw Bay, the re-occurrence of summer cyanobacterial blooms (after zebra mussels became established) reversed years of nutrient management (Vanderploeg et al., 2001). With the rapid spread of zebra mussels in North America, more attention is needed to counter the possibility that zebra mussel invasion may alter the results of nutrient control of cyanobacterial blooms.

1.4 Public health impact of cyanobacterial blooms

Cyanotoxins may be a health threat to public health via exposure routes such as contaminated drinking water. Health effects associated with toxic cyanobacteria exposure are dependent on the types of toxin, exposure route, and the level of toxin (Cheung et al., 2013). The major routes of exposure to cyanotoxins are ingestion, inhalation, and dermal contact (Codd et al., 1999). Recreational activities, involving contaminated water, may result in skin contact, inhalation, and accidental ingestion of soluble cyanotoxins or toxic

cyanobacteria cells. Toxins have been detected in air samples, as well as personal nasal swabs, near contaminated lakes (Backer et al., 2010; Wood and Dietrich, 2011). Cyanotoxins are of particular concern in drinking water because they can pass through conventional water treatment processes (Falconer et al., 1999) and be present in the distribution system and ultimately in the tap water if not properly treated; thus, exposure may occur through ingestion of drinking water or food containing cyanotoxins. Cyanotoxins, including microcystins, nodularins, and cylindrospermopsin, have been detected in drinking water (Beltran et al., 2012; Yen et al., 2011), as well as food supplements (Saker et al., 2005). Ingestion of toxins may occur through the consumption of seafood harvested from water bodies with cyanobacterial blooms (Poste et al., 2011). There may be both short- and long-term effects that result from exposure to cyanotoxins. For example, short-term health effects associated with exposure to microcystins include gastrointestinal symptoms, liver inflammation, liver failure, pneumonia, dermatitis, etc. (Lopez et al., 2008). Chronic and long-term exposure to the toxin have been associated with liver and colorectal cancers (Hernandez et al., 2009). Epidemiological data for human poisoning by cyanotoxins are very limited. Most cases of human injury, attributed to cyanobacterial toxins, have been studied retrospectively, and complete exposure data, such as the concentration of cyanotoxins, are rarely available (Kuiper-Goodman et al., 1999). Animal toxicity tests have provided valuable information on the toxicity of cyanotoxins.

1.4.1 Short-term health effects in humans

Cyanobacterial poisoning in humans and animals was first described in the nineteenth century (Hunter, 1998). Since then, a range of illnesses, associated with acute exposure to cyanobacteria, has been reported; among which hay fever-like symptoms, pruritic skin rashes and gastrointestinal symptoms, which are the most frequently reported (Stewart et al., 2006b). Some acute reaction to toxic cyanobacteria are mild, while others are described as serious acute illnesses, with symptoms such as severe headache, pneumonia, fever, myalgia, and blistering in the mouth (Stewart et al., 2006b). Gastrointestinal illness has been reliably attributed to cyanobacterial toxins in water supplies; there have been outbreaks of gastrointestinal illness coincident with either the breakdown of a natural cyanobacterial bloom or with the artificial lysis of a bloom by algaecide (Kuiper-Goodman et al., 1999). The most recent and serious known human poisoning due to cyanotoxins occurred in a Brazilian hemodialysis center, where 100 patients developed acute liver failure and 50 died (Jochimsen et al., 1998).

Recreational risk related to exposure of cyanobacteria is difficult to assess, as the numbers of exposed people are usually relatively small, their individual sensitivity to the toxin differs, the intensity and duration of exposure vary, and cyanotoxin types and concentrations also vary, giving different response rates (Koreiviene et al., 2014). Adverse effects of cyanotoxins, yielding nonspecific signs and mild symptoms can easily go undiagnosed by health care specialists. A cohort study on recreational exposure to freshwater cyanobacteria found that a significant increase in reporting of minor self-limiting symptoms, particularly respiratory symptoms, was associated with exposure to

higher levels of cyanobacteria (Stewart et al., 2006a). From 2009 to 2010, eleven freshwater harmful algal bloom-associated outbreaks were reported to the Centers for Disease Control and Prevention (CDC) by New York, Ohio, and Washington (Hilborn et al., 2014). These outbreaks resulted in at least 61 illnesses and two (3%) hospitalizations. Reported effects included dermatologic symptoms; gastrointestinal signs or symptoms; respiratory symptoms; fever; neurologic symptoms; ear symptoms; and eye irritation (Hilborn et al., 2014).

1.4.2 Long-term health effects in humans

Long-term adverse health effects could be caused by short-term exposures to toxins as well as chronic low-level exposure (Kuiper-Goodman et al., 1999). However, much less is known about the health effects of repeated low level exposure to cyanotoxins than acute poisoning (Dorr et al., 2010). Microcystins, as hepatotoxins, have been linked to liver damage and liver cancer in several epidemiological studies. Fitzgeorge et al. (1994) demonstrated that microcystins toxicity is cumulative: the effect of chronic exposure is larger than acute exposure. The high incidence of primary liver cancer in southeast China is likely related to high microcystins levels in the drinking water (Ueno et al., 1996). Researchers have identified microcystins in the serum of chronically-exposed fishermen as well as an indication of liver damage, possibly from microcystins toxicity (Chen et al., 2009). A cross-sectional investigation found out that chronic exposure to microcystins was associated with liver damage, as measured by the abnormal serum enzyme levels in children in Southwest China (Li et al., 2011). The death of a 34-year-old woman, due to

liver failure, has been suggested to be the consequence of chronic consumption of microcystins from diet supplements (Zegura et al., 2011). Another cyanotoxin, β-methylamino-L-alanine (BMAA), has been suggested as a causative agent of several neurodegenerative disorders. Analysis of brain tissues from deceased persons with amyotrophic lateral sclerosis/Parkinsonism dementia complex identified BMAA in the study groups, but not in tissues from control patients who died of causes unrelated to neurodegenerative illness (Cox et al., 2003; Murch et al., 2004). Epidemiological evidence also found a high cluster of amyotrophic lateral sclerosis in close proximity to a lake with documented cyanobacteria blooms (Caller et al., 2009), which might be related to chronic exposure to the aerosolized cyanotoxins (Stommel et al., 2013). Epidemiological studies on the chronic effect of other cyanotoxins are relatively limited. However, animal studies suggest possible chronic toxicity, such as tumor promotion and malaise, by other cyanotoxins (Falconer, 2008; Lopez et al., 2008).

1.5 Economic impact of cyanobacterial blooms

Cyanobacterial blooms commonly lead to economic losses through their negative impacts on recreational activities, tourism, and increased treatment costs in potable water supply plants. Additionally, controlling strategies, research, and monitoring programs can also lead to economic losses. In the United States alone, toxic cyanobacterial blooms result in losses in recreational, drinking, and agricultural water resources of over $2 billion annually (Dodds et al., 2009) and the overall algal bloom costs in Australia have been estimated to be between $180 and $240 million per year (Atech, 2000). Cyanobacterial

blooms can render a water body unsuitable for recreation, leading to a negative impact on local businesses that cater to recreational activities. Re-occurring blooms will then gradually cause the area to lose its attraction as a tourist destination. The economic losses to tourism, related to a toxic *Anabaena* bloom which occurred in the Darling River, Central Australia, were estimated to be around $1.5 million (Steffensen, 2008). Another bloom occurring in the Hawkesbury Nepean River, Australia, cost the tourism facilities an economic loss of $6.7 million compared to the previous year, when no bloom occurred (Steffensen, 2008).

Drinking water plants, that use cyanobacterial bloom-impacted water, usually need special treatment to ensure the safety of drinking water, leading to an increase in the treatment cost. For example, the Celina water treatment plant constructed a dual ozone/peroxide treatment system and installed granular activated carbon in order to deal with algae-associated problems from the Grand Lake St Marys in Ohio (Soward, 2011). As of October 2010, the estimated total costsassociated with treating algae problem was $12,388,700. Annually, drinking water treatment costs to remove algal toxins and algal decomposition products were estimated to be 19 million euros in the United Kingdom (Pretty et al., 2002). In events of severe cyanobacterial blooms, water treatment intakes could be closed and people will need to buy bottled water; the estimated costs could exceeded RMB 100 million for Lake Taihu (Le et al., 2010).

Monitoring programs for harmful algal blooms requires regular testing of the bloom intensity and toxin levels. The costs involved in assessing the blooms depends on the type of testing carried out and the extent of sampling. Monitoring large water bodies may

require intensive sampling, due to the fact that cyanobacterial blooms can be highly patchy and unpredictable. In Australia, about $8.7 million per year is spent annually on monitoring and contingency planning for harmful cyanobacterial blooms (Atech, 2000). In addition, treating bloom-impacted surface water through direct treatment or water catchment management are also costly. In September 2010, the Ohio EPA conducted a pilot study to examine the efficacy of alum additions on cyanobacterial blooms in three small bays of Grand Lake St Marys and the cost was $61,500 (Tetratech, 2010).

1.6 Regulations on cyanobacteria and cyanotoxins

Throughout our lifespan, humans will be inevitably be exposed to cyanotoxins. The extent of human health risk, due to cyanotoxins, depends greatly on exposure levels. Reducing human exposure to cyanotoxins may be achieved through establishment of guideline values for cyanotoxins from drinking and recreational water. The derivation of guideline values for cyanotoxins in drinking water should be based on estimating the tolerable daily intake (TDI) of the cyanotoxin. The TDI is the amount of a harmful substance that can be consumed daily over a lifetime without appreciable health risk. The guideline values can be calculated as Equation 1.1.

$$\text{Guideline} = \frac{TDI \times body\ wt \times AF}{C} \quad (1.1)$$

In Equation 1.1, body weight is assumed to be 60 kg for a human adult and allocation factor (AF) is the proportion of the TDI via drinking water. An AF of 0.8 (80% of total intake) is assumed for drinking water and C is the amount of drinking water consumed per day, assumed to be 2 liters for an adult (Codd et al., 2005). For microcystin-LR, the World Health Organization (WHO) adopted a provisional TDI of 0.04 µg per kg body weight per day based on data from a subchronic toxicity trial in mice, with supporting data from growing pigs, to establish the guidelines for microcystin-LR for drinking water exposure (Falconer et al., 1999). Then a guideline level of around 1 µg/L can be calculated from the equation. However, WHO concluded that there were insufficient data to derive a guideline value for cyanotoxins, other than microcystin-LR (Falconer et al., 1999). For recreational waters, the WHO has developed a three tier guideline for cyanobacteria blooms (World Health Organization, 2003). Potential human health risk in recreational waters is considered low at 20,000 cells/mL cyanobacteria, moderate at 20,000 to 100,000 cells/mL and relatively high at a cell concentration of over 100,000 cells/mL (visible cyanobacterial scum formation). The lowest tier of the recreational guideline is for protection of health due to the irritable or allergenic effects of cyanobacteria (World Health Organization, 2003).

Table 1.2. WHO recreational water guidelines for human health risk (modified from WHO, 2003)

Probability of Adverse Health Effects	**Cell concentration (per mL)**	**Corresponding chlorophyll-*a* concentration (µg/L)**	**Expected microcystins Level (µg/L)**
Relatively Low	<20,000 cells	<10	2-4
Moderate	20,000 - 100,000 cells	10 - 50	4-20
High	>100,000 cells (visible scum)	visible scum	>20

Even though cyanobacterial blooms have become a serious problem for water resources in the United States, no federal regulatory guidelines for cyanobacteria or their toxins in drinking water or recreational waters exist at this time. Extensive sampling in Florida indicated that cyanotoxins were often present in finished drinking water when harmful algal blooms occurred in the source waters (Williams et al., 2006), indicating the necessity and importance of establishing regulations for cyantoxins to protect public health. Many states and other jurisdictions rely on WHO guidelines to manage cyanobacteria blooms and toxins. Other states derived their own guidelines to support public health decision-making, such as posting advisories or closing water bodies

(Chorus, 2005). For example, the Ohio Environmental Protection Agency (EPA) also established two levels of algal bloom advisories for recreational water (Kasich et al., 2012) and a two level cyanotoxin threshold for drinking water (Kasich et al., 2013). In Ohio, advisories will be issued at State Park Beaches or at Public Water Systems where harmful algal blooms have been identified. Table 1.3 shows the regulation threshold for the cyanotoxins regulated in Ohio waters.

Table 1.3. Ohio regulation thresholds for microcystins, anatoxin-a, cylindrospermopsin, and saxitoxins (modified from Kasich et al., 2012 and Kasich et al., 2013).

Advisory type	**Microcystins (µg/L)**	**Anatoxin-a (µg/L)**	**Cylindrospermopsin (µg/L)**	**Saxitoxins (µg/L)**
Recreational Public Health	6	80	5	0.8
Recreational No Contact *	20	300	20	3
Drinking water Do Not Drink	1	20	1	0.2
Drinking water Do Not Use	20	300	20	3

[*] A No Contact Advisory will be issued when toxin levels exceed the recommended threshold and there are one or more probable cases of human illness or pet deaths attributable to the bloom.

1.7 Monitoring methods of cyanobacterial blooms

It is difficult to monitor cyanobacterial blooms and toxins, because the bloom toxicity can change rapidly, multiple toxins can be produced by the same bloom, and toxins detection can be difficult and expensive. Consequently, there are no standardized methods for cyanotoxin detection in water, and no national monitoring programs in the US.

There are several available methods for monitoring cyanobacterial blooms. Since many commonly occurring cyanobacteria are more often toxic than non-toxic, the simplest approach for monitoring is to assume toxicity and to monitor cyanobacteria abundance in the water. Such an approach begins with visual inspection to identify the presence of color and/or scum in surface water. Generally, the water will be discolored of brown, green, blue green, white, black, purple or red, if cyanobacteria abundance in water is 4,000 cells/ml or more (Kasich et al., 2012). However, visual inspection provides no quantitation and sometimes may be inaccurate.

A fluorescence-based method has been introduced as an early warning system for cyanobacterial blooms by measure concentrations of chlorophyll-*a* or phycocyanin. Chlorophyll-*a* measurement does not differentiate total eukaryotic phytoplankton from cyanobacteria, which is especially important for the detection of cyanobacteria in a water

body with other algae groups. Phycocyanin, which is specific to cyanobacteria, has worked well for cyanobacterial bloom detection and toxin prediction (Brient et al., 2007; Marion et al., 2012).

Another monitoring method is by qualitative identification of cyanobacterial taxa and population densities. This method provides quantification and a basis for risk assessment and regulations; however, phytoplankton identification and counting requires sample collection, preserving, transporting and special expertise. In addition, toxic and non-toxic strains from the same cyanobacterial species cannot be separated by microscopic identification.

The use of molecular genetic methods, such as quantitative polymerase chain reaction (qPCR), can target specific toxin production genes leading to the precise identification methods for toxic cyanobacteria. The large nonribosomal peptide synthetase gene cluster involved in microcystins synthesis (*mcy* A-J) has been identified and sequenced and could be used for detection and quantification of toxin producing strains (Rinta-Kanto et al., 2005).

Monitoring cyanotoxin concentrations may also be needed in some situations; however, quantitating every cyanotoxin is difficult and expensive given the number of known cyantoxins. Currently monitoring of microcystins or other known toxins has been used to assess the public health threat of blooms. Enzyme-linked immunosorbent assays (ELISA) are commonly used by government agencies for cyanotoxin monitoring. Others methods such as protein phosphatase type 2 inhibition assays (PP2IA), and high-performance liquid chromatography, coupled with ultraviolet light detection (HPLC/UV) or mass

spectrometry (HPLC/MS) are also available for monitoring cyanotoxins such as microcystins (Chorus, 2005). Although public health relevant, most cyanotoxin analyses are costly and time consuming and are not well-suited for monitoring large numbers of water bodies at regional or national levels as cyanobacterial blooms and scums are patchy and transient (Hunter et al., 2009).

Remotely-sensed data from satellites and airborne sensors have been introduced for cyanobacterial bloom monitoring, due to their large spatial coverage, and are very cost effective. Algae contain a variety of pigments, which give them their own unique spectral signature and in some cases allows for differentiation between species (Roelfsema et al., 2006). Currently most remote sensing algorithms for cyanobacterial blooms in inland waters aim at retrieval of the pigments chlorophyll-*a* or phycocyanin, as a useful proxy for cyanobacteria biomass. Studies have developed algorithms to retrieve chlorophyll-*a* and phycocyanin, based on Landsat, Medium Resolution Imaging Spectrometer (MERIS), Moderate Resolution Imaging Spectroradiometer (MODIS), etc (Becker et al., 2009; Kutser, 2004; Simis et al., 2005; Vincent et al., 2004).

Chapter 2: The effects of invasive zebra mussels on cyanobacterial communities and microcystins levels in United States lakes

2.1 Chapter overview

Zebra mussel invasion of Northern American lakes has occurred during the last century and, as one of the filter feeders in aquatic bodies, it plays an important role in occurrence of toxic cyanobacteria blooms, which is supported mostly by lake-specific or pilot studies Herein, large-scale data from the USEPA National Lake Assessment (USEPA, 2009) and the U.S. Geological Survey Aquatic Nuisance Species database were used to study the potential zebra mussel impact on cyanobacteria community and microcystins levels in American lakes. An agglomerative hierarchical clustering analysis, based on abundance of 52 genera of cyanobacteria, was used to identify cyanobacterial community structure. Based on the composition of cyanobacterial communities, the lakes were clustered into six groups. ANOVA Based on Dissimilarities tests (Adonis) showed that there was a significant difference in cyanobacterial communities between lakes located in areas with and without established zebra mussel populations. Lakes located in areas with established zebra mussels had significantly higher microcystins levels and cyanobacteria abundance, but lower concentrations of phosphorous. Since total phosphorus and cyanobacteria abundance were directly or indirectly influenced by zebra mussel establishment, they

were considered to be important factors in the pathways whereby zebra mussels influence microcystins levels. Structural equation modeling was used to confirm and estimate the effect of zebra mussels on microcystins concentrations via different pathways. The results suggest three potential pathways whereby zebra mussels influence microcystins production. The first is through phosphorus removal, which has a negative effect on microcystins production. Both the second and third pathways are dependent on zebra mussel grazing, increasing either the total abundance of cyanobacteria or selectively increasing the number of toxic cyanobacteria. The total effect of zebra mussel establishment resulted in an overall 1.40-fold increase in microcystins level, which presumably resulted from three contributing factors: 1) an 0.86-fold decrease in microcystins level through total phosphorus decrease; 2) an 1.06-fold increase through an increased cyanobacteria abundance; and 3) an 1.53-fold increase through other ways such as selective filtration that increases cyanobacteria toxicity.

2.2 Introduction

One of the major consequences of lake eutrophication is the increase in blooms of cyanobacteria accompanied by a decrease in other groups of phytoplankton, namely chrysophytes, cryptophytes, chlorophytes and diatoms (Watson et al., 1997). In addition to their impact on ecosystem diversity, cyanobacteria dominance constitutes a threat to both public and ecosystem health due to their toxin production (Carmichael et al., 2001; Cheung et al., 2013; Chorus, 2001). Cyanotoxins can poison human and animals by ingestion of contaminated water or aquatic organisms which have bioaccumulated these

toxins in their tissue (Carmichael et al., 2001). One group of commonly occurring cyanotoxins, the microcystins, can cause death from liver damage and are potential carcinogens (Sivonen and Jones, 1999; Ueno et al., 1996). Microcystins can be produced by toxic strains of a number of cyanobacterial genera, including planktonic *Microcystis*, *Nostoc*, *Oscillatoria*, *Anabaena*, *Planktothrix*, *Anabaenopsis* (Fristachi et al., 2008). Among them, *Microcystis*, *Planktothrix*, and *Anabaena* are the most common microcystins-producing cyanobacterial genera (Hisbergues et al., 2003).

Zebra mussels (*Dreissena polymorpha*) are invasive bivalves that have spread rapidly through the Laurentian Great Lakes watershed (Griffiths et al., 1991) and continue to spread throughout U.S. lakes (Bossenbroek et al., 2007). Zebra mussels are extremely efficient at filter-feeding (Gazulha et al., 2012), leading to the filtering of suspended particles (including phytoplankton) and excreting nutrients, thereby physically altering the habitat. By doing so, they are able to change the composition and abundance of planktonic communities, and thereby impact ecosystem functions in water bodies (Caraco et al., 1997; Fahnenstiel et al., 1995; Heath et al., 1995; Holland, 1993; Lei et al., 1996; Nicholls and Hopkins, 1993; Roditi et al., 1996). It has been suggested that this invasive bivalve has the potential to promote toxic blooms of cyanobacteria by selective feeding on plankton (Makarewicz et al., 1999; Vanderploeg et al., 2001) Dominance of *Microcystis aeruginosa* in low-nutrient lakes was found to be associated with zebra mussels (Raikow et al., 2004) and dense summer blooms of toxic *Microcystis* were discovered three years after their initial colonization (Lavrentyev et al., 1995). Some studies have shown that *D. polymorpha* promotes an increase in cyanobacteria densities

or their toxicity (Juhel et al., 2006; Knoll et al., 2008; Nicholls et al., 2002); whereas other studies have shown that zebra mussel decreases cyanobacteria densities (Baker et al., 1998; Bastviken et al., 1998; Pires et al., 2005; Smith et al., 1998). The negative impact of zebra mussel on cyanobacteria density is particularly true in highly eutrophic systems (Reeders et al., 1993; Sarnelle et al., 2012). The ways in which zebra mussels affect cyanobacterial blooms are not fully understood. Suggested mechanisms include: 1) selective filtration, which favors cyanobacteria, especially toxic ones (Vanderploeg et al., 2009; Vanderploeg et al., 2001); 2) nitrogen and phosphorus remineralization, which may support cyanobacterial blooms (Conroy et al., 2005; Wojtal-Frankiewicz and Frankiewicz, 2011); and 3) increased light penetration (Fishman et al., 2010).

Although significant changes in phytoplankton community structure and composition have been identified with zebra mussel invasion (Fishman et al., 2010), very few studies have focused on the effect of zebra mussels on cyanobacteria community structure and composition. To better understand the effects of zebra mussels on cyanobacterial community structure and microcystins levels in water, over one thousand lakes in the U.S. were investigated, using multivariate analysis of information obtained from the National Lake Assessment (USEPA, 2009) and the U.S. Geological Survey Aquatic Nuisance Species (USGS-ANS) databases. Structural equation modeling was also used to explore potential pathways whereby zebra mussels could influence microcystins production.

2.3 Methods

2.3.1 Data sources

2.3.1.1 Cyanobacteria data

The NLA conducted by the USEPA, Office of Water and Office of Research and Development, together with multiple organizations, provides a very comprehensive survey of lakes in the US. A total of 1,028 lakes were sampled for the NLA during the summer of 2007, representing the condition of about 50,000 lakes nationwide (USEPA, 2009). NLA measurements included indicators of: water quality (nutrients, dissolved oxygen and algal density); recreation (algal toxins and pathogens); physical habitat (lakeshore and shallow water habitat cover); and biology (phytoplankton and zooplankton, cyanobacterial abundance indicated by cells/mL, microcystins level, and trophic state indicated by nutrient level). At each lake site, crews collected samples at a single station located at the deepest point in the lake and at ten stations around the perimeter of the lake (USEPA, 2009). Single grab water samples were collected to measure nutrients, chlorophyll-a, phytoplankton, and the algal toxin, microcystins. Microcystins was measured using ELISA, which has a detection limit of 0.1μg/L. The sample for phytoplankton counting was fixed with Lugol's iodine and identified to the lowest possible taxonomic level (usually genus) (Rigosi et al., 2014).

2.3.1.2 Zebra mussel data

Information on the distribution of zebra mussels was obtained from the USGS-ANS database (http://nas.er.usgs.gov/), which catalogs new sightings of zebra mussels. The

zebra mussel data were combined with National Lake Assessment data using the 8-digit hydrologic unit code (HUC), which identify a hydrological feature such as an area of a drainage basin. USGS has been using the HUC to track non-indigenous aquatic species for over 10 years (Fuller, 1999). Since water bodies with the same HUC are hydraulically connected and geographically close, they are likely to share the same zebra mussel status; i.e., connected lakes are more likely to be invaded than non-connected lakes (Bobeldyk et al., 2005). Zebra mussel establishment observed after 2007 was not considered when combining the two datasets.

2.3.2 Statistical methods

To investigate differences of cyanobacterial community structure between lakes, agglomerative hierarchical cluster analysis was performed, based on Bray-Curtis similarity coefficient (Bray and Curtis, 1957), a commonly used method in ecological studies. The cluster analysis was used to delineate groups of lakes with similar cyanobacterial communities. Raw abundance data were log(x+1) transformed and standardized by range (subtracting the minimum and dividing by the range) prior to cluster analysis in an attempt to stabilize the variance in the data. Then, agglomerative hierarchical cluster analysis was performed by means of the Ward's linkage, which has been proven to be the most robust (Singh et al., 2011) to investigate differences in community structure. Furthermore, the similarity percentage (SIMPER) procedure was used to identify those genera that contributed most to difference between cyanobacterial communities in the groups. ANOVA Based on Dissimilarities (Adonis) was used to test

whether there was a significant difference in cyanobacteria communities between lakes located in areas that have established zebra mussels and those that have not.

Student's t-test was used to examine the effect of zebra mussel establishment on cyanobacteria-related parameters. Parameters that deviated from normal distribution, judging from normal Q-Q plot, were log transformed before the t-test. Spearman correlation was used to identity factors that were correlated with the levels of microcystins. Structural equation modeling was used to estimate the effect of zebra mussels on microcystins concentration and identify pathways whereby zebra mussels influence microcystins concentration. We estimated the total effect, the effect through decreasing total phosphorus, and the effect through cyanobacteria abundance of zebra mussel on microcystins concentration. Before regression, microcystins levels, total nitrogen levels, and total phosphorus levels were log transformed, and cyanobacteria abundance was log(x+1) transformed to meet assumptions of the model. Microcystins concentrations below the detection limit were assumed to be half of the detection limit, 0.05 μg/L, which was already done by the USEPA in the dataset.

Agglomerative hierarchical cluster analysis and Adonis were performed using R-Forge vegan package (Oksanen et al., 2007), a CRAN package for the analysis of ecological communities. Similarity Percentage (SIMPER) calculations were conducted using PAST v. 2.17c (Hammer et al., 2001). Other statistical procedures were performed using SAS 9.3 (SAS Institute Inc, Cary, NC).

2.4 Results

2.4.1 Cyanobacterial community structure in U.S. lakes

In the NLA-2007 survey, 52 cyanobacteria genera were identified, including *Anabaena, Microcystis, Oscillatoria, Planktothrix* and *Anabaenopsis,* which are potential microcystins producers (Sivonen and Jones, 1999). *Microcystis* and *Anabaena* were found in 599 (58.44 %) and 593 (57.85 %), respectively, of all lakes surveyed, suggesting that these genera were most common cyanobacterial genera in U.S. lakes.

The agglomerative hierarchical cluster analysis revealed that the lakes could be separated into six clusters with different cyanobacterial community assemblages (Figure 2.1). The clustering tree was used to identify clusters that represent cyanobacterial community assemblages. The selection of the appropriate number of clusters depends on the purpose of the analysis and cluster interpretability (Lewy and Vinther, 1994; Pelletier and Ferraris, 2000). Alternative selections in the number of clusters and cyanobacteria composition were used before making a final decision. Lakes in Cluster 1 were mainly composed of *Aphanocapsa* (12.22%), *Leptolyngbya* (12.16%), *Chroococcus* (11.91%), and *Anabaena* (10.32%); Cluster 2 contained 35.39% *Microcystis*, 20.83% *Oscillatoria*, and 12.44% *Anabaena*; Cluster 3 was predominantly *Microcystis* (64.70%); Cluster 4 was found to contain 19.77% *Anabaena*, 17.06% *Aphanizomenon*, and 14.65% *Aphanocapsa*; and Cluster 5 was composed of 61.11% *Microcystis* and 21.68% *Synechococcus* (Table 2.1). Cluster 6, consisting of 28 lakes (2.7% of the total), contained no cyanobacteria.

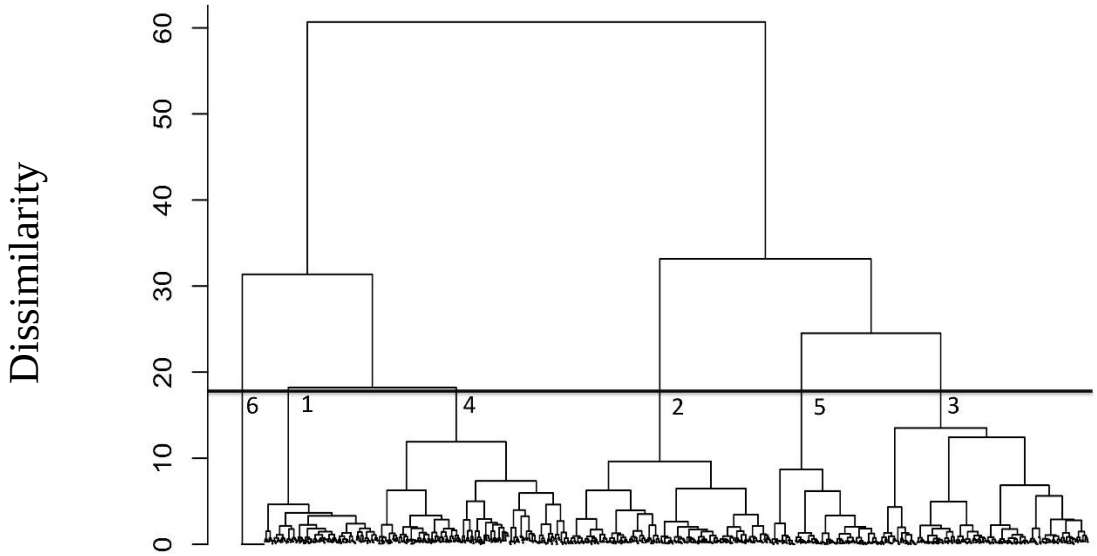


Figure 2.1. Agglomerative hierarchical clustering of cyanobacteria in lakes based on the ward's linkage and Bray–Curtis distance, with cluster numbers showing the six different clusters.

Table 2.1. Composition of the six identified lake clusters (the mean percentage of genus abundance)

Genus [a]	**Cluster 1 (%) (n=138)**	**Cluster 2 (%) (n=244)**	**Cluster 3 (%) (n=252)**	**Cluster 4 (%) (n=231)**	**Cluster 5 (%) (n=132)**	**Cluster 6** [b] **(%) (n=28)**
Anabaena	10.32	12.44	7.30	19.77	1.54	0
Coelosphaerium	7.52	1.67	0.82	3.71	1.16	0
Aphanizomenon	6.71	0.88	5.61	17.06	1.32	0
Aphanocapsa	12.22	3.93	0.63	14.65	0	0
Chroococcus	11.91	5.29	1.14	7.04	0.77	0
Leptolyngbya	12.16	0.62	0.00	1.03	0	0
Merismopedia	5.77	4.22	1.22	5.43	1.04	0
Microcystis	6.48	35.49	64.70	2.59	61.11	0
Oscillatoria	0.06	20.83	1.74	0.91	3.08	0
Phormidium	6.73	0.08	0	3.74	0	0
Synechococcus	0.00	0.58	1.16	0.77	21.68	0

[a] Only genera that have mean percentage of more than 5 in any of the clusters were listed

[b] Cluster 6 (n=28) contained no cyanobacteria

SIMPER (similarity percentages) analysis was performed to identify the extent to which each genus contributed to the dissimilarity among clusters. *Microcystis* contributed most to the dissimilarity among clusters (9.67%), followed by *Anabaena* (8.09%), *Synechococcus* (7.13%), *Chroococcus* (6.62%), *Oscillatoria* (6.48%), *Aphanizomenon* (5.71%), and *Aphanocapsa* (5.49%). The overall dissimilarity among the six clusters was 54.24%.

The distribution of the identified clusters shows a distinctive spatial clustering (Figure 2.2). Adonis supports the results of the cluster analysis and differences between clusters are highly significant ($p<0.001$). The Shannon-Weaver diversity index for each cluster also points to very distinctive cyanobacterial assemblages. In addition, the significantly different microcystins levels among clusters suggest a strong correlation between cyanobacterial community structure and microcystins level. The average Shannon-Weaver index of cyanobacteria genera was 0.80, ranging from 0 to 2.08. Lakes in Cluster 1 had the highest level of microcystins and the highest cyanobacteria biodiversity, whereas lakes in Cluster 6 had the lowest level of microcystins and had no cyanobacteria (Table 2.2). The Spearman's rank correlation between the Shannon-Weaver index of cyanobacteria genera and microcystins level was 0.221 ($p<0.001$), indicating that higher cyanobacterial biodiversity was associated with higher microcystins levels. A study on polar freshwater ecosystem also observed an association between cyanobacterial biodiversity and toxin production, suggesting the possible linkage between cyanobacteria biodiversity and microcystins level (Kleinteich et al., 2012).

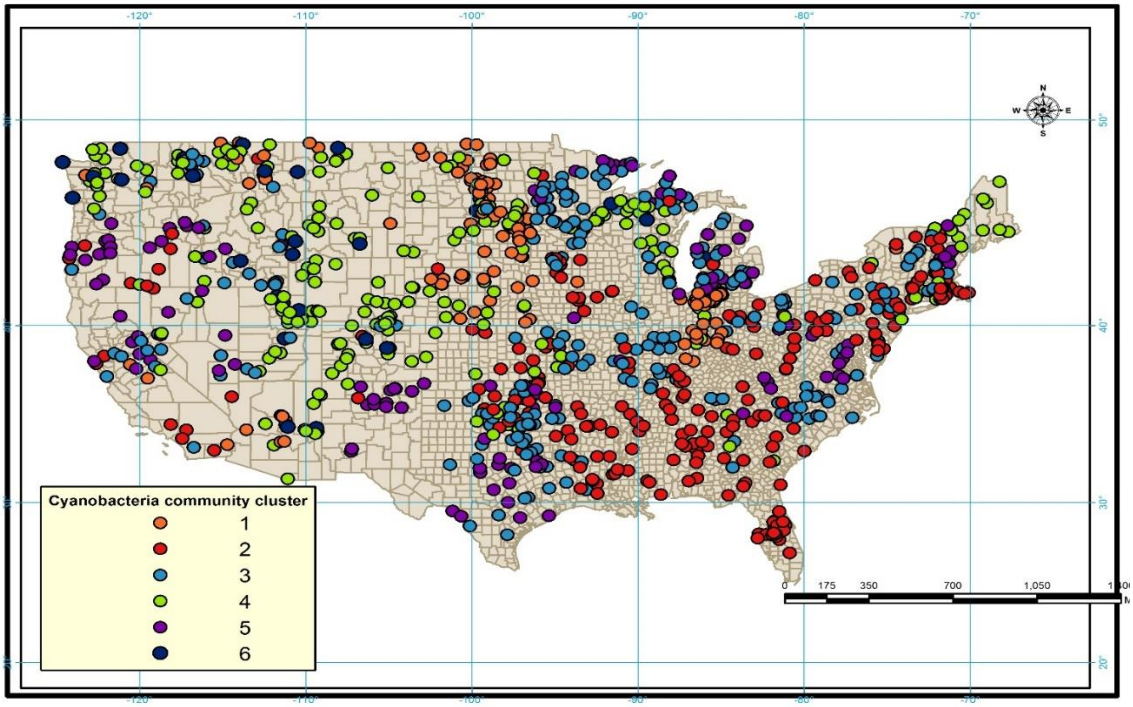


Figure 2.2. Spatial distribution of the 6 clustered lakes identified by summer cyanobacteria community structure using agglomerative hierarchical cluster analysis with the 2007 National Lake Assessment data.

Table 2.2. Characteristics of the six lake clusters in terms of microcystins level, cyanobacteria biodiversity and zebra mussel establishment

Cluster	Percentage of lakes with detected microcystins (%)	Percentage of lakes with established zebra mussel (%)	Mean Shannon-Weaver index of cyanobacteria genera	Mean microcystins level (µg/L)
1	59.42	28.26	1.18	4.88
2	34.02	14.34	1.04	0.62
3	43.25	24.21	0.69	0.70
4	25.11	7.79	0.60	0.42
5	9.85	18.94	0.51	0.25
6	14.29	3.57	NA	0.08
Total	34.05	17.46	0.80	1.10

2.4.2 Zebra mussel establishment and cyanobacterial community structure

Lake clusters are significantly associated with zebra mussel establishment (p<0.001) (Table 2.2) revealed by chi-square test. Lakes in Cluster 1 had the highest likelihood (28.26%) of being in an area where zebra mussels were established while lakes in Cluster 6 had the lowest probability (3.57%). Adonis showed there was a highly significant difference in cyanobacterial communities between lakes located in areas that had been established with zebra mussels and those that had not (p=0.001). SIMPER analysis showed that the overall dissimilarity in cyanobacterial communities was 45.71% between the lakes located in areas with established zebra mussels and the lakes without them. SIMPER analysis also showed that *Microcystis* (8.29%) contributed most to the dissimilarity, followed by *Anabaena* (8.08%), *Chroococcus* (7.03%), *Synechococcus* (6.79%), *Aphanizomenon* (5.79%), *Oscillatoria* (5.70%), and *Aphanocapsa* (5.48%).

2.4.3 Zebra mussels and cyanobacterial blooms

Among the lakes located in areas with established zebra mussels, 50.56 % had detectable microcystins, while among lakes located in areas where zebra mussels had not been established, only 30.54% of the lakes had detectable microcystins. Student's t-test showed that microcystins levels (t=-2.73, p=0.0065) and abundance of cyanobacteria (t=-2.51, p=0.0127) were significantly higher in lakes located in areas where zebra mussels had been established (Table 2.3). Box plots of the log transformed microcystins levels also indicated that lakes located in areas where zebra mussels had been established had higher microcystins levels (Figure 2.3). However, in lakes located in areas where zebra

mussels were established, the total phosphorus level was significantly lower (t=4.56, p<0.0001), indicating that zebra mussels play an important role in nutrient budget. The nitrogen levels were not significantly (p=0.4572) different based on status of zebra mussel establishment, indicating that the effect of zebra mussels on total nitrogen level was not significant. Previous studies have indicated that zebra mussels are extremely efficient in removing nutrients (N and P), especially phosphorus from lakes (Dzialowski and Jessie, 2009; Goedkoop et al., 2011; Zhu et al., 2006). In the current study, we only observed lower phosphorus levels in lakes located in zebra mussel invaded areas, which supports the conjecture that zebra mussels effectively remove phosphorus from lakes (Johengen et al., 1995). Zebra mussel establishment seemed to significantly increase the biodiversity of cyanobacteria genera (t=-2.87, p<0.001). Phytoplankton communities are sensitive to nutrient levels and the ratio of available nitrogen to phosphorus can play an important role in cyanobacterial community composition (Smith, 1983), which could be the potential cause of the different cyanobacteria community structures in lakes where zebra mussels had established and those where they had not.

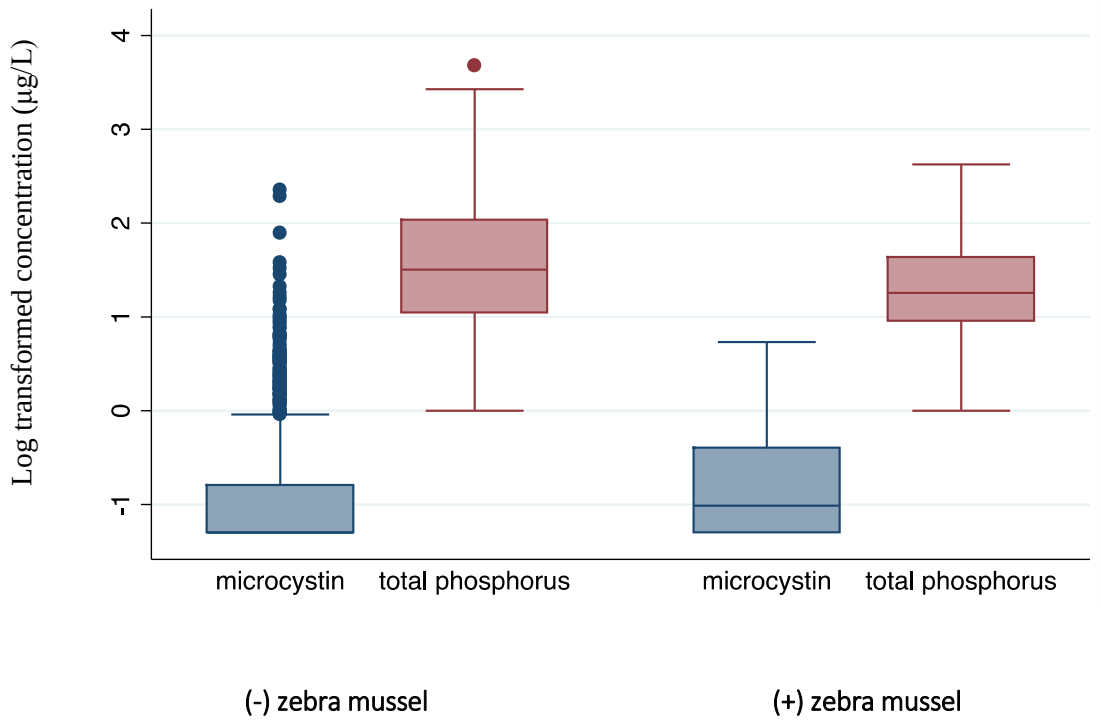


Figure 2.3. A box plot showing the comparison of average levels of microcystins and total phosphorus (log scale) between the U.S. lakes with zebra mussel establishments and without

Table 2.3. Relationship between zebra mussel establishment, cyanobacteria bloom-related parameters, and nutrient levels

Zebra mussel status of the area	**Zebra mussel not established (n=848)**	**Zebra mussel established (n=180)**	**Student's t-test**	
Variable	**Median**	**Median**	**t**	*p*
Microcystins (μg/L)	0.05	0.10	-2.73	0.0065
Nitrogen (μg/L)	576	627	-0.74	0.4572
Phosphorus (μg/L)	32	18	4.56	<0.0001
Cyanobacteria (cell/mL)	5248	6157	-2.51	0.0127
Microcystis (cell/mL)	239	508	-1.21	0.2250
Anabaena (cell/mL)	10	97	-5.64	<0.0001
Chlorophyll-*a*	8.67	6.40	0.85	0.3960
Cyanobacteria biodiversity	0.74	1.03	-2.87	<0.0001

2.3.4 Analysis of potential pathways whereby zebra mussels affect microcystins level

Zebra mussel establishment had significant relationships with total phosphorus level, cyanobacteria abundance, and microcystins level (Table 2.3). Based on the ability of zebra mussels for phosphorus removal and selective filtration, we suspected that total phosphorus levels and cyanobacteria abundance were involved in the causal pathway of increased microcystins levels in the presence of zebra mussels (Figure 2.4). High levels of phosphorus are usually directly implicated in increased microcystins levels by promoting cyanobacteria growth (Dolman et al., 2012), and increasing cyanobacterial toxicity (Davis et al., 2009; Vezie et al., 2002). Selective grazing by zebra mussels was also suspected in the increase in cyanobacteria abundance (Naddafi et al., 2007) and cyanobacteria toxicity (Vanderploeg et al., 2001), both of which could increase microcystins production. The assumption of the causal relationship was supported by our observed data, as well as the selective filtration ability of zebra mussels. Although nitrogen has also been an important factor in determining microcystins levels, it was not proposed in a pathway due to a non-significant difference in nitrogen in lakes between zebra mussel establishment and non-establishment.

Structural equation modeling confirmed the proposed causal pathway between zebra mussel establishment and microcystins level (Figure 2.4). All the direct effects in the model were highly significant ($p<0.001$). First, zebra mussel establishment led to a 1.61 times decrease in total phosphorus level, which may lead to a decrease in cyanobacteria abundance and microcystins level. Second, zebra mussel establishment led to a direct increase in cyanobacteria abundance by about 2.06 times, which may have led to the

increase in microcystins level. Third, zebra mussels have been shown to increase microcysitn levels through an indirect effect by 1.53 times (p<0.001). This pathway could be the result of selective grazing in which toxic cyanobacteria are separated, mixed with mucus to form psedofeces, and expelled (Wilson, 2008) thereby increasing the density of the toxic cyanobacteria. The sum total effect of zebra mussel establishment on microcystins level was a 1.40 times increase (Table 2.4, p=0.005*).* This total effect consisted of: a 0.86 times decrease in microcystins level through a decrease in total phosphorus, a 1.06 times increase through cyanobacteria abundance, and a 1.53 times increase through other ways such as increase in toxic cyanobacteria (Table 2.4).

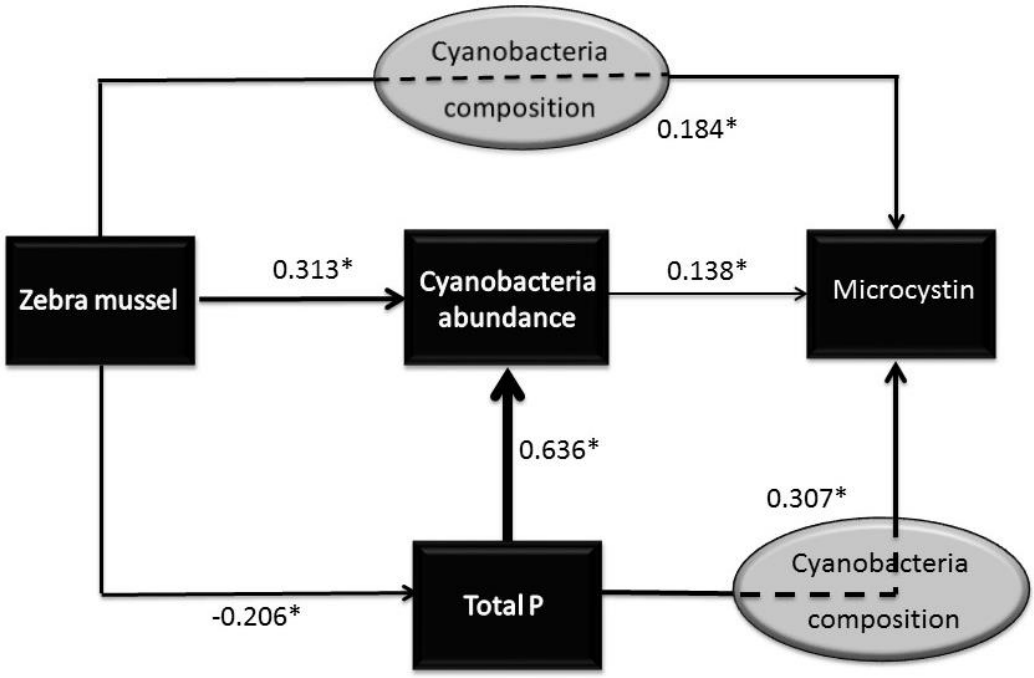


Figure 2.4. Structural equation models depicting the pathways for zebra mussel influence on microcystins level. (* indicated highly significant, p <0.001)

Table 2.4. Total effect of zebra mussel establishment on microcystin, cyanobacteria abundance and total phosphorus

Variable	Total effect (log changes)	*p* value	95% Confidence Interval
Microcystin	0.146	0.005	0.044, 0.248
Cyanobacteria abundance	0.182	<0.001	0.001, 0.363
Total phosphorus	-0.205	<0.001	-0.313, -0.098

2.5 Discussion

The influence of zebra mussel establishment on summer cyanobacterial community structure and microcystins production was assessed on a national scale utilizing data from over 1000 lakes in the United States. Zebra mussel establishment was shown to affect cyanobacterial community structure, abundance of cyanobacteria, and microcystins level; however, a limitation of this study was single sampling from a majority of the lakes.

Besides, the zebra mussel establishment status was based on the same HUC from the

USGS non-indigenous aquatic species database. Lakes located in areas where zebra mussels have invaded may not necessarily show obvious evidence of invasion, thus, we may underestimate the effects of zebra mussels by grouping the lakes based on the HUC.

Cyanobacterial community structure was different in lakes located in areas with zebra mussel establishment as opposed to those without, indicating that cyanobacterial community structure was influenced, possibly through selective grazing and nutrient removal by the zebra mussels. Lakes located in areas where zebra mussels were established had higher cyanobacteria abundance and biodiversity and lower phosphorus levels, which was consistent with observations in Saginaw Bay in terms of phosphorus level (Johengen et al., 1995). Nutrient levels play an important role in influencing cyanobacteria community composition (Xie et al., 2012). Grazing of zebra mussels on zooplankton could also lead to cyanobacteria community change as zooplankton grazing and respiration are important drivers of the phytoplankton community (Scavia and Fahnenstiel, 1987). Both nutrient cycling and selective grazing have been identified as mechanisms for the influence of zebra mussels on phytoplankton community structure and composition (Barbiero et al., 2006; Fernald et al., 2007; Fishman et al., 2010; Nicholls et al., 2002).

Lakes in the U.S. were clustered into 6 groups based on summer cyanobacteria community structure; lakes with similar cyanobacteria community structure tended to spatially cluster together. Both cyanobacterial community structure and cyanobacterial biodiversity were related to microcystins levels; the lakes that had the highest cyanobacterial biodiversity also had the highest level of microcysitn.

Microcystins levels were significantly higher in lakes located in areas where zebra mussels were established compared with lakes located in areas where they were not. A similar pattern was observed in 39 inland Michigan lakes where microcystins levels were higher in lakes with zebra mussels (Knoll et al., 2008). However, the mechanisms whereby zebra mussels influence microcystins levels are poorly understood. In the current study, higher cyanobacteria abundance and lower total phosphorus levels were observed in lakes located where zebra mussels were established. The reduction in total phosphorus after zebra mussel establishment has also been documented in the Great Lakes (Fahnenstiel et al., 1995; Mida et al., 2010). Since total phosphorus and cyanobacteria abundance were both influenced, we believe them to be important factors involved in determining microcystins levels in lakes with established zebra mussels. Structural equation modeling confirmed and quantified three possible ways whereby zebra mussel establishment could influence microcystins level (Figure 2.4). The first was by decreasing phosphorus concentration, which had a negative effect on microcystins production. The second was through an increase in cyanobacteria abundance, which had a positive effect on microcystins production. Third, zebra mussels may influence microcystins levels through other ways, such as selective filtration and ejection of toxic cyanobacteria.

In support of established data, we have demonstrated increased microcystins levels in lakes with zebra mussel establishment, showing that zebra mussel invasion have resulted in increasing toxic cyanobacteria blooms in inland lakes (Knoll et al., 2008; Vanderploeg et al., 2001). Zebra mussels have spread rapidly across the eastern half of North America

after their establishment in the 1980s (Bossenbroek et al., 2007) and their spread to the western U.S. was expected (Bossenbroek et al., 2007). With the expansion of zebra mussels to other parts of the United States, we may expect to have an increase in cyanobacteria blooms and consequently an increase in levels of microcystins, presenting a hazard to human and wildlife health and a detriment to recreational and other beneficial uses of inland lakes.

2.6 Chapter conclusions

- Cyanobacterial community structure was different in lakes located in areas where zebra mussels were established as opposed to lakes located in areas where they were not.
- Cyanobacterial community structure and cyanobacterial biodiversity were correlated with microcystins levels.
- Microcystins level and cyanobacteria abundance were significantly higher in lakes located in areas where zebra mussels were established compared with lakes located in areas where they were not.
- Total phosphorus level was significantly lower in lakes located in areas where zebra mussels were established compared with lakes located in areas where they were not; possibly due to the nutrient removal ability of zebra mussels.
- There appeared to be a positive effect of zebra mussel establishment on microcystins level. Alternatively, zebra mussel establishment can remove phosphorus and thereby suppress microcystins production.

- Three possible ways in which zebra mussel establishment could influence microcystins levels were proposed and quantified. The first was a negative effect caused by phosphorus removal, which would reduce microcystins production whereas the second and third ways were assumed to be due to increase in cyanobacteria abundance and toxic cyanobacteria, which would result in a positive effect on microcystins production.

Chapter 3: Spatial analysis of land-use impacts on nutrient concentrations and cyanobacterial blooms in USA lakes

3.1 Chapter overview

For effective control of noxious cyanobacterial blooms, understanding factors that promote cyanobacteria growth and cyanotoxin production is important. Towards this end, we used a spatial modeling approach to explore relationships among watershed land-use, nutrient concentrations, cyanobacterial abundance, and microcystins production from a dataset consisting of 1,028 U.S. lakes. As expected, we found nitrogen and phosphorus concentrations to be lower ($p<0.05$) in lakes surrounded by undeveloped watersheds (e.g., forested) than those dominated by agriculture. While we found that 98% of the lakes had cyanobacteria and 59% had *Microcystis* spp., logistic regression analysis revealed that the presence/absence of both response variables was unrelated in-lake total nitrogen and phosphorus concentrations. However, for lakes in which cyanobacteria or *Microcystis* was present, their abundances were positively related to both in-lake total nitrogen and phosphorus concentrations. We also found that 34% of our study lakes had detectable concentrations (>0.1 μg/L) of the hepatotoxins, microcystins. Through a series of analyses in which we determined the independent effect of both in-lake total nitrogen

and phosphorus concentration (after controlling for cyanobacteria abundance) on 1) the odds of having detectable microcystins levels and 2) microcystins concentration, we found that both response were more positively related to nitrogen concentration than phosphorus concentration. Additionally, after controlling for the concentration of both nutrients and cyanobacteria abundance, we found the percentage of barren land to be negatively related to microcystins concentration. Ultimately, our study suggests that efforts to reduce land-use activities that seemingly lead to nutrient runoff (e.g., agriculture) could help reduce in-lake nutrient concentrations, as well as the prevalence of toxic cyanobacteria.

3.2 Introduction

Land use, defined as "the arrangements, activities and inputs people undertake in a certain land cover type to produce, change or maintain it"(FAO/UNEP, 1999), has been shown to have significant impacts on downstream water quality (Tong and Chen, 2002). In particular, both agriculture, which has been identified as the largest source of excess nitrogen and phosphorus to both inland (Lee et al., 2009; Mitchell et al., 2009; Nielsen et al., 2012) and coastal (Bennett et al., 2001; Carpenter et al., 1998) waters, and urbanization (Foley et al., 2005) have been shown to be key contributors to degraded water quality in "downstream" aquatic ecosystems, including reduced water clarity (Crosbie and Chow-Fraser, 1999), excessive benthic algal production (Leland and Porter, 2000), and noxious cyanobacteria blooms (Foley et al., 2005). In response, changes in land use and land management practices have been recommended to improve the quality

of downstream water bodies (Changnon and Demissie, 1996). Such practices include, but are not limited to, conservation tillage (Holland, 2004), nutrient management plans (Santhi et al., 2006) and forest riparian buffers (Lowrance et al., 1997).

In addition to reducing water transparency, which can negatively affect the local economy via reduced recreational activity (e.g., boating, swimming, fishing (Steffensen, 2008) and reduced property values (Dodds et al., 2009), cyanobacteria blooms can threaten both ecological and human health. Not all cyanobacterial blooms are toxic; however, 25 – 75% of blooms have been estimated to contain harmful toxins (Bláha et al., 2009; Chorus, 2001). Such blooms are a global threat to ecological and human health (Edwards et al., 2006; Janus, 2010; Peperzak, 2003) and have resulted the deaths and of fish, and birds (Malbrouck and Kestemont, 2006).

Cyanobacterial blooms are ubiquitous throughout the United States (USA) and other countries (Fristachi et al., 2008). It is expected that cyanobacteria bloom formation may increase with continued climate change (Paerl et al., 2011a; Paerl and Huisman, 2009), especially warmer temperature and associated hydrologic changes may strongly affect the formation of harmful cyanobacteria blooms (Paerl and Paul, 2012).

Microcystis is one of the most common genera in bloom forming cyanobacteria found in freshwater systems (Fristachi et al., 2008). Species within this genus can produce toxins such as microcystins, which are the most commonly reported cyanotoxins in North America (Pelaez et al., 2010). Other commonly occurring cyanotoxins include saxitoxins and cylindrospermopsin (Pelaez et al., 2010). Microcystins in water supplies can cause gastrointestinal and hepatic illness (Byth, 1980; Teixeira Mda et al., 1993), as well as

hepatocellular carcinoma (Yu, 1995). Cyanotoxins also can cause skin rashes via dermal contact exposure route (Pilotto et al., 1997).

Human activities in the watershed (e.g., land development, urbanization, agricultural practices) often lead to excessive nutrient runoff, and have been associated the occurrence of cyanobacterial blooms (Anderson et al., 2002; Glibert et al., 2005; Glibert and Burkholder, 2006; Heisler et al., 2008; United Nations, 2006) in both developed and developing countries. Nitrogen and phosphorus, in particular, have been shown to not only promote cyanobacteria growth, but also favor the growth of toxic strains over nontoxic strains (Davis et al., 2009; Vezie et al., 2002). More attention has been given to phosphorus because some cyanobacteria have the ability to fix nitrogen from the atmosphere (Carpenter, 2008; Wang and Wang, 2009). Nutrient inputs may not be the only pathway by which land use can influence cyanobacteria growth and cyanotoxins production. Other land-use inputs, such as trace metals and organic pollutants, have been reported to play a role in cyanobacteria growth or toxin production (Lukac and Aegerter, 1993; Neilan et al., 2013; Sheng et al., 2012; Xu et al., 2013). For example, zinc and iron have been found to stimulate cyanobacteria growth (Lukac and Aegerter, 1993; Xu et al., 2013), whereas iron also has been found to suppress microcystins production (Neilan et al., 2013). Research also has suggested that polycyclic aromatic hydrocarbons have a potential to promote *Microcystis aeruginosa* growth (Zhu et al., 2012).

Herein, we sought to identify and quantify the effect of land use on cyanobacterial growth and microcystins production, through nutrient concentrations and other pathways. Although the relationship between land use and nutrient concentrations have been

explored by other studies (Abell et al., 2011; Nielsen et al., 2012), spatial autocorrelation has been a confounding factor. Spatial autocorrelation is a well-recognized concern for observational studies. Using a comprehensive dataset of over 1,000 lakes, we investigated two hypotheses that 1) certain types of land use promote cyanobacterial blooms through increased nutrient concentration; and 2) There are other significant factors of land use, in addition to nutrient concentrations, that impact cyanobacterial blooms. To test these hypotheses we quantified: 1) the effect of land use on total nitrogen and total phosphorus concentrations in downstream lakes; 2) the effect of land use and nutrient concentrations on cyanobacteria concentrations; and 3) the effect of land use, nutrient concentrations and cyanobacteria abundance on microcystins concentrations using spatial models. Ultimately, we seek to use our findings to help identify appropriate strategies for reducing the contributions land use on the formation of downstream cyanobacterial blooms.

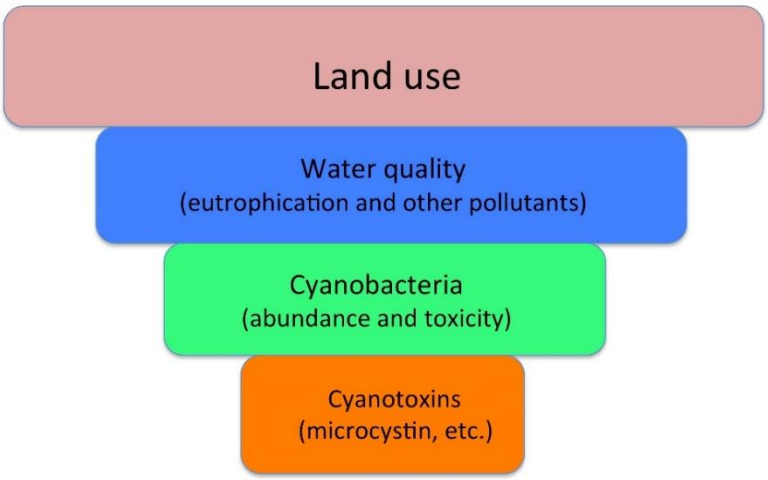


Figure 3.1. Proposed pathways by which land use can influence cyanobacteria abundance and microcystins production in lakes.

3.2 Methods

3.2.1 Data sources

We analyzed data from the National Lake Assessment database (http://water.epa.gov/type/lakes/NLA_data.cfm), conducted by the U.S. Environmental Protection Agency (EPA), Office of Water, and Office of Research and Development in collaboration with other organizations. This database consists of information on water quality, biological conditions, habitat conditions, and recreational suitability in 1,028 USA lakes during the summer of 2007. Lakes in Alaska and Hawaii, the Laurentain Great Lakes, and the Great Salt Lake were not included in the survey. Water bodies included in our dataset were natural or man-made freshwater lakes, ponds, or reservoirs that had a surface area greater than 40,469 m^2, and an average depth of at least 1 m. Single grab water samples were collected to measure nutrients, chlorophyll-a, phytoplankton, and the algal toxin, microcystins. Nondetects of microcystins were assumed to have the concentration of 0.05 μg/L, half of the detection limit, which was already done by the USEPA in the dataset. Of the 1,028 sample lakes, 933 were sampled once with the rest being sampled twice, in which case, the average of the two samples was used for this study. A detailed description of the sampling campaign can be found in the following documents: (1) Quality Assurance Project Plan (EPA 841-B-07-003); (2) Field Operations Manual (EPA 841-B-07-004).Variables included in our analyses were total nitrogen, total phosphorus, microcystins concentration, cyanobacteria abundance as well as watershed land use (based on National Land Cover Dataset [NLCD] 2001 land-use classes; Table 3.1).

Table 3.1. Land-use class definitions under NLCD 2001.

Land use classification	**Definition**
Water	All areas of open water or permanent ice/snow cover.
Developed	Areas characterized with a high percentage (>30%) of constructed materials (e.g. asphalt, concrete, buildings, etc).
Barren	Areas characterized by bare rock, gravel, sand, silt, clay, or other earthen material, with little or no "green" vegetation present regardless of its inherent ability to support life. Vegetation, if present, is more widely spaced and scrubby than that in the "green" vegetated categories; lichen cover may be extensive.
Forested	Areas characterized by tree cover (natural or semi-natural woody vegetation, generally greater than 6 meters tall); tree canopy accounts for 25-100% of the cover.

Continued

Table 3.1 continue

Shrubland	Areas characterized by natural or semi-natural woody vegetation with aerial stems, generally less than 6 meters tall, with individuals or clumps not touching to interlocking. Both evergreen and deciduous species of true shrubs, young trees, and trees or shrubs that are small or stunted because of environmental conditions are included.
Herbaceous	Upland areas characterized by natural or semi-natural herbaceous vegetation; herbaceous vegetation accounts for 75-100 percent of the cover.
Agricultural	Areas characterized by herbaceous vegetation that has been planted or is intensively managed for the production of food, feed, or fiber; or is maintained in developed settings for specific purposes. Herbaceous vegetation accounts for 75-100 percent of the cover.
Wetlands	Areas where forest or shrubland vegetation accounts for greater than 20 percent of vegetative cover and the soil or substrate is periodically saturated with or covered with water and areas where forest or shrubland vegetation accounts for greater than 20 percent of vegetative cover and the soil or substrate is periodically saturated with or covered with water.

The sample for phytoplankton counting was fixed with Lugol's iodine and concentrated via sedimentation. Phytoplankton was counted to the lowest possible taxonomic level (usually genus) (Rigosi et al., 2014). Cyanobacteria abundance (cell/mL) in each lake was calculated by summing the abundances of cyanobacteria species (formerly called blue-green algae in the National Lake Assessment database).

On average, forest covered 33.16% of lake basins (Table 3.2), followed by agricultural land (20.66%), herbaceous land (13.10%) and water area (9.28%). The percentage of water in lake basin did not include the lakes of interest.

Table 3.2. Summary of land use percentages in lake basins from 2007 NLA dataset

	Water area	Developed land	Barren	Forest	Shrub land	Herbaceous land	Agricultural land
Mean percentage	9.28	8.56	1.10	33.16	9.14	13.10	20.66
Standard deviation	12.83	13.83	5.41	29.67	18.72	21.53	24.64

3.2.2 Data analyses

We used Spearman's rank correlation to explore the relationship between watershed land use and nutrient concentrations of the lakes. Before regression, total phosphorus concentrations, total nitrogen concentrations, and micorcystins concentrations were log10 transformed to satisfy the linear assumption. Cyanobacteria abundance and *Microcystis* abundance were log10 (x+1) transformed.

Multivariate ordinary least squares (OLS) linear regression was used to quantify the relationships between land-use types and nutrient concentrations (total nitrogen and total phosphorus). The percentage of each land-use category was used as independent variables. Logistic regression was used to explore the relationships between nutrient concentrations, land use types and the occurrence of cyanobacteria and *Microcystis*. In lakes where cyanobacteria or *Microcystis* were present, OLS linear regression was used to estimate the effect of nutrient on the abundance of cyanobacteria and *Microcystis.* Logistic regression also was used to explore relationships between the presence of microcystins and land use, nutrient concentrations, and cyanobacteria abundance. When microcystins was present, OLS linear regression was used to estimate the effect of land use, nutrient concentrations and cyanobacteria abundance on microcystins concentrations. In all models, where land use categories were used as independent variables, stepwise model selection was used to find the significant land use categories. The significant level for entry was 0.15 and the significant level for elimination was 0.05.

To account for the spatial autocorrelation of our data, after each OLS linear regression, Moran's I was used to test the spatial dependence of the residuals or Pearson's residuals

from the regression. If Moran's I showed significant spatial autocorrelation, then SAR as well as CAR were employed. For each logistic regression, if Moran's I was significant for the Pearson's residuals, CAR logistic model was used to adjust for the spatial autocorrelation.

Spatial SAR was fitted using the spdep (Spatial dependence: weighting schemes, statistics and models) package by Bivand in statistical programming software R, and other statistical analyses were carried out using SAS 9.3 (SAS Institute Inc., Cary, NC). PROC GLIMMIX was be used to fit CAR models, using coordinates from USA Contiguous Equidistant projection. First, exponential covariate function was used to fit all CAR models. If the exponential function failed to capture the spatial autocorrelation, a power covariate function was used followed by, a spherical covariate function. The coordinates were extracted using ArcGIS (ESRI, 2009).

3.3 Results

3.3.1 Spearman's correlation between land-use and water quality

Results from Spearman's rank correlation indicated that agricultural land, forest land, shrubland, herbaceous land and developed land were significantly correlated with many water quality parameters (Table 3.3). With regard to nutrients, total phosphorus (ρ=-0.57; p<0.05) and total nitrogen (ρ=-0.60; p<0.05) concentrations were negatively correlated with the percentage of forested land in the watershed. By contrast, both total phosphorus (ρ=0.40; p<0.05) and nitrogen (ρ = 0.46; p<0.05) concentrations were positively correlated with the percentage of agricultural land in the watershed. Chlorophyll-*a* also

was positively related to the percentage of agricultural land ($\rho = 0.48$; $p<0.05$) and negatively related to the percentage of forested land ($\rho = -0.38$; $p<0.05$). In terms of cyanobacterial bloom parameters, the percentage of forested land was negatively related to the abundance of cyanobacteria ($\rho=-0.23$; $p<0.05$) and microcystins concentration ($\rho=-0.31$; $p<0.05$). The percentage of agricultural land also was positively correlated with cyanobacteria abundance ($\rho = 0.29$; $p<0.05$), *Microcystis* abundance ($\rho=0.12$; $p<0.05$), and microcystins concentration ($\rho=0.37$; $p<0.05$)

Table 3.3. Summary of the Spearman's rank correlation analyses of water quality variables and land-use types of the lake basins.

	Water area	Developed land	Barren	Forest	Shrub land	Herbaceous land	Agricultural land	Wetland
Total phosphorus	-0.172*	0.065*	-0.186*	-0.538 *	-0.237*	0.252*	0.402*	0.009
Total nitrogen	0.019	0.170*	-0.285*	-0.596*	-0.257*	0.170*	0.468*	0.142*
Turbidity	-0.164*	0.120*	-0.188*	-0.498*	-0.180*	0.236*	0.418*	-0.015
Dissolved organic carbon	0.148*	0.053*	-0.350*	-0.468*	-0.200*	0.191*	0.276*	0.270*
Conductivity	0.035	0.162*	-0.159*	-0.664*	-0.110*	0.334*	0.371*	0.029
Chlorophyll-*a*	-0.089*	0.270*	-0.207*	-0.382*	-0.261*	0.080*	0.479*	0.140*
Cyanobacteria	0.007	0.127*	-0.192*	-0.231*	-0.138*	0.081*	0.286*	0.103*
Microcystis	-0.064*	0.188*	-0.048	0.066*	-0.066*	-0.105*	0.122*	0.027
Microcystin	0.122*	0.173*	-0.185*	-0.309*	-0.224*	0.051	0.368*	0.175*

* indicates $p<0.05$

3.3.2 Quantifying the effect of land-use on water quality parameters

3.3.2.1 Total nitrogen

SAR and CAR were used to address the spatial autocorrelation of the residuals. The SAR model for total nitrogen included the percentage of barren, forested, and agricultural land base on p-values (Table 3.4, Model A). The simultaneous autoregressive error coefficient from the total nitrogen model was 0.714 (p<0.0001), indicating a significant spatial correlation of the error. The CAR model included the same the types of land-use as the SAR model.

These three models (OLS, SAR, and CAR) (Table 3.4, Model A), supported two key results: 1) lakes with a large amount of forested or barren land in the watershed had lower concentrations of total nitrogen; and 2) lakes with a large amount of agricultural land in the watershed had higher total nitrogen concentrations. The two spatial models (SAR and CAR) produced very similar results. According to the CAR model, for every 1% increase in agricultural land in a lake basin, the estimated total nitrogen concentration increases by 0.64%; for every 1 % increase in forest, the estimate total nitrogen concentration decreased by 1.21%; for every 1 % increase in barren land, the estimate total nitrogen concentration decreases by 1.70%.

Table 3.4. The effect of lake basin land use on lake nutrient concentrations, summaries for final stepwise selected OLS, SAR and CAR models

Model A (the effect of land use on total nitrogen concentration)						
Model type	**OLS**		**SAR**		**CAR**	
	Estimate (Standard error)	*p*	Estimate (Standard error)	*p*	Estimate (Standard error)	*p*
Intercept	3.06767 (0.03414)	<.0001	2.92848 (0.04224)	<.0001	2.9130 (0.0478)	<.0001
Barren	-0.01621 (0.00207)	<.0001	-0.00914 (0.00195)	<.0001	-0.00746 (0.00197)	0.0002
Forest	-0.00844 (0.00044)	<.0001	-0.00551 (0.00051)	<.0001	-0.00528 (0.00054)	<.0001
Agricultural land	0.00264 (0.00058)	0.0001	0.00301 (0.00195)	<.0001	0.00277 (0.00058)	<.0001
Shrub land	-0.00260 (0.00068)	0.0001	___	___	___	___
Developed land	-0.00186 (0.00087)	0.032	___	___	___	___

Continued

Table 3.4 continue

Model B (the effect of land use on total phosphorus concentration)						
	Estimate (Standard Error)	*p*	Estimate (Standard Error)	*p*	Estimate (Standard Error)	*p*
(Intercept)	1.42968 (0.06337)	<.0001	1.70330 (0.07058)	<.0001	1.69370 (0.07369)	<.0001
Barren	-0.01532 (0.00322)	<.0001	-0.01509 (0.00312)	<.0001	-0.01293 (0.00310)	<.0001
Forest	-0.00632 (0.00088)	<.0001	-0.00763 (0.00085)	<.0001	-0.00723 (0.00090)	<.0001
Agricultural land	0.00834 (0.00100)	<.0001	0.00472 (0.00094)	<.0001	0.00490 (0.00097)	<.0001
Shrub land	0.00399 (0.00107)	0.0002	___	___	_____	_____
Water area	_____	_____	-0.00409 (0.00137)	0.00281	-0.00377 (0.00132)	0.0044
Herbaceous land	0.00636 (0.00101)	<.0001	_____	_____	_____	_____

3.3.2.2 Quantifying the effect of land-use on total phosphorus concentration

The SAR and CAR models for total phosphorus included the percentage of barren, forest, agricultural land and water (Table 3.4, Model B). The simultaneous autoregressive error coefficient from the SAR model is 0.697 (p<0.0001), indicating a significant spatial autocorrelation of the error.

Similar to the results for total nitrogen, the percentages of forest and barren land in lake basins had a negative (p<0.05) relationship with total phosphorus concentrations, and the percentage of agricultural land had a positive relationship (p<0.05) with the total phosphorus concentrations according to all three of the models (Table 3, Model B). In spatial models (SAR and CAR), the percentage of water in the basin had a positive relationship (p<0.05) with total phosphorus concentration. According to CAR model, for every 1% increase in agricultural land in the lake basin, the estimated total phosphorus concentration increases by 1.13%; for every 1% increase in forest, the estimated total phosphorus concentration decreases by 1.65%; for every 1% increase in barren land, the estimated total phosphorus concentration decreases by 2.93%; for every 1% increase in water area, the estimated total phosphorus concentration decreases by 0.86%.

3.3.3 Quantifying the effect of Nutrient concentration and land-use on cyanobacteria and *Microcystis*

3.3.3.1 Quantifying the effect of Nutrient concentration and land-use on cyanobacteria and *Microcystis* presence

Cyanobacteria were detected in 1,000 of the 1,025 sampled lakes (samples from 3 lakes were lost) (97.6%). Logistic regression suggested that lake nutrient concentrations were not significantly related with the presence of cyanobacteria, while land use was significantly related with the presence of cyanobacteria (Table 3.5). The presence of cyanobacteria was negatively related to the extent of barren land, and positively related to the extent of agricultural land in lake basins (Table 3.5). The spatial models were not needed for these analyses because Moran's I was not significant (Moran's I = 0.0033, p = 0.18). *Microcystis* were found in 602 of the 1025 lakes (58.7%). Both regular logistic regression and CAR logistic regression showed that the odds of having *Microcystis* were not significantly related (p>0.10) to the concentration of total nitrogen, total phosphorus, or any land use category. The results showed that land use did not significantly influence the presence of cyanobacteria or *Microcystis* through nutrient concentrations. By contrast, the presence of cyanobacteria was negatively related with the extent of barren land (OR=0.963) and positively related with agricultural land (OR=1.041).

Table 3.5. Odds Ratio of the presence of cyanobacteria associated with total phosphorus, total nitrogen and land use, estimated by multivariate logistic regression

Independent variables	Odds ratio Point Estimate	95% Confidence Limits	
Total phosphorus	1.023	0.395	2.65
Total nitrogen	1.855	0.464	7.415
Barren	0.963	0.935	0.991
Agricultural	1.041	1.004	1.078

3.3.4 Quantifying the effect of Nutrient concentration and land-use on cyanobacteria and *Microcystis* abundance

SAR and CAR models were used for these analyses because Moran's I of residuals form OLS model was significant (p<0.05). In lakes where cyanobacteria were present, all three models showed a significant (p<0.05) positive relationship between the abundance of cyanobacteria, and total nitrogen and total phosphorus (Table 3.6). The estimated coefficients of total phosphorus were much smaller than total nitrogen in all three models,

indicating the effect of total nitrogen was bigger than total phosphorus on cyanobacteria abundance. After controlling for nutrient concentrations, barren land was negatively related to cyanobacteria abundance (Table 3.6).

In lakes where *Microcystis* was present, all three models showed that the abundance of *Microcystis* had a significant positive relationship ($p<0.05$) with total nitrogen and total phosphorus concentrations (Table 6). After controlling for nutrient concentrations, no land use category had a significant relationship with *Microcystis* abundance. Therefore, land use category was not included in the models (Table 3.7).

Results indicated that although total phosphorus and total nitrogen showed no significant relationship with the presence of cyanobacteria or *Microcysits*, they were both positively related with the abundance of cyanobacteria/*Microcystis* under the conditions that cyanobacteria/*Microcystis* were present. The estimated coefficients of total phosphorus were much smaller than the ones of total nitrogen in all the models, indicating the effect of total nitrogen was greater than total phosphorus on cyanobacteria/*Microcystis* abundance.

Table 3.6. The effect of total nitrogen, total phosphorus and land use on cyanobacteria abundance in lakes where cyanobacteria were present. A summary of results from multivariate OLS, SAR and CAR models

Model	**OLS**		**SAR**		**CAR**	
	Estimate (Standard error)	p	Estimate (Standard error)	p	Estimate (Standard error)	p
Intercept	1.405 (0.215)	<.0001	1.777 (0.247)	<.0001	1.920 (0.261)	<.0001
Total phosphorus	0.131 (0.067)	0.0575	0.231 (0.070)	0.0010	0.255 (0.071)	0.0003
Total nitrogen	0.763 (0.101)	<.0001	0.570 (0.110)	<.0001	0.503 (0.111)	<.0001
Barren	-0.025 (0.005)	<.0001	-0.022 (0.005)	<.0001	-0.020 (0.005)	0.0002

Table 3.7. The effect of total nitrogen, total phosphorus and land use on *Microcystis* abundance from in lakes where *Microcystis* was present. A summary of results from multivariate OLS SAR and CAR models

Model	**OLS**		**SAR**		**CAR**	
	Estimate (Standard error)	*p*	Estimate (Standard error)	*p*	Estimate (Standard error)	*p*
Intercept	2.032 (0.340)	<.0001	2.176 (0.3810)	<.0001	2.095 (0.401)	<.0001
Total phosphorus	0.187 (0.100)	0.0626	0.2260 (0.103)	0.0278	0.247 (0.103)	0.0171
Total nitrogen	0.428 (0.159)	0.0104	0.352 (0.171)	0.0398	0.360 (0.176)	0.0410

3.3.5 Quantifying the effect of cyanobacteria abundance, nutrient concentrations and land use on microcystins concentration

Microcystins were detected (>0.1μg/L) in 350 lakes (34.0%). The average concentration of microcystins in all the lakes was 1.02 μg/L. Logistic regression was used to explore the probability of having detectable microcystins. Since Moran's I indicated significant spatial autocorrelation in Pearson's residuals (p<0.05), a CAR logistic model also was used. Regular logistic regression and CAR logistic models generated similar results. Both models showed that the odds of having detectable microcystins related significantly (p<0.05) with nitrogen concentration, cyanobacteria abundance and several land use types (Table 3.8). Barren land and shrubland were negatively related to detectable microcystins concentrations, whereas developed and agricultural land were positively related to detectable microcystins. However, total phosphorus was not significantly related to detectable microcystins when controlled for total nitrogen, cyanobacteria abundance, and land use.

Table 3.8. Odds ratio of the presence of microcystins associated with total phosphorus, total nitrogen, cyanobacteria abundance and land use of the basin, estimated by multivariate logistic regression and CAR logistic regression

	Logistic			**CAR-logistic**		
Parameter	Odds ratio	95% Confidence Limits		Estimate	95% Confidence Limits	
Total nitrogen	10.343	5.249	20.382	10.326	5.215	20.449
Total phosphorus	0.835	0.563	1.240	0.836	0.561	1.245
Cyanobacteria	1.722	1.428	2.077	1.722	1.425	2.080
Developed	1.016	1.005	1.027	1.016	1.005	1.027
Barren	0.737	0.558	0.973	0.736	0.556	0.974
Agricultural	1.012	1.005	1.019	1.012	1.005	1.019
Shrubland	0.985	0.974	0.995	0.984	0.973	0.995

For those lakes with detectable microcystins, only OLS linear regression was used to model the concentration of microcystins because Moran's I suggested no significant

spatial autocorrelation of the residuals (p>0.05). Results from the OLS model (R^2 = 0.28) suggested that microcystins concentration was related to total nitrogen concentration, cyanobacteria abundance, and land use type (Table 3.9). For every 1% increase in total nitrogen, the estimated increase in microcystins concentration was 0.70%, according to the OLS model. The effect of total phosphorus was not significant (P = 0.24). When controlling for total phosphorus, total nitrogen, and cyanobacteria abundance, land use type was still significantly related to microcystins concentration, especially barren land and water area (Table 3.9). Barren land was negatively related to microcystins concentration whereas water area was positively related to microcystins concentration (Table 3.9).

Table 3.9. The effect of total nitrogen, total phosphorus and cyanobacteria abundance on microcystins concentration in lakes where microcystins were detected. OLS multivariate regression analyses are summarized

	Estimate (Standard Error)	P
Intercept	-2.8547 (0.2899)	<.0001
Total phosphorus	-0.0938 (0.07907)	0.2363
Total nitrogen	0.7013 (0.12312)	<.0001
Cyanobacteria	0.1457 (0.0343)	<.0001
Barren	-0.1460 (0.0691)	0.0352
Water	0.0064 (0.0021)	0.0027

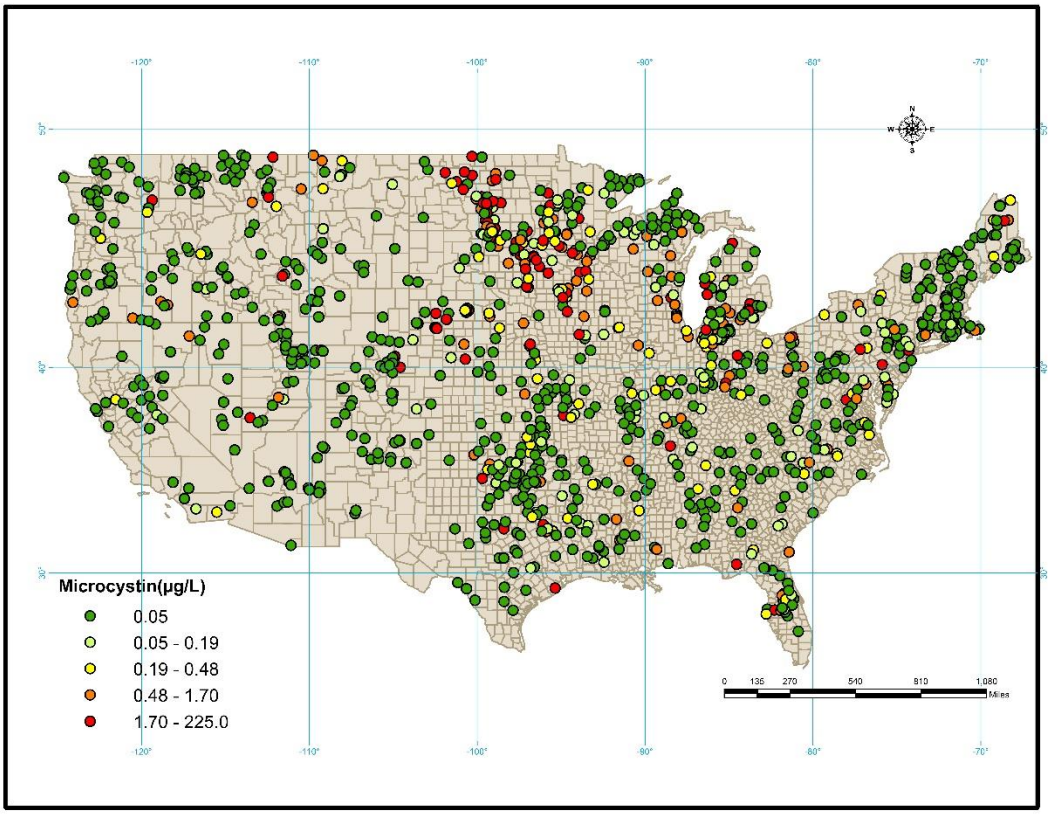


Figure 3.2. Summary of microcystins concentrations from the lakes of United States in the National Lake Assessment in the summer of 2007

3.4 Discussion

Based on our analyses, watershed land-use significantly impacts the concentrations of nutris and microcystins in USA lakes. Both total nitrogen and total phosphorus concentrations were positively related the percentage of agricultural land in the watershed, a finding that has been documented elsewhere (Nielsen et al., 2012; Tong and Chen, 2002). By contrast, both total nitrogen and total phosphorus were negatively related to the

percentage of forested and barren land in the lake basins. Forest coverage can reduce the volume of runoff and act as a buffer that benefits the water quality. Greater amounts of forests in watershed has been shown to protect surface water from eutrophication and contamination (Nielsen et al., 2012; Tong and Chen, 2002). A previous modeling study found that the total amount of nitrogen and phosphorus produced per unit area from different types of land-use were in the following order in a watershed in Ohio: agriculture> urban>forest>barren (Tong and Chen, 2002). Our models produced similar results with Tong et al. (2002). The decrease in both total nitrogen and total phosphorus corresponding to higher percentages of barren land in the basin was greater than that corresponding to higher percentage of forest. Low concentration of nitrogen and phosphorus in barren land accompanied with the low water holding capability of barren land could cause the negative relationship between barren land and nutrient levels by dilution.

Although studies have linked cyanobacteria abundance in lakes with nitrogen and/or phosphorous (Dolman et al., 2012; Wilhelm et al., 2011), the presence of cyanobacteria or *Microcystis* in the lakes was found to be unrelated to nutrient concentrations in this study. There were some lakes with high concentrations of both nitrogen and phosphorus but no cyanobacteria or *Microcystis* . For instance, Lyman Lake, Arizona had both total phosphorus concentration and total nitrogen concentration over 200 μg/L, but no cyanobacteria or toxin was found. However, after controlling for total nitrogen and phosphorus concentrations, more barren land in the basin resulted in a lower possibility of cyanobacteria presence, as well as lower cyanobacteria abundance. Barren land caused

by smelting, logging, and fires has been found to have elevated concentrations of copper (Maxwell, 1991), which can be toxic to cyanobacteria (Jardim and Pearson, 1984; Xu et al., 2013). Cultures of soils from barren sites were characterized by a low diversity of chlorophytes and a few diatoms, and no cyanobacteria (Maxwell, 1991), suggesting that barren land had a potential to suppress cyanobacteria in nearby lakes. However, none of land use categories was significantly related to the presence or abundance of *Microcystis* after controlling for nutrient concentrations. In lakes which cyanobacteria or *Microcystis* was present, both total nitrogen and total phosphorus were significantly related to the abundance of the cyanobacteria or *Microcystis*. Results showed that the effect of total nitrogen was greater than total phosphorus on cyanobacteria and *Microcystis* abundance. However, more attention has been given to phosphorus due to the nitrogen-fixing capability of some cyanobacteria (Carpenter, 2008; Wang and Wang, 2009). This result showed that controlling both nitrogen and phosphorus is important in mitigating cyanobacterial blooms. Studies have found that nitrogen fixation could supply little new nitrogen even when nitrogen fixing cyanobacteria are dominant (Ferber et al., 2004). A study in Lake Taihu, China showed that reducing both nitrogen and phosphorus input was important for controlling cyanobacterial bloom in this hypereutrophic system (Paerl et al., 2011b). The dual control of both nitrogen and phosphorus inputs is essential in reducing cyanobacteria blooms (Jeppesen et al., 2007; Kronvang et al., 2005).

Microcystins occurrence (or detection) has been found to be common in U.S. lakes. A cyanobacterial bloom survey in Wisconsin waters in the summer of 1987 found that approximately 25% of the study sites had toxic algae (Repavich et al., 1990), which was

comparable to the occurrence of toxin in the NLA survey. Cyanobacteria abundance was significantly positively related with microcystins level in this study. Greater Nitrogen concentration related with higher microcystins concentration, whereas total phosphorus concentration did not show a significant relationship with microcysitns concentration in the U.S. lakes, further supporting that nitrogen should be controlled for mitigating cyanobacteria and microcystins prevalence in the lakes. A previous study suggested reducing nitrogen could control microcystins production by suppressing *mcy*B transcription (Ginn et al., 2010), which could explain the association between nitrogen concentration and microcystins concentration. Nitrogen seems to be very important in promoting the production of microcystins since toxic *Microcystis* strains require higher nitrogen than nontoxic ones (Davis et al., 2009; Vezie et al., 2002). . Although phosphorus has been showed to play a role in promoting toxic *Microcystis* growth and microcystins production under experimental conditions (Davis et al., 2009; Jahnichen et al., 2011; Rapala et al., 1997), total phosphorus concentration did not show a significant relationship with microcystins concentrations in our study lakes when controlling for total nitrogen. Barren land showed a negative effect on microcystins concentrations, after controlling for nutrient concentrations and cyanobacteria abundance, possibly due to suppressing microcystins production with runoff containing heavy metals. Substances from barren land such as copper are toxic to cyanobacteria and suppress cyanobacteria growth (Xu et al 2013, Jardim et al, 1985). Barren land due to smelting and mining has been found to have high concentrations of Cd (Li et al., 2000; Mench et al., 2003), that could decrease microcystins concentration by down regulating the transcription of microcystins-related genes (Qian et al., 2012).

3.5 Chapter Conclusions

- Watershed land use influenced lake nutrient concentrations. More forest and less agricultural land in the watershed can protect the lakes from eutrophication.
- The occurrence of cyanobacteria or *Microcystis* in lakes was unrelated to in-lake nutrient concentrations. However, for those lakes in which cyanobacteria or *Microcystis* was present, their abundance was positively related to both total nitrogen and total phosphorus concentrations.
- Nitrogen appeared more important than phosphorus in controlling toxic cyanobacteria abundance.
- Land use types may influence on cyanobacteria and microcystins concentrations in different ways other than increased nutrients concentrations. Other ways could be water contaminates such as heavy metals that may suppress cyanobacteria growth and microcystins production.
- Barren land showed a negative relationship with cyanobacteria concentrations as well as microcystins concentrations after controlling for total nitrogen and total phosphorus. Further study is warranted to understand the mechanisms of how barren lands influence on cyanobacteria needs further study.

Chapter 4: Cyanobacteria blooms and non-alcoholic liver disease in the contiguous United States: a county level ecological study using satellite remote sensing data

4.1 Chapter overview

There is evidence suggesting that cyanobacterial toxins can cause liver damage and cancer. However, the excess risk of liver disease caused by these toxins remains uncertain, given that there is little epidemiologic research on the effects of these toxins in humans. The purpose of this study is to determine if there is an association between cyanobacteial blooms and nonalcoholic liver disease in the contiguous US. An ecological geographic study method was employed. Gender and age standardized mortality rate (SMR) of nonalcoholic liver disease was computed between 1999 and 2010 for each of the U.S. counties. A bloom coverage map was produced using estimated phycocyanin levels from MERIS images from 08/01/2005-09/30/2005. A flexibly-shaped spatial scan statistic tool was used to identify significant clusters of death from nonalcoholic liver disease. A map of local indicator of spatial association (LISA) clusters and a Bayesian spatial regression, implementing a negative binomial model, were used to analyze the relationship between cyanobacterial bloom coverage and death from nonalcoholic liver disease. Cyanobacterial blooms were found to be widely spread in the United States, including coastal areas. Significant clusters of nonalcoholic liver disease deaths were

identified in impacted coastal areas by cyanobacterial blooms. Bayesian regression analysis showed that bloom coverage is significantly related with nonalcoholic liver disease death risk. Risk of death from nonalcoholic liver disease increased by 0.3% (95% Bayesian confidence interval 0.1% to 0.5%) with each 1 % increase in bloom coverage in the affected county after adjusting for age, gender, educational level, and race. The significant association between cyanobacterial blooms and nonalcoholic liver disease in this ecological study suggested a population level health impact from cyanobacterial blooms. Observed significant association suggested the need of further investigation for establishing a causal association between acute and chronic human health effects and cyanobacterial blooms.

4.2 Introduction

An important cause of morbidity and mortality in the United States is liver disease, accounting for up to 2% of all deaths in the U.S. (Kim et al., 2002). Economically, approximately 1% of the total national health care expenditure is spent on the care of liver disease patients with the burden of liver disease appears to be on the rise (Kim et al., 2002). Globally, liver disease also constitutes a fast increasing burden to society. In the United Kingdom, liver disease is the fifth most common cause of death with increasing rates (Williams et al., 2006). The potential risk factors for nonalcoholic liver disease include age, gender, and obesity (Preiss and Sattar, 2008). Accumulating evidence suggests that nonalcoholic liver disease is rapidly becoming another important cause of hepatocellular carcinoma (Younossi et al., 2011) . Consequently, it appears very

important to identify the role of the environmental factors in the etiology of nonalcoholic liver disease.

Cyanobacteria blooms have been reported to be a severe problem in many water bodies around the world. Recent research suggests that eutrophication, coupled with climate change, promotes the proliferation and expansion of cyanobacterial harmful algal blooms worldwide (O'Neil et al., 2012). These blooms can affect water quality, produce a variety of toxins, including microcystins, nodularins and anatoxins (Dittmann and Wiegand, 2006). It has also been shown that the neurotoxic amino acid, BMAA, is widely produced by cyanobacteria (Holtcamp, 2012). Human exposure to these toxins occurs by ingestion, skin contact, and inhalation (Cheung et al., 2013). Despite the potential health risks of cyanobacterial toxins shown by animal studies, only limited epidemiological studies have been reported in humans. Microcystins, the most common and thoroughly studied cyanobacterial toxins, have been identified as liver toxins (Carmichael et al., 2001). Cyanobacterial toxins, such as microcystins, usually accumulate in vertebrate liver cells and are suggested as hepatotoxins (Wiegand and Pflugmacher, 2005). Microcystins are resistant to digestion in the gastrointestinal tract and are concentrated into the liver by an active transport system (Runnegar et al., 1995). Acute poisoning occurs by destruction of the liver architecture, leading to blood loss in the liver and hemorrhagic shock (Falconer et al., 1981). Chronic exposure to these toxins causes an ongoing active liver injury in mice (Falconer et al., 1988) and there is experimental evidence for tumor promotion by microcystins (Humpage and Falconer, 1999). Human exposure to microcystins occurs by: 1) ingestion of microcystins from tap water due to cyaonbacteiral blooms in the source

water; 2) recreational exposure through accidental ingestion or inhalation and dermal contact; and 3) ingestion of seafood with accumulated microcystins. Algal cells and waterborne toxins can be aerosolized by a bubble-bursting process via wind-driven, white-capped waves (Blanchar.Dc and Syzdek, 1972). Aerosol samples, taken during recreational activities on bloom impacted lakes, have been found with detectable levels of microcystins (Backer et al., 2008; Backer et al., 2010). Although the levels of aerosolized toxin were generally low, laboratory investigations have found that exposure of mice to microcystin-LR by the intranasal route was about 10 times more effective than by the oral route (Fitzgeorge et al., 1994). There is evidence that microcystins can be accumulated in seafood, such as fish (Pawlik-Skowronska et al., 2012). As a primary target of microcystins, liver disease has been associated with microcystins contamination. The relationship between cyanobacteria toxin and liver cancer has been observed in several epidemiology studies in developing countries (Svircev et al., 2009; Ueno et al., 1996). Deaths in Brazil have been attributed to exposure to cyanobacterial hepatotoxins (microcystins) via hemodialysis water (Carmichael et al., 2001) and chronic exposure to microcystins has been identified as a risk factor for childhood liver damage (Li et al., 2011).

Spaceborne remote sensing has been introduced to monitor algal blooms, mostly by quantifying concentration of pigments, such as chlorophyll-*a* or phycocyanin. However, as chlorophyll-*a* is common to almost all phytoplankton, its retrieval from remotely sensed data cannot be used to specifically determine the abundance of cyanobacteria where other groups of eukaryotic algae co-occur. In contrast to chlorophyll-*a*,

phycocyanin is a pigment only found at high concentrations in cyanobacterial blooms. Phycocyanin has been shown to be a better indicator of cyanobacterial blooms (McQuaid et al., 2011) and has been proposed as a rapid tool for predicting elevated microcystins levels(Marion et al., 2012) . Phycocyanin, other than chlorophyll-*a* and carotenoid, is the most measureable pigment-protein complex in *Microcystis* spp. (Ruiz-Verdu et al., 2008). The Medium Resolution Imaging Spectrometer (MERIS) was found to be suitable for retrieving phycocyanin concentrations because its band (near 620nm) can be used to detect the phycocyanin absorption peak that is also near 620 nm. A nested semi-empirical band ratio model (Simis et al., 2005) based on MERIS has been proven to statistically outperform other MERIS-based algorithms (Ruiz-Verdu et al., 2008). A previous study based on Lake Erie beaches also suggested that the semi-empirical band ratio model performed well even with relatively low phycocyanin levels (Lee et al., 2014). Although MERIS ceased its operation on 9 May 2012, due to a sudden failure in communication, it is still useful in terms of retrieving historical bloom conditions back to 2002. The advantages of using satellite images for water quality parameters include: a) a near continuous spatial coverage of satellite imagery allowing for estimates over large areas, b) a record of archived imagery giving an estimation of historical bloom condition. In other studies, the linkage between some satellite measured environmental factors and health risks showed a potential for satellite imagery use in public health studies (Hu and Rao, 2009).

The objective of this study was to examine the association between cyanobacterial blooms and nonalcoholic liver diseases in the US. The study adopted an ecological

method using aggregate disease mortality data at the county level for the Contiguous United States and MEIRS-derived data for cyanobacterial bloom coverage. Exploratory spatial analysis methods and regression models were used to link nonalcoholic liver disease mortality rates with satellite-estimated bloom coverage.

4.3 Methods

4.3.1 MERIS-estimated bloom coverage data

All MERIS L1B full resolution images covering the Contiguous United States from 08/01/2005 to 9/30/2005 were retrieved from the National Aeronautics and Space Administration (NASA)'s Goddard Space Flight Center (GSFC) Ocean Color Science Team that are available from http://oceancolor.gsfc.nasa.gov/. August and September were chosen to match the common seasonal outbreak time (late summer or early fall) of cyanobacterial blooms (http://www.cdc.gov/nceh/hsb/hab/default.htm). After downloading the data, we used the Basic ERS & Envisat (A) ATSR and MERIS (BEAM) VISAT toolbox provided by ESA and Brockmann Consult and its supplementary Regional Case-2 Water Processor (Chawira, 2012) to further refine the data in New York State. In particular, we applied the Case-2 Regional Processor (C2R) v1.5.2 to convert the top of atmosphere (TOA) radiance (archived in the original L1B data) to water leaving radiance (R_L_W) above the surface. Then, phycocyanin levels were estimated using the nested semi-empirical band ratio model (Simis et al., 2005). Detailed information on the image processing and phycocyanin was described in Lee et al, 2014. Maximum value composite technique was used to combine all images in to one big image

with each pixel being the highest value for that pixel location. This generates a cloud-free phycocyanin image for the spatial monitoring of cyanobacterial blooms in the Contiguous United States.

WHO guidelines for recreational exposure to cyanobacteria use a three-tier approach based on cyanobacterial density and chlorophyll-*a* level (World Health Organization, 2003). For protection from health outcomes, due to the irritative or allergenic effects of cyanobacterial compounds, a guideline level of 20,000 cyanobacterial cells/ml (corresponding to 10mg chl-a/litre under conditions of cyanobacterial dominance) has been derived (World Health Organization, 2003). We chose the level of 20,000 cyanobacterial cells/ml to be the threshold of significant blooms. To transform the threshold of 20,000 cyanobacteria cells/ml to a phycocyanin level, we used a linear relationship between the log transformed parameters suggested in Ahn et al. (Ahn et al., 2007) and derived a level of 4.11 µg/L as equivalent to 20,000 cells/ml of cyanobacteria. The relationship between cyanobacterial cell abundance and phycocyanin is shown in Equation 4.1.

$$\text{Log(cyanobacteria)}=0.360*\log(\text{phycocyanin})+4.08 \qquad (4.1)$$

As a precaution, a threshold of 4 µg/L phycocyanin was used as our actual threshold for identifying water bodies afflicted bycyanobacterial blooms that could have potential adverse health effects on its neighborhood. By combining the area of each county from the Cartographic county boundary downloaded from U.S. Census (http://www.census.gov/geo/maps-data/data/tiger-cart-boundary.html), and the ground spatial resolution (260 m x 290 m) of MERIS, we calculated the bloom coverage as the

percentage of county area covered by cyanobacterial blooms. The maximum value composite and the zonal statistics were performed using ArcGIS 10.0 (Esri 2011). Although the bloom coverage data is only from 2005, they represent the bloom distributions in that decade. The development of eutrophication and algal blooms is gradual over years (Millie et al., 2009; Paerl et al., 2011b). A study showed that eutrophication conditions in U.S. estuaries remained nearly the same over a decade (Bricker et al., 2008).

4.3.2 Nonalcoholic liver disease data

Nonalcoholic liver disease data (ICD-10 codes: R74.0, K71.0 – K77.8; (Sogaard et al., 2009) data at the county level were extracted for the period from 1999-2010 from the Multiple Cause of Death data in the Centers for Disease Control and Prevention (CDC) Wide-ranging Online Data for Epidemiologic Research (WONDER) online database (http://wonder.cdc.gov/mcd.html). Nonalcoholic liver disease mortality data and population-at-risk were retrieved by county, gender, and age (10 year intervals). Aggregated, nonalcoholic liver disease mortality counts and population-at-risk were also retrieved by gender and age groups for the U.S. to be used as the standard population in calculating nonalcoholic liver disease mortality rates adjusting for gender and age effects. Gender and age adjusted rates were calculated using indirect standardization for each county. Rate adjustment removes the effects of gender and age from crude rates, so as to allow meaningful comparisons across populations with different underlying race and age structures. Data from the US, during the study period, was used as the standard

population to obtain a standardized rate for each county. Some other potential confounders, such as educational level and race, were also adjusted by putting the two factors as covariates in the regression model. The percentage of people over 25 with a college degree or above was used as the indictor of educational level and the percentage of black people was used to adjust for race. The percentage of people over 25 with a college degree was from the U.S. Census Bureau, 2006-2010 American Community Survey and the percentage of black people was retrieved from the Multiple Cause of Death data in the CDC WONDER online database.

The indirect standardization first calculated the expected number of nonalcoholic liver disease deaths for each county, which was determined by the number of cases that would be expected if people in the study population had the same mortality rate as people in the standard population with the same age and gender group. Standardized mortality rates (SMRs) were calculated by dividing the observed count by the expected value. Death counts were "suppressed" when the data met the criteria for confidentiality constraints. Rates were suppressed for sub-national data representing zero to nine (0-9) deaths. All counties with suppressed data were not included thereafter in calculating standardized rates and spatial analysis and modeling. The number of counties in the study area was 2914, with 196 counties being omitted that had suppressed disease data. Consequently, the number of data points (counties) for the statistical modeling is 2914.

4.3.3 Linking Nonalcoholic liver disease data with bloom coverage

First, a flexible-shaped spatial scan statistic (FlexScan) was performed to identify spatial clusters of nonalcoholic liver disease deaths. Tango and Takahashi (Tango and Takahashi, 2005) showed that FlexScan detects irregular shaped clusters by using a limited exhaustive search that would detect arbitrarily-shaped clusters by aggregating their nearest circular neighboring areas.

Exploratory spatial data analysis and Bayesian regression models were used to assess the effect of cyanobacterial bloom on nonalcoholic liver disease (SMR) using GeoDa software (Anselin, 1995; Anselin, 2004) and WinBUGS (Lunn et al., 2000). The exploratory spatial data analysis methods were a bivariate global Moran's I statistic and local indicator of spatial association (LISA). Bivariate global Moran's I value determines the overall strength and direction of the relationship between two variables, SMR and bloom coverage in each county. LISA provides information relating to the location of spatial clusters and outliers. Local statistics are important because the magnitude of spatial autocorrelation is not necessarily uniform over the study area. The result of the LISA analysis presented a cluster map in this analysis. LISA analysis by GeoDa identified clusters of High-High nonalcoholic liver disease clusters (units of significantly high disease mortality rates surrounded by significantly high bloom coverage), Low-Low clusters (units of significantly low disease mortality rates surrounded by significantly low bloom coverage), and High-Low or Low-High outliers. Significance was tested by comparison to a reference distribution obtained by random permutations; 999 permutations were used to determine significance level for the differences between

spatial units. Spatial contiguity was assessed as Queen's contiguity that defines spatial neighbors as those areas with shared borders and vertexes.

A preliminary negative binomial regression analysis was carried out in STATA 13.0 (Stata Corp., College Station, TX, USA) to assess the relationship between nonalcoholic liver disease deaths and bloom coverage, adjusting for educational level and race. Negative binomial regression was used instead of Poisson regression because of the overdispersed data. Thereafter, Bayesian negative binomial models were fitted in WinBUGS (Lunn et al., 2000) to examine the association between nonalcoholic liver disease deaths and bloom coverage using a conditional autoregressive (CAR) process. Basically, spatial random effects were used at a county level to account for spatial correlation present in the data. Markov Chain Monte Carlo simulation (MCMC) was applied to estimate model parameters (Gelfand and Smith, 1990). After the initial burn-in of 5,000 iterations, another 10,000 iterations were used for the summaries of the posterior distribution of the parameters. It was assumed that the observed counts of nonalcoholic liver disease death (*Yi*) in county *i* follow a negative binomial distribution with parameters *pi* and *r*, that is, Y*i* ~ NB (p_i, r), where p_i relates to average number of cases via the formula (μi) = *pit*/*r* where r is the over-dispersion parameter. We modeled the average number of deaths (μi) as a function of potential risk factors as in Equation 4.2:

$$\text{Log}\,(\mu i) = \log Ei + \alpha + \boldsymbol{X}i\ \boldsymbol{*\beta}\ + ei + \ \varnothing i \qquad (4.2)$$

ui denotes expected number of deaths in county *i.* α is the incidence rate when all covariates have zero value, ***X****i* is a vector of covariates in county *i*, $\boldsymbol{\beta}$ a vector of the regression coefficients, e*i* is the unobserved (i.e., uncorrelated) heterogeneity, and $\varnothing i$ is

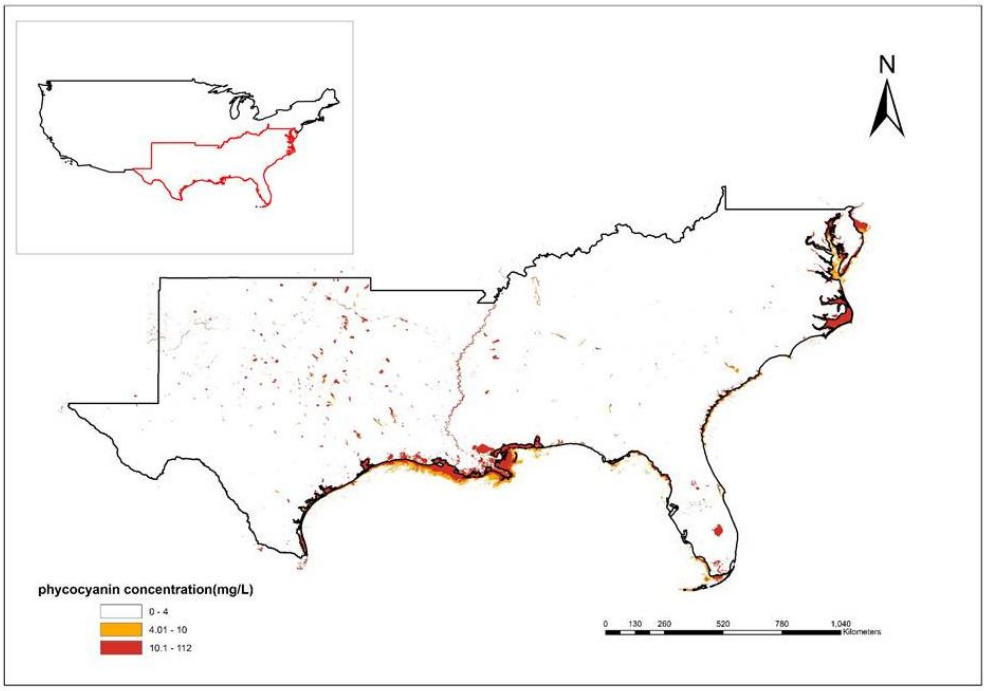
the structured spatial random effect. County-specific random effects were modeled via a conditional autoregressive (CAR) process, which implies that each *øi,* conditional on the neighbors, follows a normal distribution with a mean equal to the average of neighboring spatial effects and variance inversely proportional to the number of neighbors.

4.4 Results

4.4.1 The spatial distribution of cyanobacterial blooms in the contiguous U.S.

Based on the estimated phycocyanin concentrations from MERIS, cyanobacterial blooms were widely spread in U.S. water bodies, including lakes and rivers (Figure 4.1). From the maximum value composited image, it was observed that a large part of Lake Erie was covered by cyanobacterial bloom, mostly in the western basin. Other parts of the Great Lakes, such as Saginaw Bay, also showed significant bloom coverage. The largest lakes in the Contiguous U.S. (i.e. Great Salt Lake, Lake of the Woods, Lake Oahe, Lake Okeechobee, and Lake Pontchartrain) were all afflicted with cyanobacterial blooms. Coastal areas in Texas, Louisiana, North Carolina, Virginia, Maryland and Delaware also had significant cyanobacterial blooms. Based on our satellite estimations, the occurrence of cyanobacterial blooms in the U.S. was shown to be a common and serious problem in U.S. waters. When the data were aggregated at the county level, it was observed that counties in coastal areas, as well as counties in the mid-north areas, have substantial bloom coverage; overall, 1,949 counties showed some bloom coverage (Figure 4.2).

(A) Southern part of the US

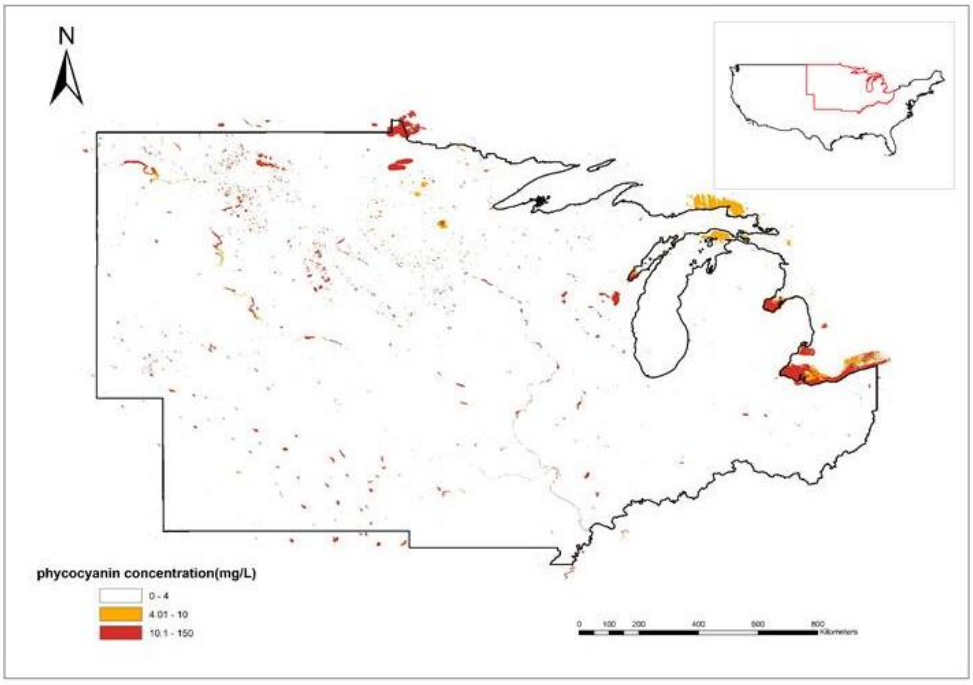


Continued

Figure 4.1. The spatial distribution of cyanobacteria blooms in the Contiguous U.S. in 2005 as estimated by MERIS; (A) Southern part of the US; (B) Midwestern part of the US; (C) Western part of the US; (D) Northeastern part of the US.

Figure 4.1 Continued

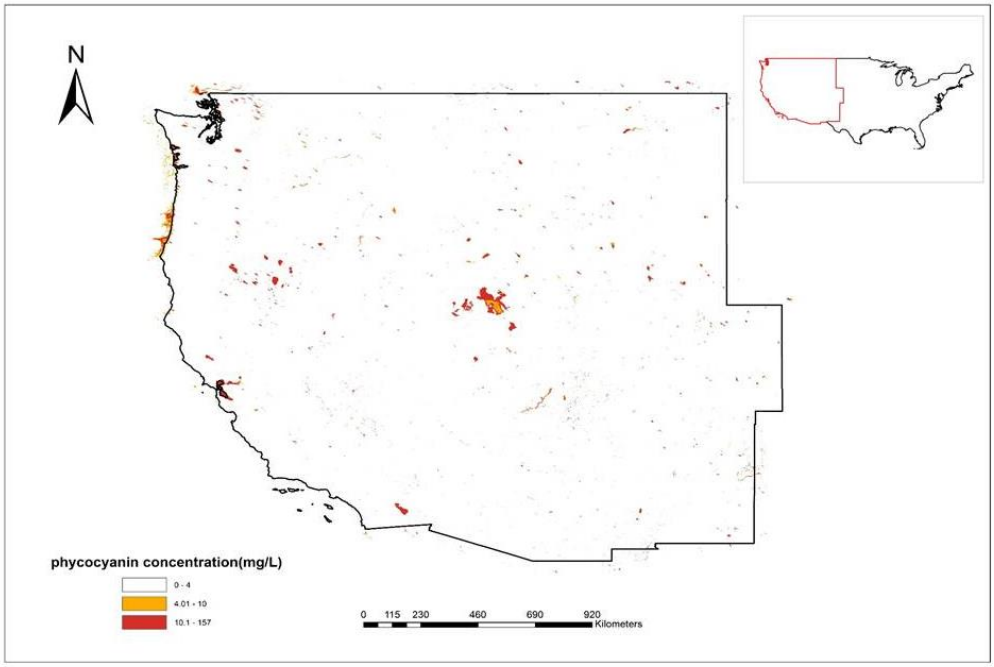
(B) Midwestern part of the US



Continued

Figure 4.1 Continued

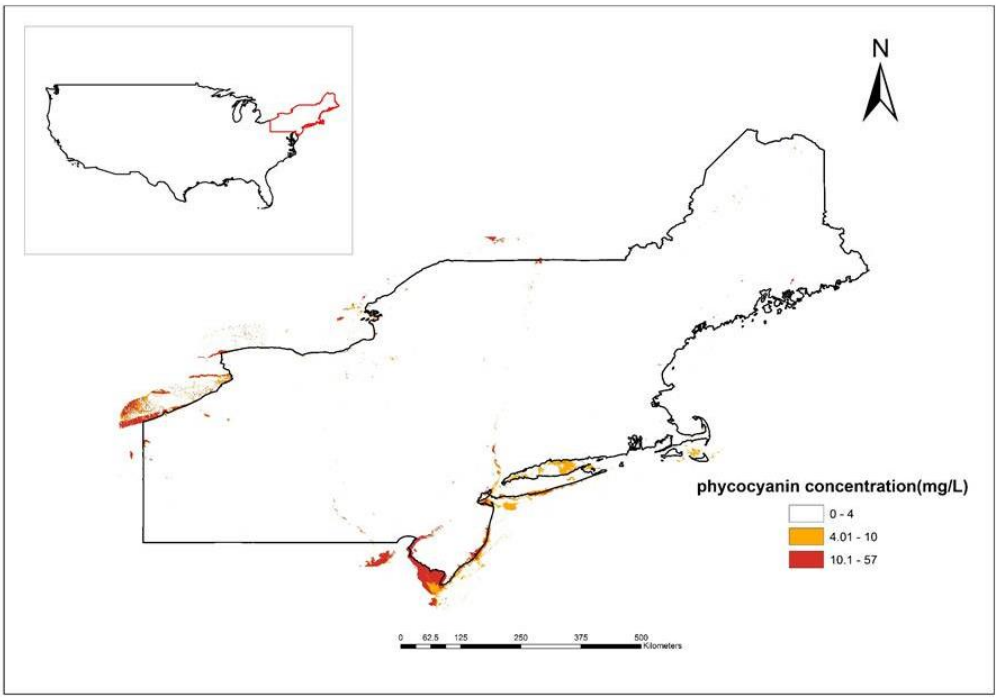
(C) Western part of the US



Continued

Figure 4.1 Continued

(D) Northeastern part of the US



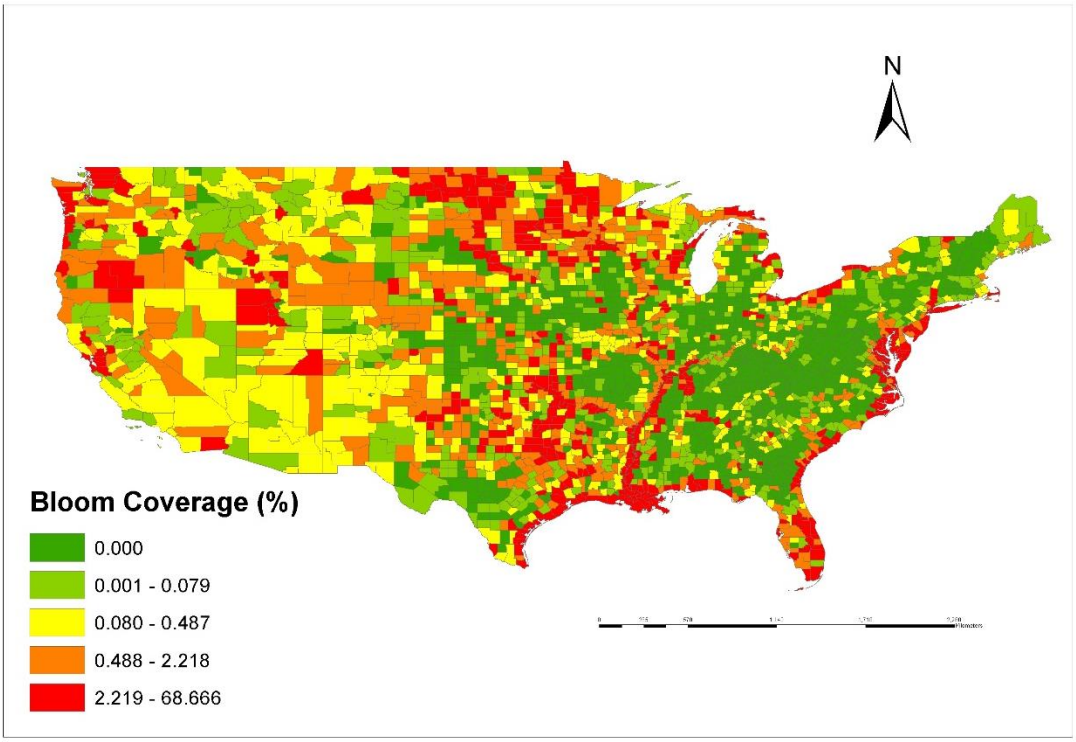


Figure 4.2. Bloom coverage area (percentage by county) in the U.S. in 2005 as estimated by MERIS.

4.4.2 Spatial clusters of nonalcoholic liver disease

In total, there were 773,828 nonalcoholic liver disease deaths in the U.S. from 1999 to 2010; spatial variations in the nonalcoholic liver disease mortality were observed (Figure 4.3). FlexScan identified 65 significant spatial clusters of nonalcoholic liver disease (p<0.01), which included 432 counties. There were some significant clusters along the coastal areas. The most likely clusters were located along the coastal area of Texas and included 14 counties (p=0.001). Around Lake Erie, where cyanobacteria blooms have developed for years, two significant clusters were identified. Counties in the clusters also

showed higher bloom coverage than counties from the non-clusters according to Wilcoxon signed-rank test (p<0.001).

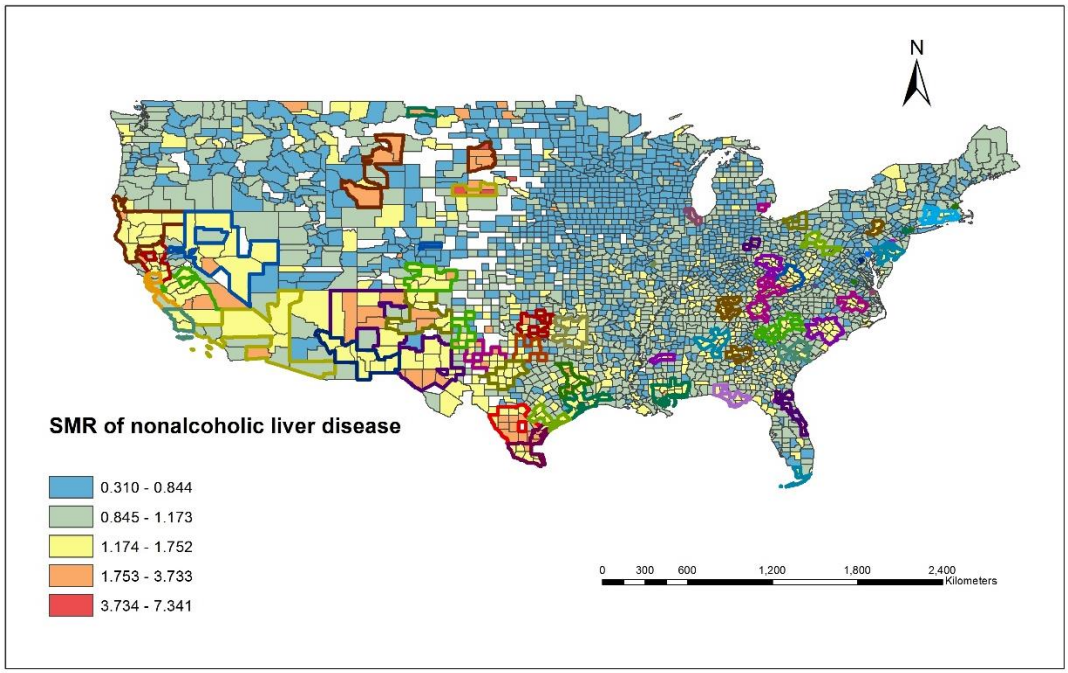


Figure 4.3. FlexScan identified significant clusters of nonalcoholic liver disease deaths counts from 1999 to 2010.

4.4.3 Exploratory spatial analysis on the relationship between nonalcoholic liver disease and bloom coverage

The global Moran's I value is 0.001 (p=0.001), indicating an overall positive spatial correlation of nonalcoholic liver disease SMR and bloom coverage, although the relationship is not very strong. The bivariate LISA cluster map is shown in Figure 4.4

(permutations = 999, $p<0.05$). The map shows local patterns of spatial correlation at the county level between SMR and average bloom coverage for its neighbors. Significant clusters, as well as outliers, are color coded by type of spatial autocorrelation. The High-High and Low-Low counties represent spatial clusters, while the High-Low and Low-High counties represent spatial outliers. The clusters were observed in those places with significant positive spatial relationships between the two variables, while the outliers showed significant negative spatial relationships. There were significant clusters around coastal areas near Texas. The spatial outlier locations, especially areas with high SMR but low bloom coverage, need further investigation to see if other risk factors, such as obesity or viral infections, may be contributing factors to the SMR in the outliers.

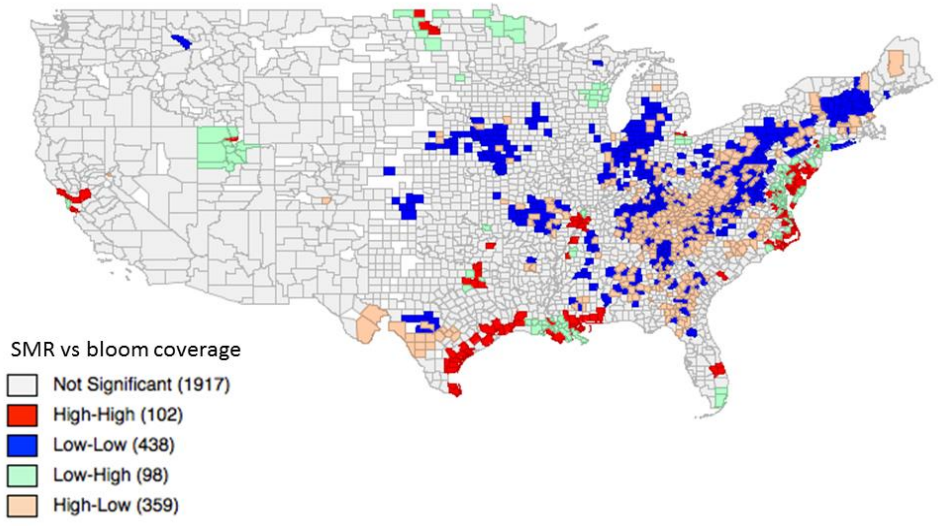


Figure 4.4. Bivariate LISA cluster map of nonalcoholic liver disease and cyanobacterial bloom coverage

4.4.4 Bayesian regression of nonalcoholic liver disease on bloom coverage

Bayesian regression revealed a significant relationship between nonalcoholic liver diseases and bloom coverage using a negative binomial model (this model accounted for the spatial correlated parttern of the data). According to this model, risk for nonalcoholic liver disease death increased 0.3% (95% Bayesian confidence interval 0.1% to 0.5%) for each 1 % increase in the bloom coverage of the county; adjusting for age, gender, educational level, and race (Table 4.1). The results show that bloom coverage was a significant factor influencing the nonalcoholic liver disease rate.

Table 4.1. Model estimates of the ajusted association between cyanobacterial bloom coverage and nonalcholic liver disease mortality in the Coutigious U.S. by Baysian negative binomial regression

Variable	Risk Ratio Estimate	95% credible interval
County bloom coverage	1.003	(1.001,1.005)
Percentage of adults with higher degree	0.385	(0.350, 0.422)
Percentage of black population percentage	1.60	(1.46,1.78)

4.5 Discussion

We estimated the overall spatial distribution of cyanobacterial bloom in the U.S. using satellite remote sensing. The cyanobacterial blooms were widely spread in the US, indicating a serious environmental problem in a wide area. Most large lakes in the U.S.

showed cyanobacterial blooms. Harmful cyanobacterial blooms may be more common than previously estimated (Fristachi et al., 2008). Coastal areas are especially vulnerable to cyanobacteria blooms. In the Contiguous United States, 1949 counties show at least some blooms in their water bodies (note that due to the limitation of spatial resolution, small ponds could not be assessed). For exposure monitoring of algal blooms and their toxins, remote sensing has proven to be a useful, quick, and cheap method for evaluation of large areas. Currently, number of states regularly monitor cyanobacteria and cyanotoxins in water bodies (e.g. New York State implemented the "Citizens Statewide Lake Assessment Program" to monitor lake conditions, including harmful algal blooms and Ohio regularly monitors microcystins levels at recreational beaches). Remote sensing techniques can serve as a supplement to *in situ* monitoring of water bodies with more extensive coverage. In the United States, the distribution of nonalcoholic liver disease mortality seems to vary geographically, which could be the result of possible environmental risk factors. In the contiguous US, we have identified 65 spatial clusters with high mortality rates of nonalcoholic liver disease; counties in the spatial clusters also showed higher cyanobacterial bloom coverage than counties in the non-clusters, indicating that an environmental risk is associated with cyanobacterial bloom and could be contributing to the spatial clusters of nonalcoholic liver disease. By Bayesian spatial regression, we found a significant positive relationship between the relative risk of nonalcoholic liver disease mortality and cyanobacterial bloom coverage after adjusting for gender, age, race, and educational level. There was an excess risk of nonalcoholic liver disease mortality in areas with high bloom coverage. The results show that spatial distribution of cyanobacterial bloom was associated with nonalcoholic liver disease

mortality, suggesting that cyanobacterial blooms are an important risk factor. Cyanobacterial blooms may produce toxins such as microcystins have been shown to be liver toxin (Li et al., 2011). Living in bloom areas makes it more likely for persons to be exposed to excess microcystins through inhalation, recreational exposure, or ingestion of contaminated food or water. Cyanobacterial blooms have been implicated as a potential risk factor for amyotrophic lateral sclerosis (Torbick et al., 2014). Further studies are needed to investigate the level of exposure through different exposure routes that are sufficient to cause disease.

This study is an ecological study showing the possible association between nonalcoholic liver disease and spatial distribution of cyanobacterial blooms. Although such a study is generally suitable to show an association, it is not suitable to prove or disprove an etiological cause and effect. However, it can be used for hypothesis generation and testing. The significant association shown herein provides evidence for a potential health risk from cyanobacterial blooms, but more epidemiological research should be done in order to more accurately assess the risk. Exposures and possible health effects (both acute and chronic) of cyanobacteria and their toxins need to be evaluated more extensively, especially now that global warming will be more favorable for bloom-forming cyanobacteria than current conditions (Paerl and Paul, 2012). In developed countries, where people are collecting water from surface sources to drink, the environmental conditions may need more attention as microcystins are highly stable in water and are resistant to boiling. Thus, more actions should be taken in order to control the blooms.

There are several strengths of this study. First, this was an ecological study, which is particularly useful when disease data at the individual level are not available and individual levels of exposure are difficult to obtain (Best, 1999). Second, the statistically significant positive association between nonalcoholic liver disease mortality rates and cyanobacterial coverage can be taken as an indication of a potential health effect. This association would justify the need for further studies to investigate the biological mechanism(s) of the adverse effect of cyanobacterial toxins on human health, especially liver damage and liver disease. Third, the data show that satellites offer tremendous spatial coverage and provide a great resource for regional environmental monitoring, pollution event warnings, and environmental health studies. Satellite-estimated environmental factors could be used for studying potential health risks as demonstrated in this study of association between satellite bloom data and liver disease mortality.

However, there are several limitations in this study. First, while we adjusted for effects of gender, age, race, and educational level, we did not consider other potential confounding factors (e.g. obesity and diet). Second, the use of aggregated data and therefore inferences based on the analysis cannot be directly transferred to an individual level. An inherent limitation of an ecological study is that it does not have the ability to distinctively incorporate individual information. Satellite measurements do not represent individual exposure due to differences in diet, recreational activities, etc. MERIS had a spatial resolution of around 300m, which is limiting the ability to assess small lakes and ponds, which could lead to some biases in the estimation of exposure levels. In addition, the population-based ecological study does not consider population dynamics during the

study period (e.g. people may have migrated during the study period) and the residence at time of death may not be the location where the disease was initiated. According to the U.S. 2000 census data, between March 1999 and March 2000, 43.4 million Americans moved and 39% of all moves were cross county (Schachter, 2001).

4.6 Chapter Conclusions

- This ecological study in the Contiguous U.S. using satellite data and data of multiple causes of death found a significant positive association between nonalcoholic liver disease mortality risk and cyanobacteria bloom coverage.
- We identified clusters of nonalcoholic liver disease mortality in clusters in those counties that also had higher bloom coverage.
- The evidence for excess nonalcoholic liver disease in areas with high cyanobacterial coverage suggests that more attention should be centered on the public health impact of harmful cyanobacterial blooms.
- Additionally, remote sensing could be used to assess the distribution of algal blooms over large areas and serve as a primary warning to protect public health.

Chapter 5: Satellite remote sensing of two drinking water intakes in Lake Erie for cyanobacteria population and toxins using two MODIS-based indicators

5.1 Chapter Overview

The growth of mass populations of toxin-producing cyanobacteria, such as *Microcystis aeruginosa*, and the production of their toxins (microcystins) are serious concerns for the ecological health of lakes, such as Lake Erie. The toxins are significant health hazards and could contaminate tap water if it is not properly treated. In this study, water from the intakes of two water plants along Lake Erie (Toledo and Painesville) were collected and examined in concert with the performance of satellite-based cyanobacteria bloom indicators from the Moderate Resolution Imaging Spectroradiometer (MODIS) from May to October in 2013. Good correlations were observed between toxic cyanobacteria, microcystins and MODIS-retrieved bloom indicators for the Toledo water plant intake, where blooms were much more severe, but not for the Painesville water plant intake point in central Lake Erie. The microcystins level showed a Spearman's correlation of 0.815(p<0.05) with MODIS-retrieved chlorophyll-a concentration for the Toledo intake point inwestern Lake Erie. Both total and toxic *Microcystis* abundance showed significant positive correlations with MODIS-retrieve chlorophyll-a for the Toledo plant, as well as

the two locations combined. This finding demonstrates the potential for satellite remote sensing for detection and monitoring of cyanobacterial blooms as a preliminary warning for protection of human health from regional bloom-impacted waters.

5.2 Introduction

Cyanobacteria can be found throughout the world in inland and coastal waters. They are very fast growing under conditions of high nutrient levels and often form blooms during warm summer and autumn weather. These blooms are a serious concern, because they can have adverse environmental, economic, and public health impacts (de Figueiredo et al., 2004; Steffensen, 2008). Some of cyanobacteria can produce toxins that have neurotoxic, hepatotoxic, and tumor promoting properties (Codd et al., 2005; Li et al., 2011). These toxins include microcystins, saxitoxins, cylindrospermopsin, nodularins and anatoxins (Sinclair and Hall, 2008) of which microcystins are the most commonly reported cyanobacterial toxins in North America (Pelaez et al., 2010) . Exposure routes by these toxins are ingestion of contaminated water, skin contact and inhalation (Codd et al., 1999). In recent decades, the incidence and intensity of these blooms, as well as the associated economic costs, have increased worldwide (O'Neil et al., 2012). Of more concern, recent studies suggest that future environmental changes, including rising water temperatures and climate-induced eutrophication, will be more favorable for bloom-forming cyanobacteria (Michalak et al., 2013; Paerl et al., 2011a). *Microcystis* blooms in the Laurentian Great Lakes, especially in Lake Erie, have been observed almost annually in recent years (Michalak et al., 2013; Rinta-Kanto et al., 2009). Among the Great Lakes,

Lake Erie is the shallowest and most nutrient-rich, making it more vulnerable to harmful algal blooms (HAB). It has 1402 kilometers of shoreline that stretches along the borders of Michigan, Ohio, Pennsylvania, New York, and Ontario and provides drinking water to over 11 million people (Environmental Protection Agency, 2004). According to the report by the Ohio Lake Erie Commission, there are 27 lake-fed water treatment plants along Ohio's North Coast (Ohio Lake Erie Commission, 2004). In Toledo and other communities in western Lake Erie, microcystins and algae were found in the intake water that was subsequently treated to make the water safe for drinking; the cost of treatment for Toledo alone is $3-4 million a year (Associated Press, 2013).

Due to the severe adverse impacts of cyanobacterial blooms, the monitoring of blooms and toxins is especially important to provide timely warnings for protection of public health. However, cyanotoxin concentrations are rarely routinely monitored in recreational and finished drinking water, due to the costly testing procedures. Additionally, traditional monitoring methods typically require that water samples are collected in the field and returned to the laboratory so that the lab work, including counting cyanobacteria, can be performed; this process can be time-consuming as well as labor intensive. Due to the limitations of traditional monitoring methods, the frequency of sampling frequency and the number of sampling locations for cyanobacterial blooms are usually limited.

To ensure public health, there is a clear need to develop monitoring systems for cyanobacterial blooms and their toxins in Lake Erie and other water bodies; a timely warning can help to avoid recreational exposure. In addition, this warning also alerts water treatment plant to implement special treatment procedures to ensure drinking water

safety. Because of the unique spectral characteristics of cyanobacterial blooms, spaceborne remote sensing has been introduced to monitor algal blooms in Lake Erie (Stumpf et al., 2012; Vincent et al., 2004), mostly by quantifying pigment concentration such as chlorophyll-a*a* or phycocyanin. The major advantage of using satellite imagery for determining water quality parameters is the near continuous spatial coverage in a time-saving manner. Many operational programs now started to incorporate satellite remote sensing into their detection methods (Stumpf et al., 2009; Wynne et al., 2013). Currently, Experimental Lake Erie Harmful Algal Bloom Bulletin by the National Centers for Coastal Ocean Science and Great Lakes Environmental Research Laboratory uses a MODIS-based Cyanobacteria Index (CI) for monitoring cyanobacterial blooms in Lake Erie. However, remote sensing is only used as a preliminary indicator due to the lack of well-established relationship between satellite observation and *in situ* data. To date, only a few peer-reviewed articles have evaluated performance of satellite remote sensing for monitoring toxins and toxin-producing cyanobacteria for cyanobacterial blooms in aquatic systems.

In the present study, we examined the performance of two satellite based indicators for the monitoring of cyanobacterial blooms for two drinking water intakes in Lake Erie. Due to the dominance of *Microcystis* in Lake Eire (Brittain et al., 2000), we focused our cyanobacteria monitoring on *Microcystis* and toxin producing *Microcystis*. By examining the association between satellite indicators and tested cyanobacterial bloom indicators such as pigment concentration, cyanobacteria abundance and toxins level, we can assess the usefulness of satellite indicators for building public health meaningful monitoring

system. In events of intensive blooms, drinking water plants could be more prepared with the assistance of the monitoring system.

5.2 Materials and Methods

5.2.1 Study site

All field and laboratory methods were performed using water samples collected from the intake points of two water treatment plants in Toledo and Painesville, Ohio (Figure 5.1). The Toledo water plant draws water from the western basin of Lake Erie, east of the Toledo city area, where massive cyanobacterial blooms usually occur. This location is about 3 miles from the shore (City of Maumee, 2013) as indicated as the center of the box in Figure 5.1. The Painesville water treatment plant draws its water from the central basin, where the blooms are much milder. Weekly samples were collected from Painesville water treatment plant intake (41.784º, -81.301 º) from late May to the end of October, 2013; weekly samples were collected from the Toledo water treatment plant intake (41.738 º, -83.303º) during the same period.

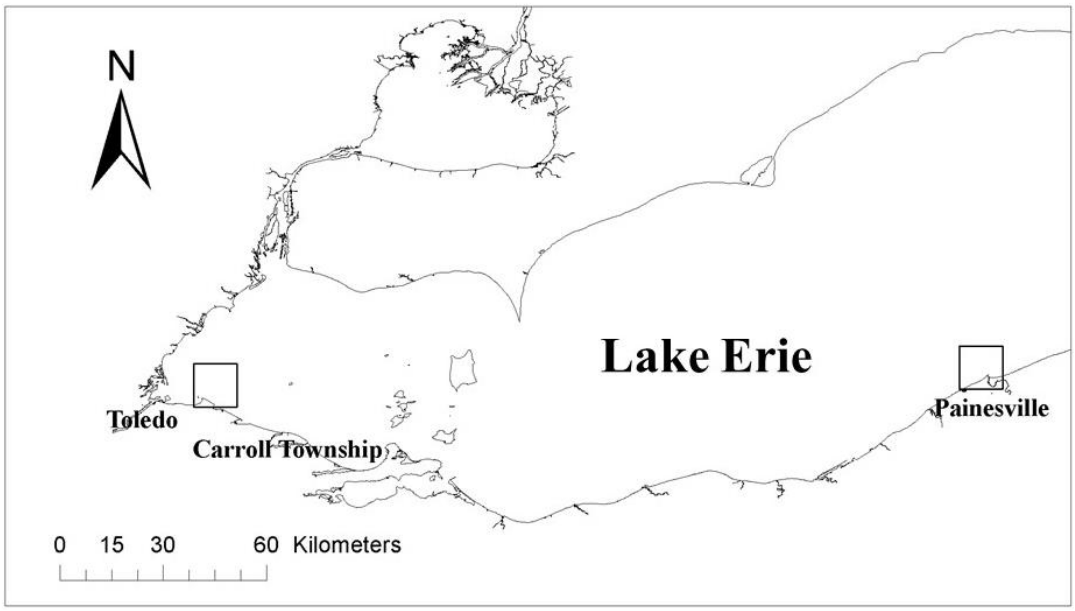


Figure 5.1. Map showing the intake locations of Toledo and Painesville water plants

5.2.2 Water quality measurements

Water quality parameters such as water temperature, turbidity and pH were measured *in situ* and obtained from the water plants. Both chlorophyll-*a* and phycocyanin (PC) were measured using a two-channel handheld Aquaflour™ flourometer (Turner Designs®, Sunnyvale, CA) *in vivo* as described in our previous study (Marion et al., 2012). Water samples were sent to The Ohio State University main campus laboratory (Columbus, OH) on ice for analysis for quantitative polymerase chain reaction (qPCR), nutrient test and ELISA tests. Colorimetric methods were used for the determination of nitrate by the dimethylphenol method (Method 10206, Hach Company, Loveland, CO) or the cadmium reduction method (Method 8192, Hach Company, Loveland) and total phosphorus with

the U.S. Environmental Protection Agency approved acid persulfate digestion method (Method 8190, PhosVer® 3, Hach Company, Loveland, CO). Heterotrophic plate count (HPC) test was performed using Environmental Protection Agency approved standard method 9215.

5.2.3 Toxin measurements

Microcystins concentrations were quantified using the EPA-validated Microcystins/Nodularins (ADDA) ES, ELISA kit in a 96-well format following the instructions of the manufacturer (catalog number PN520011ES, Abraxis®, Warminster, PA). Water samples went through a freeze/thaw procedure five times to effectively rupture the cells and release the toxins. For each multi-well plate, a total of six standards were used in duplicate to develop plate-specific standard curves (R^2 Range = 97.2% - 99.6%). All samples along with positive and negative controls were evaluated in duplicate. Samples with microcystins level over 5 µg/L exceeds the range of detection and were diluted for microcystins quantification. Samples with microcystins below 0.10 µg/L were considered "non-detects" according to manufacturer's instructions. For statistical analyses, the 'non-detects' were assigned a value of 0.05 µg/L (1/2 of the detection limit) based on the information provided by ELISA manufacturer (http://www.abraxiskits.com/uploads/products/docfiles/278_Microcystin%20PL%20ADDA%20users%20R120214.pdf , Abraxis, Warminster, PA).

5.2.4 Quantification of *Microcystis* PC-IGS and *mcy*B genetic abundance

Water samples (200mL) were filtered through an Isopore™ polycarbonate membrane (0.4 μm, Millipore). Metagenomic DNAs on sample filters were extracted using the xanthogenate-sodium dodecyl sulfate (XS) DNA extraction method with slight modification (Tillett and Neilan, 2000). Briefly, sample filters were incubated in 1 mL of XS buffer at 70 °C for 2 h; then the incubation mixture was vortexed for 10 s and put on ice for 30 min. After centrifugation at 13,200 rpm for 15 min at 4 °C, the supernatant was transferred to a new tube and mixed with an equal volume of 100% isopropanol. Subsequently, the mixture was applied to the column in the DNeasy® Blood & Tissue Kit (Qiagen, Valencia, CA) and centrifuged for 1 min at 10,000 rpm. After the sequential AW1/AW2 washing steps, DNA in the column was eluted with 100 μl of TE buffer and stored at -20 °C for real-time PCR analysis.

Quantification of *Microcystis* PC-IGS and *mcy*B genetic abundance was performed on a Bio-Rad C1000 Touch Thermal Cycler (USA) machine; the primers and probes specific for *Microcystis* PC-IGS and *mcy*B genes were described previously (Kurmayer and Kutzenberger, 2003). The 5-end and 3-end of probe in the present study was labeled with the 6-carboxyfluorescein (6-FAM) reporter and the Minor Groove Binding non-fluorescent (MGB-NFQ) quencher (Life Technologies, Foster City, USA). Each PCR mixture (20 μl) contained TaqMan universal PCR master mix (10 μL) (Life Technologies, Foster City, USA); *Microcystis* PC-IGS-specific primers (188F/254R, 300 nM) or *Microcystis mcy*B -specific primers (30F/108F, 900 nM); *Microcystis* PC-IGS-specific TaqMan probe (100 nM) or *Microcystis mcy*B –specific probe (250 nM) and diluted

DNA template (2 μL). The PCR profile was initial cycle of 50 °C for 2 min and 95 °C for 10 min and 50 cycles of at 95 °C for 30 s, 56 °C for 1 min, and 72 °C for 30 s.

Constructions of standard curves to quantify *Microcystis* PC-IGS and *mcy*B genetic abundance were prepared as following: 398-bp *Microcystis* PC-IGS and 470-bp *Microcystis mcy*B fragements were amplified from *Microcystis aeruginosa* NIES843 genomic DNA. The primer set specific for *Microcystis* PC-IGS and *mcyB* fragments were PCIGSseqF/ PC-IGSseqR (5-TGCGCGAAACCTATGTAGC-3 and 5-GAATTGGCTTTTTCGGTC-3, respectively) and mcyB_548/mcyB_1017 (5-GGAGAATGCGGTCTTCAGAG-3 and 5-TAACCGGGGCAATCAGTTAG-3), respectively. PCR mixtures (20 μl) were prepared according to the manufacturer's instructions (DreamTaq polyermase, Thermo Scientific, USA). The PCR profiles were: one initial cycle of 95 °C for 10 min; 35 cycles of at 95 °C for 30 s, 55 °C for 30s, and 72 °C for 1 min, and one cycle of 72 °C for 10 min, which were performed on MultiGene Thermal Cycler (Labnet, Edison, NJ, USA). After product size confirmation and purification, the amplicons of *Microcystis* PC-IGS and *mcy*B fragments were cloned into pGEM-T vector (Promega, USA) according to the manufacturer's instructions. Subsequently, the ligation mixture was transformed into competent *Escherichia coli* cells through traditional heat-shocking protocol. The plasmids (pGEM-T-PCIGS and pGEM-T-*mcy*B) from the positive transformants were isolated with Qiaprep Miniprep Kit (USA). After sequencing confirmed the right clone of *Microcystis* PC-IGS and *mcyB* fragments, plasmid concentration (copies/μl) was quantified with the Quant-iT dsDNA kit.

Subsequently, a series of plasmid diluted standards, together with unknown samples, were analyzed by real-time PCR to establish standard curves.

5.2.5 Satellite remote sensing

Satellites have provided an effective means of monitoring algal blooms and satellite remote sensing has been used worldwide to estimate water quality The National Oceanic and Atmospheric Administration (NOAA) has conducted routine monitoring of Lake Erie for cyanobacterial blooms each summer for several years. The Harmful Algal Blooms in Lake Erie – Experimental HAB Bulletin at NOAA Center of Excellence for Great Lakes and Human Health provides cyanobacterial monitoring and forecasting results based on satellite remote sensing. Before the MERIS stopped sending data on April 8, 2012, the Bulletin used a Cyanobacterial Index (CI) that was calculated using the spectral shape around 681 nm band (Stumpf et al., 2012). The spectral shape is determined as a nominal second derivative around the band of interest. After MERIS stopped its service, the Experimental HAB Bulletin started using The Moderate-resolution Imaging Spectroradiometer (MODIS) as an alternative for monitoring cyanobacterial blooms in Lake Erie. The CI by MODIS is defined as the negative of the spectral shape around 678 nm, as shown in Equation 5.1.

$$CI = -\{Rrs(\lambda) - Rrs(\lambda^-) - \{Rrs(\lambda^+) - Rrs(\lambda^-)\} \times \frac{(\lambda - \lambda^-)}{(\lambda^+ - \lambda^-)}\}\} \qquad (5.1)$$

In Equation 5.1, λ = 678 nm (MODIS band 14), λ^+ = 748 nm (band 15), and λ^- = 667 nm (band 13). Level 2 reflectances are used for the analysis, these are normalized water-

leaving reflectances for pixels identified as water. Although the spectral shape is called CI (Stumpf et al., 2012), it is not selectively specific for cyanobacteria, but could be used for detecting other algal blooms (Hu et al., 2005; Moreno-Madrinan and Fischer, 2013).

The MODIS L1A data preprocessed by The National Aeronautics and Space Administration (NASA)'s Goddard Space Flight Center (GSFC) Ocean Color Science Team are available from http://oceancolor.gsfc.nasa.gov/. After downloading the data, SeaDAS (SeaWiFS Data Analysis System) version 6.4 were used to further refine the data in coastal regions. In particular, the Multi-scattering with 2-band model selection and shortwave-infrared (SWIR) correction (Wang and Shi, 2007) was applied to convert the raw radiances in counts in the original L1A data to water leaving reflectance above the surface. The turbid water index developed by Wang and Shi (Wang and Shi, 2007) is computed prior to the atmospheric correction for the identification of the productive and/or turbid waters where the SWIR algorithm can be operated. The Multi-scattering with 2-band model selection and SWIR correction method use the standard (NIR) algorithm in non-turbid oceans, whereas for turbid waters the products are obtained using the SWIR method. In addition, the level 2 process also produces chlorophyll-a concentrations based on OC3 algorithm expressed as Equation 5.2 (O'Reilly et al., 2000).

$$Chla = 10^{0.283 - 2.753R + 1.475R^2 + 0.659R^3 - 1.403R^4}, \text{ where } R = \log_{10}\left(\frac{Rrs443 > Rrs488}{Rrs551}\right) \quad (5.2)$$

Next, CI was calculated using the model given in Equation 5.1 at the study area. Then unreliable data were excluded using level 2 flags for every image, such as land mask, cloud mask and the pixels where high viewing angle or sun zenith or straylight or high-

glint contaminated. To prevent potential regional irregularity in the reflectance data, CI as well as chlorophyll-*a* for all pixel identified as water were spatially smoothed a spatial box covering (shown in Figure 5.1) surrounding our sampling point.

5.3 Results

5.3.1 *Microcystis* cells, pigments and toxins

Environmental data collected from the intake at the two water treatment plant are summarized in Table 5.1. The estimated microcystins levels and toxic gene copy numbers in samples collected from Toledo and Painesville water plant intakes in 2013 are presented in Figure 5.2. Although toxic *Microcystis* is known to have spread throughout the lake, the *Microcystis* bloom masses in the past decade have been observed in the western basin (Brittain et al., 2000; Rinta-Kanto et al., 2009; Rinta-Kanto et al., 2005). Our observations also support previous studies that western Lake Erie experienced more *Microcystis* blooms than did central Lake Erie. Harmful algal bloom-related parameters including chlorophyll-*a*, PC, PC-IGS, *mcy*B, and microcystins were significantly higher in intake water at Toledo than at Painesville from May to October in 2013 (t test, $p<0.05$).

Time series of the qPCR results and the toxins level were shown in Figure 5.2. A detectable level of microcystins was observed from July 23 to October 23 with the maximum (8.87 µg/L) occurring on September 4 at the Toledo sampling point. However, microcystins levels were not detectable during our study period from the Painesville samples. The temporal trend for toxic cyanobacteria at the two locations also differed in

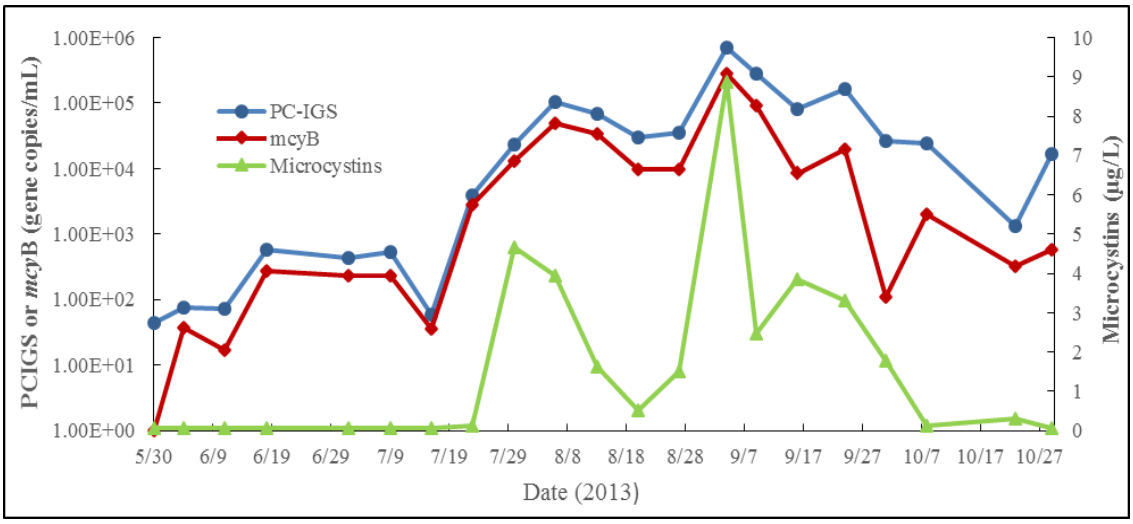
2013. At the Toledo water plant intake, both the PC-IGS and *mcy*B levels peaked in early September and showed a decreasing trend thereafter. The PC-IGS levels at the Toledo water plant intake ranged from 4.5×10 to 7.07×10^5 gene copies/mL with the highest was observed on Sep 4th. The *Mcy*B levels at the Toledo intake ranged from 0 to 2.87×10^5 gene copies/mL with the highest level was also observed on Sep 4th. The correlation between PC-IGS and *mcy*B at the Toledo intake was especially high (Spearman's correlation coefficient is 0.914, $p<0.05$). The Spearman's correlation between micorcystins and *mcy*B was 0.823 ($p<0.05$) at this location, indicating that the abundance of toxic *Microcystis* is a good indicator of the toxin level. In 2013, the relative abundance of toxic *Microcystis* at the Toledo intake, determined by the ratio of the copy numbers of *mcy*B genotypes to the total *Microcystis*, was between 0 and 71% in Toledo.

In contrast, at the Painesville water plant intake, both the PC-IGS and *mcy*B level peaked in October, near the end of the study period. The PC-IGS in Painesville water plant intake ranged from 1.44 to 6.20×10^3gene copies/mL with the highest observed on Oct 13. The *Mcy*B levels at the Painesville intake ranged from 0 to 1.02×10^3 gene copies/mL with the highest level also observed on Oct 13. The correlation between PC-IGS and *mcy*B at the Painesville intake was high, although lower than the Toledo observation (Spearman's correlation coefficient was 0.713, $p<0.05$). At Painesville, the relative abundance of toxic *Microcystis* was 0 to 69.9%.

Table 5.1. Summary of environmental parameters at the intakes of Toledo and Painesville water plant

Variables	Toledo Mean ± SE	Painesville Mean ± SE
microcystins (µg/L)	1.60 ± 0.50	0.05± 0
phycocyanin (mg/m^3)	59.22 ± 10.17	15.44 ± 1.59
chlorophyll-*a* (mg/m^3)	12.47 ± 1.20	1.84 ± 0.24
water temperature (^{o}C)	20.80 ± 0.74	19.20 ± 0.64
pH	8.43 ± 0.10	8.00 ± 0.03
turbidity (NTU)	15.8 ± 3.09	6.33 ± 0.94
total phosphorus (mg/L)	0.17 ± 0.03	0.11 ± 0.03
nitrate-N (mg/L)	0.68 ± 0.16	0.18 ± 0.04
Total organic carbon (µg/L)	4.07 ± 0.15	2.42±0.05
Heterotrophic plate count (CFU/ml)	8013 ± 1052	279 ± 72

(A)



(B)

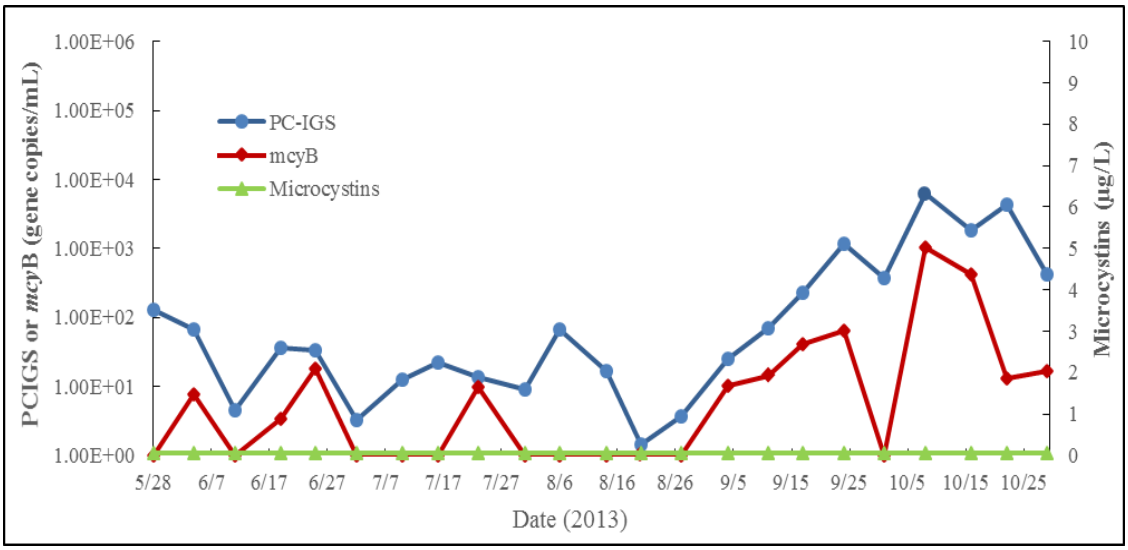


Figure 5.2. Temporal trends of PC-IGS, *mcy*B and microcystins at (A) Toledo and (B) Painesville water plant intake point from May to October in 2013

5.3.2 Relationship between environmental factors and HAB related parameters

The data for Toledo and Painesville were examined separately, so that any anomalies might become apparent. Correlations between microcystins concentration, the abundance of *Microcystis* genotypes and limnological parameters were estimated using Spearman's correlation (Table 5.2). Correlations between microcystins and environmental factors were not available at Painesville due to the fact that no microcystins were detected. In Toledo, microcystins concentration yielded a positive correlation with pH, total organic carbon and HPC. In both Toledo and Painesville locations, the total chlorophyll-*a* concentration yielded a significant correlation with *Microcystis* abundance ($p<0.05$). In Toledo, the phycocyanin pigment showed a significant positive correlation ($p<0.05$) with both microcystins levels and *Microcystis* abundance as measured by PC-IGS. There was no significant correlation between water temperature and HAB-related parameters in either location. Water from the Toledo area had higher pH values than Painesville (T test, $p<0.05$) and had a significant relationship with microcytins, *Microcystis* abundance, and toxic *Microcystis*. Total organic carbon, as well as HPC, also showed a significant positive correlation with microcytins, *Microcystis* abundance and toxic *Microcystis* in Toledo.

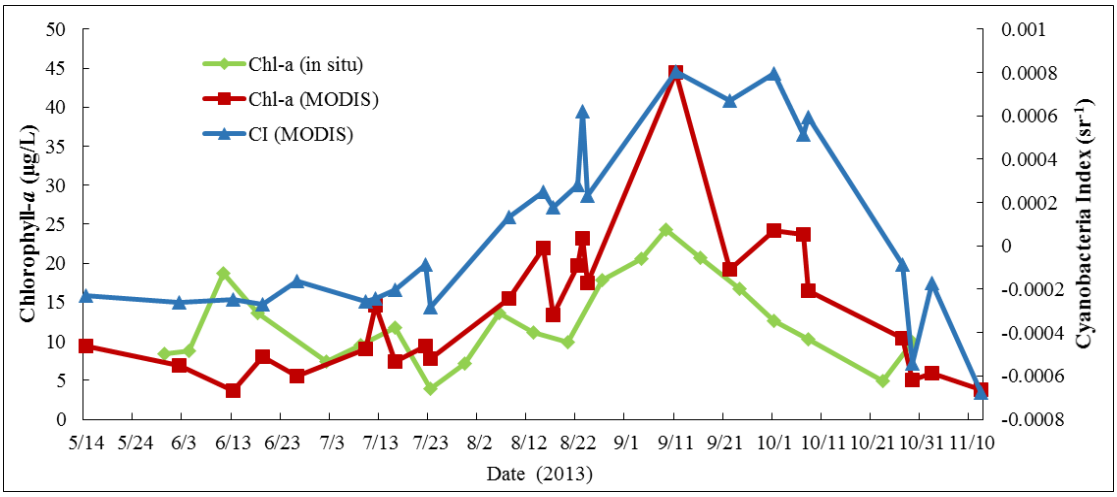
Table 5.2. Spearman's correlations between microcystins concentration, the abundance of *Microcystis* genotypes and limnological parameters

	Microcystins		PC-GIS		*mcy*B	
	Toledo (N=21)	Painesville	Toledo (N=21)	Painesville (N=23)	Toledo (N=21)	Painesville (N=23)
Chl-a	0.396	NA	0.570*	0.587*	0.371	0.448*
Phycocyanin	0.437*	NA	0.669*	0.352	0.403	0.232
pH	0.702*	NA	0.800*	0.348	0.632*	0.109
Water temperature	0.178	NA	0.115	-0.333	0.285	-0.046
turbidity	0.213	NA	0.384	0.147	0.140	0.082
total phosphorus	0.340	NA	0.429	-0.153	0.311	-0.071
nitrate-N	-0.171	NA	-0.367	-0.323	-0.124	-0.468*
Total organic carbon	0.797*	NA	0.727*	-0.200	0.586*	-0.151
HPC	0.540*	NA	0.725*	0.007	0.613*	0.006
PC-IGS	0.868*	NA	1	1	0.914*	0.713*
*mcy*B	0.823*	NA	0.914*	0.713*	1	1

5.3.3 Retrieval of chlorophyll-*a* and CI using remote sensing

Chlorophyll-*a* concentration was retrieve using the built-in algorithm in SeaDAS (O'Reilly et al. 2000). After masking unreliable pixels and spatial smoothing, the MODIS-retrieved chlorophyll-a concentration was obtained during the study period near the Toledo and Painesville water plant intakes. The comparison between the *in situ* measurement and MODIS-retrieved chlorophyll-a concentrations are shown in Figure 5.3. MODIS-retrieved chlorophyll-a concentrations were generally higher than our *in situ* measurements. One possible reason for this difference is our sampling depth. Our *in situ* samples were taken near the bottom of the lake from the intakes of the water plants, whereas satellite remote sensing retrieved chlorophyll-a concentration near the surface. The trend of chlorophyll-*a* and CI retrieved from MODIS matched well with our molecular measurements of PC-IGS and *mcy*B in Toledo. MODIS-derived CI showed significant blooms from late July to October that peaked in early September for areas near Toledo based on a threshold of zero (Wynne et al., 2013). MODIS-derived CI indicated only mild blooms near Painesville in early July, which did not match well with our molecular measurements.

(A)



(B)

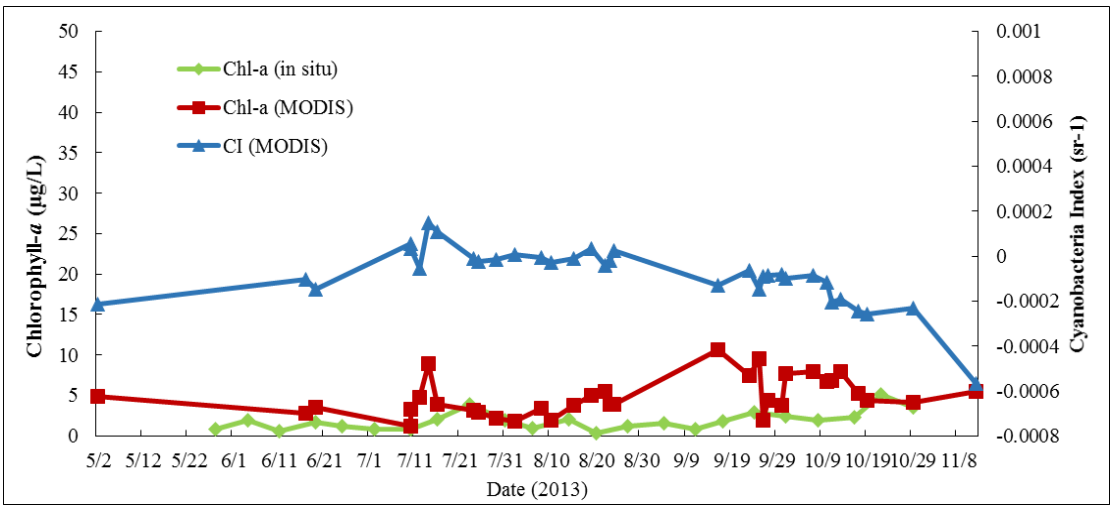


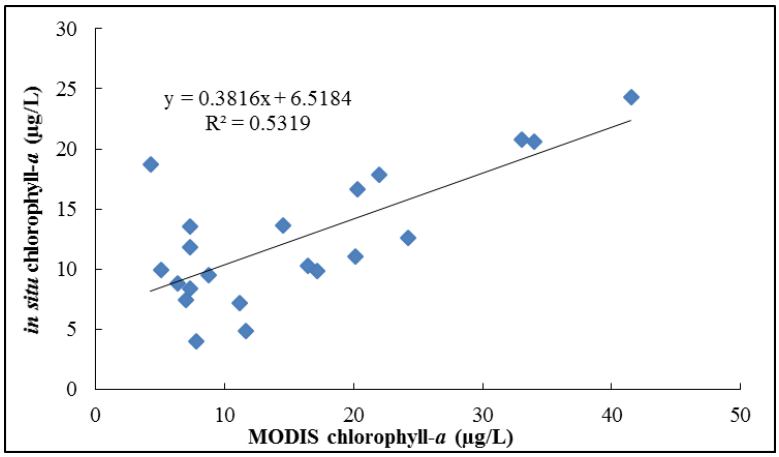
Figure 5.3. Temporal trends of MODIS-retrieved chlorophyll-a, cyanobacteria index, and in situ measured chlorophyll-a at Toledo and Painesville water plant intake points from May to October in 2013: (A) Toledo; (B) Painesville

5.3.4 Compare MODIS measured chlorophyll-*a* and CI with in situ HAB related measurements

The time series of MODIS-measured HAB indicators, such as chlorophyll-a and CI, did not match perfectly with the time series of our *in situ* measurements due the cloud coverage. For making statistically meaningful comparisons, linear interpolation was used to get the time series of MODIS measurements to match the *in situ* measurements.

In situ-measured chlorophyll-*a* showed statistically significant relationship with MODIS-measured concentration of chlorophyll-*a* (R^2=0.5319, p<0.05) at the Toledo sampling point (Figure 5.4). However, the relationship was not significant for observations at Painesville, where the *in situ* chlorophyll-*a* concentrations were much lower than those from Toledo, demonstrating that the match between MODIS- and *in situ*-measured chlorophyll-*a* was more reliable in areas with higher chlorophyll-a concentrations.

(A)



(B)

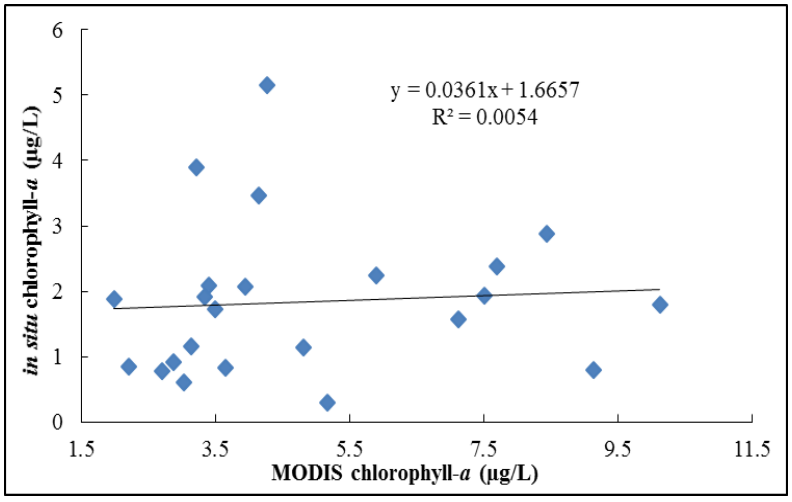


Figure 5.4. Scatter plots showing the relationship between MODIS-retrieved chlorophyll-*a* with in situ measured chlorophyll-*a* at (A) Toledo and (B) Painesville water plant intake points from May to October, 2013. The solid lines represent the best-fit linear regression models.

Spearman's correlation between MODIS measurements and *in situ* measured HAB parameters also showed that both MODIS-derived chlorophyll-*a* and CI correlated well with all HAB parameters, including levels of microcystins, *mcy*B, PC-IGS, phycocyanin, and chlorophyll-a ($p<0.05$) at Toledo and that was possible to estimate the levels of microcystins from the MODIS-measured concentrations of chlorophyll-*a* (Figure 5.5, log (microcystins) =0.0637*MODIS chlorophyll-*a* -1.4065, $R^2=0.6178$). In contrast, MODIS-measured chlorophyll-*a* showed significant correlations with concentrations of PC-IGS and *mcy*B, but not with concentrations of pigment at Painesville. MODIS-measured CI showed negative correlation with *in situ*-measured HAB parameters at Painesville, indicating that CI from MODIS did not perform well when algal levels were relatively low. When considering the entire lake data set from Lake Erie, MODIS-retrieved chlorophyll-*a* also showed significant correlations with all HAB parameters ($p<0.05$), but CI from MODIS had significant correlations with only the level of microcystins.

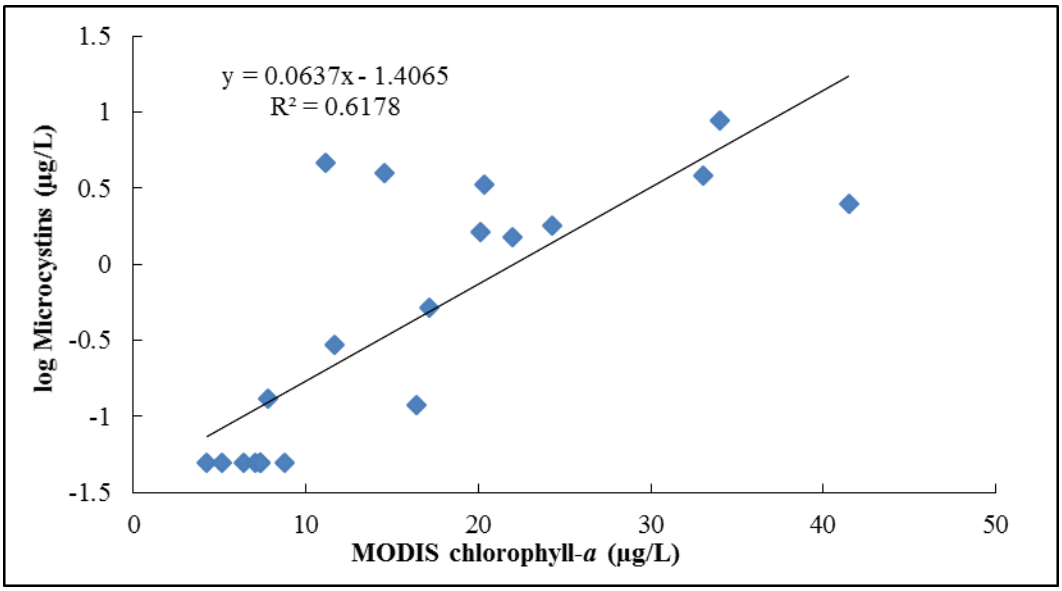


Figure 5.5. Scatter plot showing the relationship between MODIS-retrieved chlorophyll-*a* with in situ-measured levels of microcystins (log transformed) at Toledo water plant intake points from May to October, 2013. The solid lines represent the best-fit linear regression model.

Table 5.3. Spearman correlation between MODIS measurements and in situ measurements at Toledo and Painesville water intake points.

	Toledo		Painesville		Over all	
	CI (MODIS)	Chl-a (MODIS)	CI (MODIS)	Chl-a (MODIS)	CI (MODIS)	Chl-a (MODIS)
CI (MODIS)	1		1		1	
Chl-a (MODIS)	0.903*	1	-0.328	1	0.397*	1
Chl-a (*in situ*)	0.564*	0.523*	-0.288	0.253	0.176	0.699*
Phycocyanin	0.691*	0.595*	-0.395	-0.165	0.248	0.552*
PC-IGS	0.734*	0.853*	-0.701*	0.570*	0.255	0.827*
*mcy*B	0.508*	0.699*	-0.523*	0.551*	0.240	0.805*
Microcystins	0.722*	0.815*	NA	NA	0.618*	0.775*

5.4 Discussion

Although chlorophyll-*a* is common in phytoplankton, it can still be used as an indicator for cyanobacteria blooms in lakes where cyanobacteria is known to dominate, such as Lake Erie. Both the chlorophyll-*a* algorithm and Cyanobacteria Index have been proven to be useful and provide a good indication of the toxin level in western Lake Erie.

However, chlorophyll-*a* outperformed CI in this study by showing higher correlations with *Microcystis* abundance and microcystins levels in both western Lake Erie and the entire lake data set. For MODIS CI, the threshold for indicating the presence of a bloom is suggested as above zero. Based on the CI, the Toledo area was covered by blooms from August to October, during which the toxin levels were relatively high. However, no *in situ* measurements, such as microcystins or *Microcystis* abundance, were high at Painesville, although the CI was above the zero level in late July, 2013. For MODIS-retrieved chlorophyll-*a*, a reasonable threshold is 10 µg/L, which is suggested as an indicator of moderate risk associated with a dominant cyanobacterial bloom. Judging by this threshold, the lake area around Toledo was consistently covered by blooms from August to October, which matched the period when microcystins levels were detectable. At Painesville, the chlorophyll-*a* concentration, as determined with MODIS, only went above 10 µg/L one time (September).

One thing to note is that our water samples were taken from near the bottom of the lake, while satellite observations were occurred on the surface of the lake. Although the western part of Lake Eire is shallow, it is likely that *Microcystis* were more abundant near the surface. Differences in concentrations of chlorophyll-*a* retrieved by MODIS and our in *situ* measurements may have been due to a higher abundance of *Microcystis* near the surface. However, the excellent correlations between MODIS-retrieved chlorophyll-*a* and HAB-related parameters indicate that satellite observations are useful for monitoring cyanobacterial blooms and toxins for the whole water column in Lake Erie, possibly due to shallowness of the water. Based on the above observations, it appears feasible to use

remote sensing to monitor bloom-associated health risks for lake water below the surface, which is commonly used as a source for drinking water.

In summary, *Microcystis* and toxic *Microcystis* were observed in both western and central Lake Erie with western Lake Erie has more serious *Microcystis* blooms. During the summer, the raw water at the Toledo water plant contained microcystins at a level higher than the WHO drinking water guidelines of 1 μg/L, leading to expensive additional water treatment processes to ensure the removal of microcystins in the drinking water. Although neither Toledo nor Painesville water plant has been reported levels of microcystins over 1 μg/L, Carroll Township (which is about 30 kilometers east of Toledo and also draws water from western Lake Erie) issued a warning on September 5, 2013, that their treated water still contained 3.56 μg/L microcystins during the time that our Toledo observations showed a peak of *Microcystis* blooms (Figure 5.2).

Toxic *Microcystis* blooms are a threat to human health mainly due to the toxins they produce. Therefore, a rapid monitoring method is needed that provides an indication of toxin levels; such a method is important to protect the public health from both recreational and drinking water exposure. The Environmental Protection Agency (EPA) does not require regular monitoring of micorcystins in drinking water, but several public water systems near western Lake Erie, including Toledo and Carroll Township, have been voluntarily monitoring weekly for microcystins (Henry, 2013). However, weekly monitoring of toxins may not be sufficient during bloom period and could potentially miss days with high toxin levels. A reliable and rapid monitoring method could provide information for those water plants drawing water from a bloom covered lake to adjust

their monitoring frequency and treatment strategies to remove toxins. Remote sensing is one method that could be used as an aidin assessment of risk to human health by measuring concentration of chlorophyll-*a* and CI that are indicative of microcystins levels in bloom areas; MODIS is a practical and useful system for routine screening and monitoring *Microcystis* blooms (images are available twice daily from Aqua and Terra).

5.5 Chapter Conclusions

- Western Lake Erie cyanobacterial blooms prevailed from late July to early October in 2013, while the bloom was less prevalent in central Lake Erie.
- MODIS-based cyanobacteria indicators included chlorophyll-*a* and Cyanobacteria Index. Both worked well in western Lake Erie for detecting blooms and both showed good correlations with *Microcystis* abundance, toxic *Microcystis* abundance, and microcystins levels.
- In central Lake Erie, where blooms is mild, levels of MODIS-retrieved chlorophyll-*a* correlated well with abundance of both *Microcystis* and toxic *Microcystis*, while the MODIS-retrieved Cyanobacteria Index failed to show any correlation.
- Overall, MODIS-retrieved chlorophyll-*a* performed better than MODIS-retrieved Cyanobacteria Index in Lake Erie for monitoring *Microcystis* blooms and microcystins levels.
- Satellite-based monitoring for cyanobacterial blooms has a great potential for protection of public health around Lake Erie and similar water bodies.

Conclusion

This study suggested that the problem of toxic cyanobacterial blooms will become worse if the potential promoting factors, such as zebra mussel invasion and land-use changes, were not properly controlled. Combined with the influence of climate change, more toxic cyanobacterial blooms are expected in the future. Zebra mussel invasion is able to change the cyanobacterial community structure and increase the toxin levels in inland water bodies. Our current study, based on over 1,000 lakes, showed that cyanobacterial community structure was different in lakes located in areas where zebra mussels were established as opposed to lakes located in areas where they were not. Microcystins levels and cyanobacteria abundance were also significantly higher in lakes located in areas where zebra mussels were established compared with lakes located in areas where they were not. Zebra mussel invasion, as a promoting factors of toxic cyanobacterial blooms and toxins production should be controlled.

Another risk factor, land-use in the lake basin, was also invested using the same dataset for over 1,000 lakes. This study showed that land-use in the basin influenced lake nutrient concentrations and that higher nutrient concentrations in lake water greatly promote the growth of cyanobacteria and increase the level of microcystins. Increased forest and less agricultural land in the watershed can protect the lakes from eutrophication and cyanobacterial blooms. This study also indicated that nitrogen appeared to be more

important than phosphorus in controlling toxic cyanobacteria abundance. A dual strategy, controlling both nitrogen and phosphorus should be considered for future controlling of toxic cyanobacterial blooms.

Our epidemiological study in the Contiguous U.S., using satellite remote sensing, indicated that cyanobacterial bloom coverage was a potential risk factor for mortality from nonalcoholic liver disease. Risk of death from nonalcoholic liver disease increased significantly with an increase in bloom coverage in affected counties after adjusting for age, gender, educational level, and race. Clusters of nonalcoholic liver disease mortality were identified in those counties that had higher bloom coverage. More attention should be centered on the public health impact of toxic cyanobacterial blooms, due to their increasing occurrence. This study contained herein also suggested the usefulness of satellite remote sensing for the application in public health-related studies.

We also monitored the toxic cyanobacterial bloom situations in two drinking water plant intakes in Lake Erie during 2013 and examined the performance of two MODIS-based indicators for monitoring cyanobacterial blooms for the purpose of ensuring drinking water safety. Our observations showed that MODIS-based cyanobacteria indicators, including both chlorophyll-*a* and Cyanobacteria Index, worked well in western Lake Erie for detecting blooms and both showed good correlation with *Microcystis* abundance, toxic *Microcystis* abundance, and microcystins levels. However, for central Lake Erie, where blooms are mild and microcystins levels are undetectable, the MODIS-retrieved Cyanobacteria Index failed to show any correlation with bloom related parameters, while levels of MODIS-retrieved chlorophyll-*a* correlated well with abundance of both

Microcystis and toxic *Microcystis*. Overall, this study suggests that satellite-based monitoring systems have a great potential for the protection of public health around Lake Erie and similar water bodies.

References

Abell JM, Ozkundakci D, Hamilton DP, Miller SD. Relationships between land use and nitrogen and phosphorus in New Zealand lakes. Marine and Freshwater Research 2011; 62: 162-175.

Ahn C-Y, Joung S-H, Yoon S-K, Oh H-M. Alternative alert system for cyanobacterial bloom, using phycocyanin as a level determinant. Journal of Microbiology 2007; 45: 98-104.

Albert S, O'Neil JM, Udy JW, Ahern KS, O'Sullivan CM, Dennison WC. Blooms of the cyanobacterium *Lyngbya majuscula* in coastal Queensland, Australia: disparate sites, common factors. Marine Pollution Bulletin 2005; 51: 428-437.

Algermissen D, Mischke R, Seehusen F, Gobel J, Beineke A. Lymphoid depletion in two dogs with nodularin intoxication. Veterinary Record 2011; 169: 15.

Anderson DM, Glibert PM, Burkholder JM. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 2002; 25: 704-726.

Anselin L. Local Indicators of Spatial Association-Lisa. Geographical Analysis 1995; 27: 93-115.

Anselin L. Exploring spatial data with GeoDaTM: a workbook. Center for Spatially Integrated Social Science, Urbana 2004.

Associated Press. Algae blooms in Lake Erie becoming a threat to drinking water, 2013.

Atech. Cost of algal blooms. Report to Land and Water Resources Research and Development Corporation, Canberra, 2000.

Backer LC, Carmichael W, Kirkpatrick B, Williams C, Irvin M, Zhou Y, et al. Recreational exposure to low concentrations of microcystins during an algal bloom in a small lake. Marine Drugs 2008; 6: 389-406.

Backer LC, McNeel SV, Barber T, Kirkpatrick B, Williams C, Irvin M, et al. Recreational exposure to microcystins during algal blooms in two California lakes. Toxicon 2010; 55: 909-921.

Baker SM, Levinton JS, Kurdziel JP, Shumway SE. Selective feeding and biodeposition by zebra mussels and their relation to changes in phytoplankton composition and seston load. Journal of Shellfish Research 1998; 17: 1207-1213.

Barbiero RP, Rockwell DC, Warren GJ, Tuchman ML. Changes in spring phytoplankton communities and nutrient dynamics in the eastern basin of Lake Erie since the invasion of *Dreissena* spp. Canadian Journal of Fisheries and Aquatic Sciences 2006; 63: 1549-1563.

Bastviken DTE, Caraco NF, Cole JJ. Experimental measurements of zebra mussel (*Dreissena polymorpha*) impacts on phytoplankton community composition. Freshwater Biology 1998; 39: 375-386.

Becker RH, Sultan MI, Boyer GL, Twiss MR, Konopko E. Mapping cyanobacterial blooms in the Great Lakes using MODIS. Journal of Great Lakes Research 2009; 35: 447-453.

Beltran E, Ibanez M, Sancho JV, Hernandez F. Determination of six microcystins and nodularin in surface and drinking waters by on-line solid phase extraction-ultra high pressure liquid chromatography tandem mass spectrometry. Journal of Chromatography A 2012; 1266: 61-68.

Bennett EM, Carpenter SR, Caraco NF. Human impact on erodible phosphorus and eutrophication: A global perspective. BioScience 2001; 51: 227–234.

Best N. Bayesian ecological modeling. In: Lawson A, Biggeri A, Böhning D, Lesaffre E, Viel JF, Bertollini R, editors. Disease mapping and risk assessment for public health. John Wiley & Sons Ltd, West Sussex, England, 1999, pp. 193-201.

Bláha L, Babica P, Marsalek B. Toxins produced in cyanobacterial water blooms - toxicity and risks. Interdisciplinary Toxicology 2009; 2: 36-41.

Blanchar.Dc, Syzdek LD. Concentration of bacteria in jet drops from bursting bubbles. Journal of Geophysical Research 1972; 77: 5087-5099.

Bobeldyk AM, Bossenbroek JM, Evans-White MA, Lodge DM, Lamberti GA. Secondary spread of zebra mussels (*Dreissena polymorpha*) in coupled lake-stream systems. Ecoscience 2005; 12: 339-346.

Bonilla S, Aubriot L, Soares MCS, Gonzalez-Piana M, Fabre A, Huszar VLM, et al. What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*? Fems Microbiology Ecology 2012; 79: 594-607.

Bossenbroek JM, Johnson LE, Peters B, Lodge DM. Forecasting the expansion of zebra mussels in the United States. Conservation Biology 2007; 21: 800-810.

Bray JR, Curtis JT. An ordination of the upland forest communities of Southern Wisconsin. Ecological Monographs 1957; 27: 326-349.

Bricker SB, Longstaf B, Dennison W, Jones A, Boicourt K, Wicks C, et al. Effects of nutrient enrichment in the nation's estuaries: A decade of change. Harmful Algae 2008; 8: 21-32.

Brient L, Lengronne M, Bertrand E, Rolland D, Sipel A, Steinmann D, et al. A phycocyanin probe as a tool for monitoring cyanobacteria in freshwater bodies. Journal of Environmental Monitoring 2007; 10: 248-255.

Brittain SM, Wang J, Babcock-Jackson L, Carmichael WW, Rinehart KL, Culver DA. Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. Journal of Great Lakes Research 2000; 26: 241-249.

Byth S. Palm Island mystery disease. Medical Journal of Australia 1980; 2: 40-42.

Caller TA, Doolin JW, Haney JF, Murby AJ, West KG, Farrar HE, et al. A cluster of amyotrophic lateral sclerosis in New Hampshire: A possible role for toxic cyanobacteria blooms. Amyotrophic Lateral Sclerosis 2009; 10: 101-108.

Caraco NF, Cole JJ, Raymond PA, Strayer DL, Pace ML, Findlay SEG, et al. Zebra mussel invasion in a large, turbid river: Phytoplankton response to increased grazing. Ecology 1997; 78: 588-602.

Carmichael WW. A status report on planktonic cyanobacteria (blue green algae) and their toxins. Cincinnati, OH: Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 1992.

Carmichael WW, Azevedo SMFO, An JS, Molica RJR, Jochimsen EM, Lau S, et al. Human fatalities from cyanobacteria: Chemical and biological evidence for cyanotoxins. Environmental Health Perspectives 2001; 109: 663-668.

Carpenter SR. Phosphorus control is critical to mitigating eutrophication. Proceedings of the National Academy of Sciences of the United States of America 2008; 105: 11039-11040.

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications 1998; 8: 559-568.

Changnon SA, Demissie M. Detection of changes in streamflow and floods resulting from climate fluctuations and land use-drainage changes. Climatic Change 1996; 32 411–421.

Chawira M. Monitoring blue-green algae in the IJsselmeer using remote sensing and in-situ measurements. Master of Science. University of Twente, Enschede, The Netherlands, 2012.

Chen J, Xie P, Li L, Xu J. First identification of the hepatotoxic microcystins in the serum of a chronically exposed human population together with indication of hepatocellular damage. Toxicological Sciences 2009; 108: 81-89.

Cheung MY, Liang S, Lee J. Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health. Journal of Microbiology 2013; 51: 1-10.

Chorus I. Cyanotoxins occurrence, causes, consequences. Springer Berlin Heidelberg, Berlin, Heidelberg, 2001.

Chorus I. Current approaches to cyanotoxin risk assessment, risk management and regulations in different countries. Deutschland Umweltbundesamt: Federal Environment Agency, 2005.

City of Maumee. City of Maumee water quality report 2013. 2013.

Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA, Metcalf JS. Cyanobacterial toxins, exposure routes and human health. European Journal of Phycology 1999; 34: 405-415.

Codd GA, Morrison LF, Metcalf JS. Cyanobacterial toxins: risk management for health protection. Toxicol Appl Pharmacol 2005; 203: 264-72.

Conroy JD, Edwards WJ, Pontius RA, Kane DD, Zhang HY, Shea JF, et al. Soluble nitrogen and phosphorus excretion of exotic freshwater mussels (*Dreissena* spp.): potential impacts for nutrient remineralisation in western Lake Erie. Freshwater Biology 2005; 50: 1146-1162.

Cox PA, Banack SA, Murch SJ. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proceedings of the National Academy of Sciences of the United States of America 2003; 100: 13380-13383.

Crosbie B, Chow-Fraser P. Percentage land use in the watershed determines the water and sediment quality of 22 marshes in the Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences 1999; 56: 1781-1791.

Davis TW, Berry DL, Boyer GL, Gobler CJ. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms Harmful Algae 2009; 8: 715-725.

de Figueiredo DR, Azeiteiro UM, Esteves SM, Goncalves FJM, Pereira MJ. Microcystin-producing blooms - a serious global public health issue. Ecotoxicology and Environmental Safety 2004; 59: 151-163.

Dittmann E, Wiegand C. Cyanobacterial toxins-occurrence, biosynthesis and impact on human affairs. Molecular Nutrition and Food Research 2006; 50: 7-17.

Dodds WK, Bouska WW, Eitzmann JL, Pilger TJ, Pitts KL, Riley AJ, et al. Eutrophication of U.S. freshwaters: analysis of potential economic damages. Environmental Science and Technology 2009; 43: 12-19.

Dolman AM, Rucker J, Pick FR, Fastner J, Rohrlack T, Mischke U, et al. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. Plos One 2012; 7.

Dorr FA, Pinto E, Soares RM, Azevedo SMFDE. Microcystins in South American aquatic ecosystems: Occurrence, toxicity and toxicological assays. Toxicon 2010; 56: 1247-1256.

Dzialowski AR, Jessie W. Zebra mussels negate or mask the increasing effects of nutrient enrichment on algal biomass: a preliminary mesocosm study. Journal of Plankton Research 2009; 31: 1437-1440.

Edwards M, Johns DG, Leterme SC, Svendsen E, Richardson AJ. Regional climate change and harmful algal blooms in the northeast Atlantic. Limnology and Oceanography 2006; 51: 820-829.

Environmental Protection Agency. Lake Erie lakewide management plan, 2004.

Fahnenstiel GL, Bridgeman TB, Lang GA, McCormick MJ, Nalepa TF. Phytoplankton productivity in Saginaw Bay, Lake Huron: Effects of zebra mussel (*Dreissena polymorpha*) colonization. Journal of Great Lakes Research 1995; 21: 465-475.

Falconer I, Bartram J, Chorus I, Kuiper-Goodman T, Utkilen H, Burch M, et al. Safe levels and safety practices. In: Chorus I, Bartram J, editors. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management, London, 1999.

Falconer IR. Health effects associated with controlled exposures to cyanobacterial toxins. Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs 2008; 619: 607-612.

Falconer IR, Jackson ARB, Langley J, Runnegar MT. Liver pathology in mice in poisoning by the Blue-Green Alga *Microcystis aeruginosa*. Australian Journal of Biological Sciences 1981; 34: 179-187.

Falconer IR, Smith JV, Jackson ARB, Jones A, Runnegar MTC. Oral toxicity of a bloom of the cyanobacterium *Microcystis aeruginosa* administered to mice over periods up to 1 year. Journal of Toxicology and Environmental Health 1988; 24: 291-305.

FAO/UNEP. Terminology for Integrated Resources Planning and Management. Food and Agriculture Organization/United Nations Environmental Programme, Rome, Italy and Nairobi, Kenya., 1999.

Ferber LR, Levine SN, Lini A, Livingston GP. Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? Freshwater Biology 2004; 49: 690-708.

Fernald SH, Caraco NF, Cole JJ. Changes in cyanobacterial dominance following the invasion of the zebra mussel *Dreissena polymorpha*: Long-term results from the Hudson River Estuary. Estuaries and Coasts 2007; 30: 163-170.

Fishman DB, Adlerstein SA, Vanderploeg HA, Fahnenstiel GL, Scavia D. Phytoplankton community composition of Saginaw Bay, Lake Huron, during the zebra mussel (*Dreissena polymorpha*) invasion: A multivariate analysis. Journal of Great Lakes Research 2010; 36: 9-19.

Fitzgeorge R, Clark S, Keevil C. Routes of intoxication. In: Codd GA, Jefferies TM, Keevil CW, Potter P, editors. Detection methods for cyanobacterial toxins. Royal Society of Chemistry, Cambridge, 1994, pp. 69–74.

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, et al. Global consequences of land use. Science 2005; 309: 570-574.

Fristachi A, Sinclair JL, Hall S, Boyer G, Burkholder J, Burns J, et al. Occurrence of cyanobacterial harmful algal blooms workgroup report. Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. 619. Springer Press, New York, 2008, pp. 45-103.

Fuller P. USGS develops a drainage-based system to track ANS introductions. Aquatic Nuisance Species Digest 1999; 3: 32-34.

Galloway JN, Cowling EB. Reactive nitrogen and the world: 200 years of change. AMBIO: A Journal of the Human Environment 2002; 31: 64-71.

Gazulha V, Mansur MCD, Cybis LF, Azevedo SMFO. Feeding behavior of the invasive bivalve Limnoperna fortunei (Dunker, 1857) under exposure to toxic cyanobacteria *Microcystis aeruginosa*. Brazilian Journal of Biology 2012; 72: 41-49.

Gelfand AE, Smith AFM. Sampling-based approaches to calculating marginal densities. Journal of the American Statistical Association 1990; 85: 398-409.

Ginn HP, Pearson LA, Neilan BA. NtcA from *Microcystis aeruginosa* PCC 7806 Is autoregulatory and binds to the microcystin promoter. Applied and Environmental Microbiology 2010; 76: 4362-4368.

Glibert PM, Anderson DA, Gentien P, Grane´ li E, Sellner KG. The global, complex phenomena of harmful algal blooms. Oceanography 2005; 18: 136–147.

Glibert PM, Burkholder JM. The complex relationships between increasing fertilization of the Earth, coastal eutrophication, and HAB proliferation. In: Granéli E, Turner J, editors. The Ecology of Harmful Algae. Springer-Verlag, New York, 2006, pp. 341–354.

Goedkoop W, Naddafi R, Grandin U. Retention of N and P by zebra mussels (*Dreissena polymorpha* Pallas) and its quantitative role in the nutrient budget of eutrophic Lake Ekoln, Sweden. Biological Invasions 2011; 13: 1077-1086.

Graham JL, Loftin KA, Meyer MT, Ziegler AC. Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the Midwestern United States. Environmental Science and Technology 2010; 44: 7361-8.

Griffiths DJ, Saker ML. The Palm Island mystery disease 20 years on: a review of research on the cyanotoxin cylindrospermopsin. Environmental Toxicology 2003; 18: 78-93.

Griffiths RW, Schloesser DW, Leach JH, Kovalak WP. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. Canadian Journal of Fisheries and Aquatic Sciences 1991; 48: 1381-1388.

Hammer O, Harper DAT, Ryan PD. Past: Paleontological statistics software package for education and data analysis. Paleontología Electrónica 2001; 4: 1-9.

Heath RT, Fahnenstiel GL, Gardner WS, Cavaletto JF, Hwang SJ. Ecosystem-level effects of zebra mussels (*Dreissena polymorpha*): An enclosure experiment in Saginaw Bay, Lake Huron. Journal of Great Lakes Research 1995; 21: 501-516.

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, et al. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 2008; 8: 3-13.

Henry T. Carroll Township's scare with toxin a 'wake-up call' Water plant shut over lethal microcystin from algae. The Toledo Blade, 2013.

Hernandez JM, Lopez-Rodas V, Costas E. Microcystins from tap water could be a risk factor for liver and colorectal cancer: A risk intensified by global change. Medical Hypotheses 2009; 72: 539-540.

Hilborn ED, Roberts VA, Backer L, DeConno E, S. Egan J, Hyde JB, et al. Algal bloom–associated disease outbreaks among users of freshwater lakes — United States, 2009-2010. Morbidity and Mortality Weekly Report (MMWR) 2014; 63: 11-15.

Hisbergues M, Christiansen G, Rouhiainen L, Sivonen K, Borner T. PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. Archives of Microbiology 2003; 180: 402-410.

Hitzfeld BC, Lampert CS, Spaeth N, Mountfort D, Kaspar H, Dietrich DR. Toxin production in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. Toxicon 2000; 38: 18.

Hoeger SJ, Schmid D, Blom JF, Ernst B, Dietrich DR. Analytical and functional characterization of microcystins [Asp^3]MC-RR and [Asp^3,Dhb^7]MC-RR:

Consequences for risk assessment? Environmental Science and Technology 2007; 41: 2609-2616.

Holland JM. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agriculture Ecosystems & Environment 2004; 103: 1-25.

Holland RE. Changes in Planktonic Diatoms and Water Transparency in Hatchery Bay, Bass-Island Area, Western Lake Erie since the Establishment of the Zebra Mussel. Journal of Great Lakes Research 1993; 19: 617-624.

Holtcamp W. The emerging science of BMAA: do cyanobacteria contribute to neurodegenerative disease? Environmental Health Perspectives 2012; 120: A110-A116.

Horst GP, Sarnelle O, White JD, Hamilton SK, Kaul RB, Bressie JD. Nitrogen availability increases the toxin quota of a harmful cyanobacterium, *Microcystis aeruginosa*. Water Research 2014; 54: 188-198.

Hu CM, Muller-Karger FE, Taylor C, Carder KL, Kelble C, Johns E, et al. Red tide detection and tracing using MODIS fluorescence data: A regional example in SW Florida coastal waters. Remote Sensing of Environment 2005; 97: 311-321.

Hu ZY, Rao KR. Particulate air pollution and chronic ischemic heart disease in the eastern United States: a county level ecological study using satellite aerosol data. Environmental Health 2009; 8.

Humpage AR, Falconer IR. Microcystin-LR and liver tumor promotion: Effects on cytokinesis, ploidy, and apoptosis in cultured hepatocytes. Environmental Toxicology 1999; 14: 61-75.

Hunter PD, Tyle AN, Gilvear D, J, Willby NJ. Using remote sensing to aid the assessment of human health risks from blooms of potentially toxic cyanobacteria. Environmental Science and Technology 2009; 43: 2627-2633.

Hunter PR. Cyanobacterial toxins and human health. Journal of Applied Microbiology Symposium Supplement 1998; 27: 35S-40S.

IPCC. Working Group III Report. Climate Change 2007,United Nations Environmental Programme. Intergovernmental Panel on Climate Change, New York 2007.

IUCN. 100 of the world's worst invaders. Global invasive species database. International Union for Conser vation of Nature, Gland, Switzerland, 2005.

Jahnichen S, Long BM, Petzoldt T. Microcystin production by *Microcystis aeruginosa*: Direct regulation by multiple environmental factors. Harmful Algae 2011; 12: 95-104.

Janus LL. Climate change impacts from a water supply perspective. In: George G, editor. Impact of climate change on European lakes. Springer The Netherlands, 2010, pp. 469-491.

Jardim WF, Pearson HW. A study of the copper-complexing compounds released by some species of cyanobacteria. Water Research 1984; 18: 985-989.

Jeppesen E, Sondergaard M, Meerhoff M, Lauridsen TL, Jensen JP. Shallow lake restoration by nutrient loading reduction - some recent findings and challenges ahead. Hydrobiologia 2007; 584: 239-252.

Jochimsen EM, Carmichael WW, An JS, Cardo DM, Cookson ST, Holmes CE, et al. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. The New England Journal of Medicine 1998; 338: 873-8.

Johengen TH, Nalepa TF, Fahnenstiel GL, Goudy G. Nutrient changes in Saginaw Bay, Lake Huron, after the establishment of the zebra mussel (*Dreissena polymorpha*). Journal of Great Lakes Research 1995; 21: 449-464.

Juhel G, Davenport J, O'Halloran J, Culloty S, Ramsay R, James K, et al. Pseudodiarrhoea in zebra mussels *Dreissena polymorpha* (Pallas) exposed to microcystins. Journal of Experimental Biology 2006; 209: 810-816.

Kanoshinaa I, Lipsb U, Leppänenc J-M. The influence of weather conditions (temperature and wind) on cyanobacterial bloom development in the Gulf of Finland (Baltic Sea). Harmful Algae 2003; 2: 29-41.

Kasich JR, Taylor M, Nally SJ. Public water system harmful algal bloom response strategy. Ohio Environmental Protection Agency, 2013.

Kasich JR, Taylor M, Nally SJ, Zehringer J, Wymyslo TE. State of Ohio harmful algal bloom response strategy for recreational waters. Ohio Environmental Protection Agency, Ohio Department of Natural Resources, 2012.

Kim WR, Brown RS, Terrault NA, El-Serag H. Burden of liver disease in the United States: Summary of a workshop. Hepatology 2002; 36: 227-242.

Kleinteich J, Wood SA, Kupper FC, Camacho A, Quesada A, Frickey T, et al. Temperature-related changes in polar cyanobacterial mat diversity and toxin production. Nature Climate Change 2012; 2: 356-360.

Knoll LB, Sarnelle O, Hamilton SK, Kissman CEH, Wilson AE, Rose JB, et al. Invasive zebra mussels (*Dreissena polymorpha*) increase cyanobacterial toxin concentrations in low-nutrient lakes. Canadian Journal of Fisheries and Aquatic Sciences 2008; 65: 448-455.

Koreiviene J, Anne O, Kasperoviciene J, Burskyte V. Cyanotoxin management and human health risk mitigation in recreational waters. Environmental Monitoring and Assessment 2014; 186: 4443-59.

Kronvang B, Jeppesen E, Conley DJ, Sondergaard M, Larsen SE, Ovesen NB, et al. Nutrient pressures and ecological responses to nutrient loading reductions in Danish streams, lakes and coastal waters. Journal of Hydrology 2005; 304: 274-288.

Kuiper-Goodman T, Falconer I, Fitzgerald J. Human health aspects. In: Chorus I, Bartram J, editors. Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management. E & FN Spon on behalf of the World Health Organization, London and New York, 1999, pp. 41–112.

Kurmayer R, Kutzenberger T. Application of real-time PCR for quantification of microcystin genotypes in a population of the toxic cyanobacterium *Microcystis* sp. Applied and Environmental Microbiology 2003; 69: 6723-6730.

Kutser T. Quantitative detection of chlorophyll in cyanobacterial blooms by satellite remote sensing. Limnology and Oceanography 2004; 49: 2179-2189.

Lavrentyev PJ, Gardner WS, Cavaletto JF, Beaver JR. Effects of the zebra mussel (*Dreissena polymorpha* Pallas) on protozoa and phytoplankton from Saginaw Bay, Lake Huron. Journal of Great Lakes Research 1995; 21: 545-557.

Le C, Zha Y, Li Y, Sun D, Lu H, Yin B. Eutrophication of lake waters in China: Cost, causes, and control. Environmental Management 2010; 45: 662-668.

Lee SW, Hwang SJ, Lee SB, Hwang HS, Sung HC. Landscape ecological approach to the relationships of land use patterns in watersheds to water quality characteristics. Landscape and Urban Planning 2009; 92: 80-89.

Lei J, Payne BS, Wang SY. Filtration dynamics of the zebra mussel, *Dreissena polymorpha*. Canadian Journal of Fisheries and Aquatic Sciences 1996; 53: 29-37.

Leland HV, Porter SD. Distribution of benthic algae in the upper Illinois River basin in relation to geology and land use. Freshwater Biology 2000; 44: 279-301.

Lewis WM, Wurtsbaugh WA, Paerl HW. Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. Environmental Science and Technology 2011; 45: 10300-10305.

Lewy P, Vinther M. Identification of Danish North Sea trawl fisheries. ICES Journal of Marine Science 1994; 51: 263-272.

Li Y, Chaney R, Siebielec G, Kerschner BA. Response of four turfgrass cultivars to limestone and biosolid-compost amendment of a Zinc and Cadmium contaminated soil at Palmerton, Pensylvania. Journal of Environental Quality 2000; 29: 1440-1447.

Li Y, Chen JA, Zhao Q, Pu CW, Qiu ZQ, Zhang RP, et al. A cross-sectional investigation of chronic exposure to microcystin in relationship to childhood liver damage in the Three Gorges Reservoir Region, China. Environmental Health Perspectives 2011; 119: 1483-1488.

Lopez CB, Jewett EB, Dortch Q, Walton BT, Hudnell HK. Scientific assessment of freshwater harmful algal blooms. Interagency working group on harmful algal

blooms, hypoxia, and human health of the joint subcommittee on Ocean Science and Technology. Washington, DC., 2008.

Lowrance R, Altier LS, Newbold JD, Schnabel RR, Groffman PM, Denver JM, et al. Water quality functions of Riparian forest buffers in Chesapeake Bay watersheds. Environmental Management 1997; 21: 687-712.

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

Lunn DJ, Thomas A, Best N, Spiegelhalter D. WinBUGS-a Bayesian modelling framework: concepts, structure, and extensibility. Statistics and Computing 2000; 10: 325-337.

Makarewicz JC, Lewis TW, Bertram P. Phytoplankton composition and biomass in the offshore waters of Lake Erie: Pre- and post-*Dreissena* introduction (1983-1993). Journal of Great Lakes Research 1999; 25: 135-148.

Malbrouck C, Kestemont P. Effects of microcystins on fish. Environmental Toxicology and Chemistry 2006; 25: 72-86.

Marion JW, Lee J, Wilkins JR, Lemeshow S, Lee C, Waletzko EJ, et al. In vivo phycocyanin flourometry as a potential rapid screening tool for predicting elevated microcystin concentrations at eutrophic lakes. Environmental Science & Technology 2012; 46: 4523-4531.

Maxwell CD. Floristic changes in soil algae and cyanobacteria in reclaimed metal-contaminated land at Sudbury, Canada. Water Air and Soil Pollution 1991; 60: 381-393.

McQuaid N, Zamyadi A, Prévost M, Bird DF, Dorner S. Use of in vivo phycocyanin fluorescence to monitor potential microcystin-producing cyanobacterial biovolume in a drinking water source. Journal of environmental monitoring : JEM 2011; 13: 455-463.

Mench M, Bussiere S, Boisson J, Castaing E, Vangronsveld J, Ruttens A, et al. Progress in remediation and revegetation of the barren Jales gold mine spoil after *in situ* treatments. Plant and Soil 2003; 249: 187-202.

Michalak AM, Anderson EJ, Beletsky D, Boland S, Bosch NS, Bridgeman TB, et al. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proceedings of the National Academy of Sciences of the United States of America 2013; 110: 6448-6452.

Mida JL, Scavia D, Fahnenstiel GL, Pothoven SA, Vanderploeg HA, Dolan DM. Long-term and recent changes in southern Lake Michigan water quality with implications for present trophic status. Journal of Great Lakes Research 2010; 36: 42-49.

Millie DF, Fahnenstiel GL, Bressie JD, Pigg RJ, Rediske RR, Klarer DM, et al. Late-summer phytoplankton in western Lake Erie (Laurentian Great Lakes): bloom distributions, toxicity, and environmental influences. Aquatic Ecology 2009; 43: 915-934.

Mitchell A, Reghenzani J, Faithful J, Furnas M, Brodie J. Relationships between land use and nutrient concentrations in streams draining a 'wet-tropics' catchment in northern Australia. Marine and Freshwater Research 2009; 60: 1097-1108.

Moreno-Madrinan MJ, Fischer AM. Performance of the MODIS FLH algorithm in estuarine waters: a multi-year (2003-2010) analysis from Tampa Bay, Florida (USA). International Journal of Remote Sensing 2013; 34: 6467-6483.

Murch SJ, Cox PA, Banack SA, Steele JC, Sacks OW. Occurrence of beta-methylamino-L-alanine (BMAA) in ALS/PDC patients from Guam. Acta Neurologica Scandinavica 2004; 110: 267-269.

Naddafi R, Pettersson K, Eklov P. The effect of seasonal variation in selective feeding by zebra mussels (*Dreissena polymorpha*) on phytoplankton community composition. Freshwater Biology 2007; 52: 823-842.

Neilan BA, Pearson LA, Muenchhoff J, Moffitt MC, Dittmann E. Environmental conditions that influence toxin biosynthesis in cyanobacteria. Environmental Microbiology 2013; 15: 1239-1253.

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

Nicholls KH, Hopkins GJ. Recent changes in Lake Erie (North Shore) phytoplankton: cumulative impacts of phosphorus loading reductions and the zebra mussel introduction. Journal of Great Lakes Research 1993; 19: 637-647.

Nielsen A, Trolle D, Sondergaard M, Lauridsen TL, Bjerring R, Olesen JE, et al. Watershed land use effects on lake water quality in Denmark. Ecological Applications 2012; 22: 1187-1200.

O'Neil JM, Davis TW, Burford MA, Gobler CJ. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae 2012; 14: 313-334.

O'Reilly JE, Maritorena S, Siegel D, O 'Brien M, Toole D, Greg Mitchell B, et al. Ocean color chlorophyll a algorithms for SeaWiFS, OC2, and OC4: Version 4. In: O'Reilly JE, editor. SeaWiFS Postlaunch Calibration and Validation Analyses, Part 3. NASA Tech. Memo. 11. NASA Goddard Space Flight Center, Greenbelt, Maryland, 2000, pp. 9-23.

Ohio Lake Erie Commission. State of Lake Report: Lake Erie quality index, 2004.

Oksanen J, Kindt R, Legendre P, O'Hara RB. Vegan: community ecology package. 2007.

Orlova M, Golubkov S, Kalinina L, Ignatieva N. *Dreissena polymorpha* (Bivalvia : Dreissenidae) in the Neva Estuary (eastern Gulf of Finland, Baltic Sea): Is it a biofilter or source for pollution? Marine Pollution Bulletin 2004; 49: 196-205.

Paerl H. Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum. Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs 2008; 619: 217-237.

Paerl HW, Hall NS, Calandrino ES. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. Science of the Total Environment 2011a; 409: 1739-1745.

Paerl HW, Huisman J. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environmental Microbiology Reports 2009; 1: 27-37.

Paerl HW, Paul VJ. Climate change: links to global expansion of harmful cyanobacteria. Water Research 2012; 46: 1349-1363.

Paerl HW, Scott JT. Throwing fuel on the fire: synergistic effects of excessive nitrogen inputs and global warming on harmful algal blooms. Environmental Science and Technology 2010; 44: 7756-7758.

Paerl HW, Xu H, McCarthy MJ, Zhu GW, Qin BQ, Li YP, et al. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. Water Research 2011b; 45: 1973-1983.

Pawlik-Skowronska B, Toporowska M, Rechulicz J. Simultaneous accumulation of anatoxin-a and microcystins in three fish species indigenous to lakes affected by cyanobacterial blooms. Oceanological and Hydrobiological Studies 2012; 41: 53-65.

Pearson L, Mihali T, Moffitt M, Kellmann R, Neilan B. On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. Marine Drugs 2010; 8: 1650-80.

Pelaez M, Antoniou MG, He X, Dionysios D. Dionysiou, Armah A. de la Cruz, Katerina Tsimeli, et al. Sources and occurrence of cyanotoxins worldwide. In: Fatta-Kassinos D, editor. Xenobiotics in the Urban Water Cycle: Mass Flows, Environmental Processes, Mitigation and Treatment Strategies, 2010.

Pelletier D, Ferraris J. A multivariate approach for defining fishing tactics from commercial catch and effort data. Canadian Journal of Fisheries and Aquatic Sciences 2000; 57: 51-65.

Peperzak L. Climate change and harmful algal blooms in the North Sea. Acta Oecologica-International Journal of Ecology 2003; 24: S139-S144.

Pilotto LS, Douglas RM, Burch MD, Cameron S, Beers M, Rouch GJ, et al. Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities. Australian and New Zealand Journal of Public Health 1997; 21: 562-566.

Pires LMD, Bontes BM, Van Donk E, Ibelings BW. Grazing on colonial and filamentous, toxic and non-toxic cyanobacteria by the zebra mussel *Dreissena polymorpha*. Journal of Plankton Research 2005; 27: 331-339.

Pires LMD, Van Donk E. Comparing grazing by *Dreissena polymorpha* on phytoplankton in the presence of toxic and non-toxic cyanobacteria. Freshwater Biology 2002; 47: 1855-1865.

Poste AE, Hecky RE, Guildford SJ. Evaluating microcystin exposure risk through fish consumption. Environmental Science and Technology 2011; 45: 5806-5811.

Preiss D, Sattar N. Non-alcoholic fatty liver disease: an overview of prevalence, diagnosis, pathogenesis and treatment considerations. Clinical Science 2008; 115: 141-150.

Pretty JN, Mason CF, Nedwell DB, Hine RE. A preliminary assessment of the environmental costs of the eutrophication of fresh waters in England and Wales. University of Essex, Colchester UK, 2002.

Qian HF, Pan XJ, Chen J, Zhou DM, Chen ZG, Zhang L, et al. Analyses of gene expression and physiological changes in *Microcystis aeruginosa* reveal the phytotoxicities of three environmental pollutants. Ecotoxicology 2012; 21: 847-859.

Raikow DF, Sarnelle O, Wilson AE, Hamilton SK. Dominance of the noxious cyanobacterium *Microcystis aeruginosa* in low-nutrient lakes is associated with exotic zebra mussels. Limnology and Oceanography 2004; 49: 482-487.

Rapala J, Sivonen K, Lyra C, Niemela SI. Variation of microcystins, cyanobacterial hepatotoxins, in Anabaena spp. as a function of growth stimuli. Applied and Environmental Microbiology 1997; 63: 2206-2212.

Reeders HH, Vaate ABD, Noordhuis R. Potential of the zebra mussel (*Dreissena polymorpha*) for water quality management. In: Nalepa TF, Schloesser DW, editors. Zebra Mussels: Biology, Impact, and Control Lewis Publishers, Boca Raton, FL, 1993, pp. 439–451.

Reichwaldt ES, Ghadouani A. Effects of rainfall patterns on toxic cyanobacterial blooms in a changing climate: between simplistic scenarios and complex dynamics. Water Research 2012; 46: 1372-93.

Repavich WM, Sonzogni WC, Standridge JH, Wedepohl RE, Meisner LF. Cyanobacteria (blue-green algae) in wisconsin waters: acute and chronic toxicity. Water Research 1990; 24: 225-231.

Rigosi A, Carey CC, Ibelings BW, Brooks J. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. Limnology and Oceanography 2014; 59: 99-114.

Rinta-Kanto JM, Konopko EA, DeBruyn JM, Bourbonniere RA, Boyer GL, Wilhelm SW. Lake Erie *Microcystis*: Relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. Harmful Algae 2009; 8: 665-673.

Rinta-Kanto JM, Ouellette AJ, Boyer GL, Twiss MR, Bridgeman TB, Wilhelm SW. Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. Environmental Science and Technology 2005; 39: 4198-205.

Roditi HA, Caraco NF, Cole JJ, Strayer DL. Filtration of Hudson River water by the zebra mussel (*Dreissena polymorpha*). Estuaries 1996; 19: 824-832.

Roelfsema CM, Phinn SR, Dennison WC, Dekker AG, Brando VE. Monitoring toxic cyanobacteria *Lyngbya majuscula* (Gomont) in Moreton Bay, Australia by integrating satellite image data and field mapping. Harmful Algae 2006; 5: 45-56.

Ruiz-Verdu A, Simis SGH, de Hoyos C, Gons HJ, Pena-Martinez R. An evaluation of algorithms for the remote sensing of cyanobacterial biomass. Remote Sensing of Environment 2008; 112: 3996-4008.

Runnegar M, Berndt N, Kaplowitz N. Microcystin uptake and inhibition of protein phosphatases: effects of chemoprotectants and self-inhibition in relation to known hepatic transporters. Toxicology and Applied Pharmacology 1995; 134: 264-272.

Saker ML, Jungblut AD, Neilan BA, Rawn DFK, Vasconcelos VM. Detection of microcystin synthetase genes in health food supplements containing the freshwater cyanobacterium Aphanizomenon flos-aquae. Toxicon 2005; 46: 555-562.

Santhi C, Srinivasan R, Arnold JG, Williams JR. A modeling approach to evaluate the impacts of water quality management plans implemented in a watershed in Texas. Environmental Modelling & Software 2006; 21: 1141-1157.

Sarnelle O, White JD, Horst GP, Hamilton SK. Phosphorus addition reverses the positive effect of zebra mussels (*Dreissena polymorpha*) on the toxic cyanobacterium, *Microcystis aeruginosa*. Water Research 2012; 46: 3471-3478.

Scavia D, Fahnenstiel GL. Dynamics of LAKE MICHIGAN phytoplankton: mechanisms controlling epilimnetic communities. Journal of Great Lakes Research 1987; 13: 103-120.

Schachter J. Geographical mobility march 1999 to march 2000. U.S. Department of Commerce, Economics and Statistics Administration, U.S. Census Bureau 2001.

Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson MJ, et al. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences of the United States of America 2008; 105: 11254-11258.

Scott JT, McCarthy MJ. Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. Limnology and Oceanography 2010; 55: 1265-1270.

Sheng H, Liu H, Wang CY, Guo HC, Liu Y, Yang YH. Analysis of cyanobacteria bloom in the Waihai part of Dianchi Lake, China. Ecological Informatics 2012; 10: 37-48.

Simis SGH, Peters SWM, Gons HJ. Remote sensing of the cyanobacterial pigment phycocyanin in turbid inland water. 50, Limnology and Oceanography, 2005, pp. 237-245.

Sinclair JL, Hall S. Occurrence of cyanobacterial harmful algal blooms workgroup report. Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs 2008; 619: 45-103.

Singh W, Hjorleifsson E, Stefansson G. Robustness of fish assemblages derived from three hierarchical agglomerative clustering algorithms performed on Icelandic groundfish survey data. ICES Journal of Marine Science 2011; 68: 189-200.

Sivonen K, Jones J. Cyanobacterial toxins. In: Chorus I, Bartram J, editors. Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management. E & FN Spon on behalf of the World Health Organization, London and New York, 1999, pp. 41–112.

Sivonen K, Kononen K, Carmichael WW, Dahlem AM, Rinehart KL, Kiviranta J, et al. Occurrence of the hepatotoxic cyanobacterium *Nodularia spumigena* in the Baltic Sea and structure of the toxin. Appl Environ Microbiol 1989; 55: 1990-5.

Smith TE, Stevenson RJ, Caraco NF, Cole JJ. Changes in phytoplankton community structure during the zebra mussel (*Dreissena polymorpha*) invasion of the Hudson River (New York). Journal of Plankton Research 1998; 20: 1567-1579.

Smith VH. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 1983; 221: 669-671.

Sogaard KK, Horvath-Puho E, Gronbaek H, Jepsen P, Vilstrup H, Sorensen HT. Risk of venous thromboembolism in patients with liver disease: a nationwide population-based case-control study. American Journal of Gastroenterology 2009; 104: 96-101.

Soward TE. Evaluation of cancer from exposure to cyanotoxins in drinking water at Grand Lake Saint Marys. Boonshoft School of Medicine. Master of Public Health Program. Wright State University, Dayton, 2011.

Steffensen DA. Economic cost of cyanobacterial blooms. Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs 2008; 619: 855-865.

Stewart I, Webb PM, Schluter PJ, Fleming LE, Burns JW, Jr., Gantar M, et al. Epidemiology of recreational exposure to freshwater cyanobacteria--an international prospective cohort study. BMC Public Health 2006a; 6: 93.

Stewart I, Webb PM, Schluter PJ, Shaw GR. Recreational and occupational field exposure to freshwater cyanobacteria – a review of anecdotal and case reports, epidemiological studies and the challenges for epidemiologic assessment. Environmental Health: A Global Access Science Source 2006b; 5: 1-13.

Stommel EW, Field NC, Caller TA. Aerosolization of cyanobacteria as a risk factor for amyotrophic lateral sclerosis. Medical Hypotheses 2013; 80: 142-145.

Strickland T, Fisher L, Korleski C. Ohio Lake Erie phosphorus task force final report Ohio Environmental Protection Agency, 2010.

Stumpf RP, Tomlinson MC, Calkins JA, Kirkpatrick B, Fisher K, Nierenberg K, et al. Skill assessment for an operational algal bloom forecast system. Journal of Marine Systems 2009; 76: 151-161.

Stumpf RP, Wynne TT, Baker DB, Fahnenstiel GL. Interannual variability of cyanobacterial blooms in Lake Erie. Plos One 2012; 7.

Svircev Z, Krstic S, Miladinov-Mikov M, Baltic V, Vidovic M. Freshwater cyanobacterial blooms and primary liver cancer epidemiological studies in Serbia.

Journal of Environmental Science and Health Part C-Environmental Carcinogenesis & Ecotoxicology Reviews 2009; 27: 36-55.

Tango T, Takahashi K. A flexibly shaped spatial scan statistic for detecting clusters. International Journal of Health Geographics 2005; 4: 11.

Teixeira Mda G, Costa M, C., de Carvalho VL, Pereira Mdos S, Hage E. Gastroenteritis epidemic in the area of the Itaparica Dam, Bahia, Brazil. Bulletin of the Pan American Health Organization 1993; 27: 244-53.

Tetratech. Recommended actions for Grand Lake St. Marys, Ohio, 2010.

Tillett D, Neilan BA. Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria. Journal of Phycology 2000; 36: 251-258.

Tong STY, Chen WL. Modeling the relationship between land use and surface water quality. Journal of Environmental Management 2002; 66: 377-393.

Torbick N, Hession S, Stommel E, Caller T. Mapping amyotrophic lateral sclerosis lake risk factors across northern New England. International Journal of Health Geographics 2014; 13.

Ueno Y, Nagata S, Tsutsumi T, Hasegawa A, Watanabe MF, Park HD, et al. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis 1996; 17: 1317-1321.

United Nations. Water: a shared responsibility. United Nations World Water Development Report 2. World Water Assessment Programme, 2006.

USEPA. National Lakes Assessment: A collaborative survey of the Nation's lakes. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, D.C., 2009.

Vanderploeg HA, Johengen TH, Liebig JR. Feedback between zebra mussel selective feeding and algal composition affects mussel condition: did the regime changer pay a price for its success? Freshwater Biology 2009; 54: 47-63.

Vanderploeg HA, Liebig Jr, Carmichael WW, Agy MA, Johengen TH, Fahnenstiel GLNTF. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Ottawa: Scient. Information and Publ. Branch, 2001.

Vezie C, Rapala J, Vaitomaa J, Seitsonen J, Sivonen K. Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis* strains and on intracellular microcystin concentrations. Microbial Ecology 2002; 43: 443-454.

Vincent RK, Qin XM, McKay RML, Miner J, Czajkowski K, Savino J, et al. Phycocyanin detection from LANDSAT TM data for mapping cyanobacterial blooms in Lake Erie. Remote Sensing of Environment 2004; 89: 381-392.

Wang HJ, Wang HZ. Mitigation of lake eutrophication: Loosen nitrogen control and focus on phosphorus abatement. Progress in Natural Science 2009; 19: 1445-1451.

Wang M, Shi W. The NIR-SWIR combined atmospheric correction approach for MODIS ocean color data processing. Optics Express 2007; 15: 15722-33.

Watson SB, McCauley E, Downing JA. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. Limnology and Oceanography 1997; 42: 487-495.

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

Wilhelm SW, Farnsley SE, LeCleir GR, Layton AC, Satchwell MF, DeBruyn JM, et al. The relationships between nutrients, cyanobacterial toxins and the microbial community in Taihu (Lake Tai), China. Harmful Algae 2011; 10: 207-215.

Williams CD, Burns JW, Chapman AD, Pawlowicz M, Carmichael W. Assessment of cyanotoxins in Florida's surface waters and associated drinking water resources. Final Annual Report to the Florida Harmful Algal Bloom Task Force. Florida Marine Research Institute, St. Petersburg, Florida, 2006.

Wilson DC. Quagga mussels and their effects on the aquatic system. Prepared for the Colorado River Regional Sewer Coalition, June 2008 2008.

Wojtal-Frankiewicz A, Frankiewicz P. The impact of pelagic (*Daphnia longispina*) and benthic (*Dreissena polymorpha*) filter feeders on chlorophyll and nutrient concentration. Limnologica 2011; 41: 191-200.

Wood SA, Dietrich DR. Quantitative assessment of aerosolized cyanobacterial toxins at two New Zealand lakes. Journal of Environmental Monitoring 2011; 13: 1617-1624.

World Health Organization. Guidelines for safe recreational water environments. Geneva, 2003.

Wynne TT, Stumpf RP, Briggs TO. Comparing MODIS and MERIS spectral shapes for cyanobacterial bloom detection. International Journal of Remote Sensing 2013; 34: 6668-6678.

Xie L, Rediske RR, Hong Y, O'Keefe J, Gillett ND, Dyble J, et al. The role of environmental parameters in the structure of phytoplankton assemblages and cyanobacteria toxins in two hypereutrophic lakes. Hydrobiologia 2012; 691: 255-268.

Xu H, Paerl HW, Qin BQ, Zhu GW, Gao G. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnology and Oceanography 2010; 55: 420-432.

Xu H, Zhu GW, Qin BQ, Paerl HW. Growth response of *Microcystis* spp. to iron enrichment in different regions of Lake Taihu, China. Hydrobiologia 2013; 700: 187-202.

Ye C, Shen ZM, Zhang T, Fan MH, Lei YM, Zhang JD. Long-term joint effect of nutrients and temperature increase on algal growth in Lake Taihu, China. Journal of Environmental Sciences-China 2011; 23: 222-227.

Yen HK, Lin TF, Liao PC. Simultaneous detection of nine cyanotoxins in drinking water using dual solid-phase extraction and liquid chromatography-mass spectrometry. Toxicon 2011; 58: 209-218.

Younossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. Clinical Gastroenterology and Hepatology 2011; 9: 524-U109.

Yu SZ. Primary prevention of hepatocellular carcinoma. Journal of Gastroenterology and Hepatology 1995; 10: 674-682.

Zegura B, Straser A, Filipic M. Genotoxicity and potential carcinogenicity of cyanobacterial toxins - a review. Mutation Research-Reviews in Mutation Research 2011; 727: 16-41.

Zhu B, Fitzgerald DG, Mayer CM, Rudstam LG, Mills EL. Alteration of ecosystem function by zebra mussels in Oneida Lake: Impacts on submerged macrophytes. Ecosystems 2006; 9: 1017-1028.

Zhu XZ, Kong HL, Gao YZ, Wu MF, Kong FX. Low concentrations of polycyclic aromatic hydrocarbons promote the growth of *Microcystis aeruginosa*. Journal of Hazardous Materials 2012; 237: 371-375.

Appendix A: Statistical methods used in analysis

A.1 Hierarchical agglomerative cluster analysis

For ecologists, cluster analysis is used to find groups of similar community structures. Clustering helps bring out some features hidden in the complicated data. Ecologists have used multivariate statistical methods, such as cluster analysis to analyze species data for many decades. Hierarchical agglomerative clustering algorithms start by treating each object as a cluster of one and then the closest two objects are joined to form a new cluster. Then this process is repeated until there's only one cluster. Hierarchical analyses lead to clustering trees, of which the branching structure indicates the similarities among the objects (Borcard et al., 2011). The clustering tree shows the level where clusters were joined together, and the sites within each cluster.

A.2 Structural equation models

Structural Equation Modeling is a very general, very powerful analysis technique and a widely used tool for causal inference. Structural equation modeling allows examination

of a set of relationships between several variables (Ullman, 2006). Structural equation models can often visualize by path diagrams. Diagrams are fundamental to structural equation models because they present the hypothesized set of relations.

A.3 Spatial autoregressive models

Most observational data of species abundance data and environmental data are spatially autocorrelated. Failing to account for the spatial correlation may highlight spurious associations (Lennon, 2000). Statistical models such as ordinary least square regression (OLS) or logistic regression usually assume the residuals to be independent, which may not be proper for data that have obvious spatial patterns. Moran's I can be used to test spatial dependence of the residuals from multivariate linear regression. Several models have been developed to take spatial autocorrelation into consideration, such as conditional autoregressive model (CAR) and the simultaneously autoregressive model (SAR).

A.3.1 Simultaneously autoregressive model (SAR)

For normally distributed data, a SAR model can be used to account for the spatial autocorrelation (Dormann et al., 2007). Simultaneous autoregressive models assume that the response at one location can be partially explained by its neighborhood responses. One type of SAR, spatial error model assumes the autoregressive process is found in the

error term. This assumption is appropriate if spatial autocorrelation is not fully explained by the included explanatory variables. Spatial error model can be expressed as:

$$Y = X\beta + u,\ u = \lambda Wu + e \qquad \text{(A.1)}$$

In equation A.1, W is a spatial weight matrix describing the spatial linkage among spatial units, u is the error term and λ is the simultaneous autoregressive error coefficient.

A.3.2 Conditional autoregressive model (CAR)

Conditional autoregressive models can be used in normally distributed data as well as other distributions that can be used in generalizing linear regression, such as logistic models or Poisson models. CAR assumes the distribution of the value at any given location is conditional on the level of neighboring values. The covariance of the errors at two locations is a function of the distance between them. The covariance function of the distance can be exponential, power, spherical and others.

A.4 Negative binomial regression

Poisson regression has been widely used for modeling count data. However, Poisson regression requires the mean and variance to be equal for the data. The negative binomial regression is an alternative to Poisson regression that allows the mean and variance to be different. Negative binomial regression is widely used in epidemiological studies involving count data (Fang et al., 2012; Feudtner et al., 2001).

A.5 Bayesian regression

In recent years, there have been enormous advances in the use of Bayesian methodology for analysis of epidemiologic data. The task of Bayesian analysis is to build a model for the relationship between parameters and observed data, and then calculate the probability distribution of parameters conditional on the observed data. Recent developments in Markov chain Monte Carlo methodology facilitate the implementation of Bayesian analyses of complex data sets such as data with spatial autocorrelation (Dunson, 2001). The Markov chain Monte Carlo method is a simulation method for sampling from posterior distributions and computing the parameters of interest.

References

Borcard D, Gillet F, Legendre P. Numerical Ecology with R, 2011.

Dormann CF, McPherson JM, Araujo MB, Bivand R, Bolliger J, Carl G, et al. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. Ecography 2007; 30: 609-628.

Dunson DB. Commentary: practical advantages of Bayesian analysis of epidemiologic data. American Journal of Epidemiology 2001; 153: 1222-6.

Fang F, Fall K, Mittleman MA, Sparen P, Ye W, Adami HO, et al. Suicide and cardiovascular death after a cancer diagnosis. The New England Journal of Medicine 2012; 366: 1310-8.

Feudtner C, Hays RM, Haynes G, Geyer JR, Neff JM, Koepsell TD. Deaths attributed to pediatric complex chronic conditions: national trends and implications for supportive care services. Pediatrics 2001; 107: E99.

Lennon JJ. Red-shifts and red herrings in geographical ecology. Ecography 2000; 23: 101-113.

Ullman JB. Structural equation modeling: reviewing the basics and moving forward. Journal of Personality Assessment 2006; 87: 35-50.