ENVIRONMENTAL, SPATIAL AND TEMPORAL EFFECTS ON MICROBIAL COMPOSITION IN LAKE ERIE

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By

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CHAPTER 1: General Introduction

Microorganisms are small in size; however, they are exceptionally abundant and ubiquitous in diverse environments. For every milliliter of lake or seawater there is approximately 1 million bacteria, and for every gram of soil there is approximately 1 billion bacteria. Collectively, microorganisms account for about half of the total biomass on earth (Avesaath and Pinto, 2009). Microorganisms mediate numerous biogeochemical processes and are key regulators for global carbon, nitrogen and phosphorus cycling.

Therefore, microorganisms are often viewed as the liaisons of recycling nutrients and remineralization of organic matter in aquatic systems. In addition, microbes also play a key role in processes that make carbon and energy available to higher trophic levels (Burns and Galbraith, 2007 and Cotner and Biddanda, 2002). This “microbial loop” (Azam et al., 1983) has failed to be incorporated in the classic food web despite having changed the paradigm of food webs. Consistent with the large number of different functions that microbes carry out, their taxonomic compositions are highly diverse in natural environments. Studies have revealed significant correlations between the structures and diversity of microbial communities with the conditions of environments they inhabit (Lindström, 2000; Pommier et al., 2007; Wei et al., 2008; Fuhrman, 2009).

Factors that Regulate Microbial Community Structures

In aquatic environments, major factors that drive the changes of the structure and diversity of bacterioplankton communities include a number of abiotic factors, such as pH, water temperature, water chemistry, nutrient concentrations and spatial differences (Yannarell et al.
2003; Lindström et al., 2005; Wei et al., 2008). Water column mixing and dissolved oxygen concentrations have also been found to influence structures and diversity of bacterioplankton communities (Shade et al., 2010). Spatial variations of these factors often result in changes of microbial community structures across different lakes (Lindström, 2000; Van Der Gucht et al., 2001; Yannarell and Triplett, 2005). A study that examined 30 northern and southern Wisconsin lakes observed spatial variations according to lake location (North and South), type (drainage vs seepage), pH and water clarity (Yannarell and Triplett, 2005). Another study by Lindström et al. (2005) examined bacterial community compositions across 15 temperate lakes in northern Europe and identified a different set of environmental factors (including pH, hydrological retention time and water temperature) to be most important in regulating bacterial community structures.

Seasonal transition can also be profound in changing environmental conditions, and in turn the microbial community structures and diversity. This is especially evident in temperate lakes, which typically experience big changes in water temperature during the spring and summer months and high nutrient loading from the watershed during spring flooding events (Lymer et al., 2008). Seasonal fluctuations of bacterial community structures have been found to be correlated with water temperature, dissolved organic carbon (DOC), ammonia, nitrogen and phosphorus loading, increased Chl a concentrations, pH, and hydrological retention time (Crump and Hobbie, 2005; Lindström et al., 2005; Yannarell and Triplett, 2005; Boucher et al., 2006; de Figueiredo et al., 2010; Giaramida et al., 2013).

In addition to natural spatial and temporal changes to environmental conditions, anthropogenic impact, such as watershed land-use practices, can significantly change water quality. For example, nutrient loading from agriculture- or industry-impacted areas in Otago,
New Zealand typically hosted different bacterial taxa found in 45 different water bodies (swamps, ponds, riverine wetlands, estuaries, reservoirs, shallow lakes and deep lakes) (Burns and Galbraith, 2007). The trophic status of lakes reflects the level of labile nutrient availability and has been found to impact both microbial community structures and diversities (Lindström, 2000; Yannarell et al., 2003; Eiler and Bertilsson, 2004). Oligotrophic lakes generally experience a higher microbial diversity than eutrophic systems and have a high relative abundance of heterotrophic bacteria to material and energy fluxes, while in eutrophic systems, high relative abundance of heterotrophic bacteria to phytoplankton is more important (Cotner and Biddanda, 2002). *Actinobacteria* and *Betaproteobacteria* dominate oligotrophic lakes (Newton et al., 2011). Eutrophication status changes bacterial community compositions by augmenting the growth of *Cyanobacteria* and *Actinobacteria* (Eiler and Bertilsson, 2004). One study by de Figueiredo et al. (2010), reported that eutrophic bacterioplankton communities are adapted to high water temperature, pH, conductivity, soluble reactive phosphorus (SRP) and chlorophyll-*a* (*Chl a*) concentrations. *Actinobacteria*, *Betaproteobacteria*, *Alphaproteobacteria* and *Cyanobacteria* might dominate such communities.

In addition to the above environmental factors that provide bottom-up controls, the activity and structure of bacterial communities are also closely regulated by the “top-down controls” from their grazers (Scheffer et al., 1993; Muylaert et al., 2002). Grazing by zooplankton has been found to decrease bacterial diversity and bacterial abundance in Lake Plußsee (Germany) (Höfle et al., 1999). Scheffer et al. (1993) have observed that shallow lakes can be clear (oligotrophic) by high zooplankton grazing on phytoplankton biomass or they can be turbid (eutrophic) with high phytoplankton biomass due to failure of zooplankton community’s regulation on primary productivity.
CyanoHAB Impacted Environments

Eutrophication of lakes, ponds and bays can lead to establishment of harmful cyanobacteria (blue-green algae) blooms (CyanoHABs) during warm weather. CyanoHABs have become a problem that plagues most surface water systems of the world (Bláha et al., 2009; Okano et al., 2009). Increases in nitrogen and phosphorus input due to anthropogenic eutrophication, along with global climate change issues such as increasing temperature, higher atmospheric carbon dioxide concentrations and elevating ultra-violet instabilities all contribute to the development of CyanoHABs (Beardal and Raven, 2004; Bláha et al., 2009). Bloom formations can also be augmented by other factors such as decreased turbulence, stagnant water, and increased pH (Codd et al., 2005; Bláhová et al., 2008).

In freshwater systems, cyanobacteria blooms commonly consist of Microcystis, Anabaena, Nodularia, Aphanizomenon, Plankothrix and Nostos, which all have the ability to produce cyanotoxins (Carmichael, 1992; Edwards et al., 2008). Studies have revealed that at least 70% of CyanoHABs are toxin-producing (Ho et al., 2006; Okano et al., 2009). Major groups of cyanotoxins produced by CyanoHABs include anatoxins, microcystins, nodularins and saxitoxins (Carmichael, 1997; Hitzfeld et al., 2000; Landsberg, 2002). Production of specific toxins tend to be limited to one or a few species (Glibert et al., 2005). It is also found that some species can be toxic in one location and non-toxic in another (Glibert et al., 2005), probably due to differences in toxin production gene sequences or their gene expression activities (Dittmann et al., 1996; Landsberg, 2002). Both toxic and nontoxic strains exist within species found in certain cyanobacterial genera, such as Anabaena, Microcystis, Plankothrix, Aphanizomenon, and Nodularia (Sellner et al., 1994; Tillett et al., 2000; Lyra et al., 2001; Landsberg, 2002; Rouhiainen et al., 2004; Glibert et al., 2005; Christiansen et al., 2008). Toxin producing strains
have been found to adapt better to environmental conditions than non-toxin producers; specifically, increased UVB radiation and nitrogen limitation play a role in the success of toxin producers (Ding et al., 2013; Ermilova and Forchhammer, 2013; Boopathi and Ki, 2014). However, environmental conditions that favor toxic strains over non-toxin producing strains of cyanobacteria are still unclear.

The most pervasive cyanotoxins found in aquatic systems are microcystins (MCs) (Welker and Von Döhren, 2006; Lawton et al., 2011). MCs are monocyclic heptapeptide hepatotoxins. MCs have five determined non-protein amino acids and two variable protein amino acids (Bourne et al., 1996). Over 90 MC variants have been identified (Bourne et al., 1996; Ho et al., 2006; Bláha et al., 2009; Okano et al., 2009). The most studied MC variant is microcystin-LR (MC-LR) for its high occurrence in water and it is considered the most toxic (Carmichael, 1997; Hitzfeld et al., 2000; Landsberg, 2002). Specifically, MC-LR contains leucine and argenine in the variable protein amino acid positions (See Figure 1) (Lone et al., 2015). MC-LR toxicity mechanism is the inhibition of serine/threonine protein phosphatases, which leads to cytoskeletal degradation and cytolysis of hepatocytes and promotion of tumors (Honkanen et al., 1990; MacKintosh et al., 1990; Dietrich and Hoeger, 2005; Bláha et al., 2009). The World Health Organization has set 1μg L⁻¹ of MC-LR as the limit in drinking water and 20 μg L⁻¹ for recreational water (WHO, 1998).

**Consequences of CyanoHABs**

Exposure to cyanotoxins can occur through drinking water, skin absorption or consumption of toxin producing cells (Camargo and Alonso, 2006), which can cause skin irritation, gastrointestinal illness and, at high levels, tumor promotion in livers. Chronic exposure to low traces of MCs in drinking water has been linked to liver tumors (Okano et al., 2009).
to the blooming of cyanobacteria in drinking water reservoirs, MC-contaminated drinking water has become a global issue (Okano et al., 2009). A MC contamination accident of water used for dialysis led to the deaths of 50 patients at the Brazil Dialysis Center (Jochimsen et al., 1998). The 1979 outbreak of Palm Island Disease, has also been linked to contaminated drinking water by algal toxins (Hawkins et al., 1985).

As a consequence to health advisories, recreational water use is also impacted by the CyanoHABs, such as fishing, swimming and boating, which can negatively impact local economics. For example, Grand Lake St. Mary’s (Celina, Ohio), a lake that has been heavily affected by CyanoHABs and MCs, economy has been significantly impacted, recently. Many local small businesses, marinas and boat dealers have been forced to close, since recreational activities during strong bloom events were highly discouraged. Since 2009, Grand Lake St. Mary’s State Park has experienced about 250,000 dollars decline in park revenue, while the surrounding area’s revenue has experienced a 35-45 million dollars decrease (Davenport and Drake, 2011).

CyanoHABs also have significant ecological impacts, including changes in biodiversity, increased water turbidity, which limits light availability for benthic primary producers (diatoms), toxin bioaccumulation in organisms in higher trophic levels, disturbances in biotic relationships and changes in oxygen concentrations (Bláha et al., 2009). Many zooplankton species have selective grazing that favor non-harmful algal bloom competitors, although CyanoHAB species may not always release toxins (Bouvy et al., 2001). Zooplankton preferentially graze on the non-HAB algae, but due to competition for limiting nutrients, CyanoHAB species become nutrient stressed thus triggering toxin production (Bouvy et al. 2001 and Mitra & Flynn 2006). Zooplankton grazing fails to control the CyanoHAB species due to these toxins, therefore
CyanoHAB growth goes unchecked and community diversity is lost (Bouvy et al., 2001). These uncoupled top-down and bottom-up controls on cyanobacteria may be influencing community structure and the microbial loop, by increasing bacterial biomass (Bouvy et al., 2001; Muylaert et al., 2002; Mitra and Flynn, 2006). CyanoHABs can also affect organisms that are not immediately involved in predator-prey interactions with cyanobacteria. Heterotrophic bacteria also experience a bloom due to CyanoHABs high production of organic carbon that eventually leads to decreased oxygen levels, which can also be the driver of decreased diversity (Eiler and Bertilsson, 2004).

Decreases in bacterioplankton, phytoplankton and zooplankton diversity have been recorded during a toxin producing CyanoHAB. This may be caused by direct exposure to the toxins (Christoffersen et al., 2002), or chemical signaling and growth inhibitors/stimulators that are associated with these blooms (Giaramida et al., 2013). Responses, such as alternate feeding patterns at different trophic levels may also explain these decreases in community diversity, which leads to a distorted food web (Kaebernick et al., 2000). Invertebrates, such as mussels, cladocerans and copepods have suffered from toxic CyanoHABs via decreased feeding and decreased fecundity thus affecting their life history. Zooplankton and fish have been observed to avoid highly concentrated harmful algal blooms (Fiedler, 1982).

Due to bioaccumulation and bio-magnification of cyanotoxins (Camargo and Alonso, 2006), significant mortalities of fish have been found in CyanoHAB impacted freshwaters, including blue gill, bream, carp, catfish, eel, perch, pike, and trout (Landsberg, 2002). Berry et al. (2008) recorded that MC concentrations in Lake Erie yellow perch livers were 125 times higher than muscle tissue per unit dry weight. For a human who consumes fish, the WHO suggests no more than 0.04 µg of MC per kilogram of body weight per day (Bartram, 1999). The
EPA suggests that no higher than 0.003 μg of MC per kg of body weight per day should be consumed (EPA 2006). Berry et al. (2008) found that yellow perch muscle tissue was safe to consume during bloom events, but to avoid consumption of livers due to toxin build in fatty tissues.

All these negative impacts of CyanoHABs on individual groups of organisms can collectively destruct the food web structures of freshwater lakes. Sometimes, the consequence can even be seen beyond freshwater systems. Recently, there have been incidences where Californian sea otters have had hepatic damage due to MC poisoning caused by MC toxin build up in lower trophic levels due to freshwater runoff entering marine interfaces (Miller et al., 2010).

**Mechanisms for Microcystin Removal**

Due to their cyclic structures and presence of unusual amino acids, MCs are highly stable and naturally resistant to stresses, such as pH, temperature, sunlight and certain enzymes under natural ranges (Carmichael, 1994; Rapala et al., 2005; Okano et al., 2009). Immense advances in existing treatments that are efficient in MC removal, such as ozonation and UV radiation, are all involved in high energy cost (Edwards et al., 2008), and successful MC degradation is compromised under activated carbon in hyper-eutrophic water bodies (Lawton and Robertson, 1999). The activation of carbon absorption and ozone oxidation effectively removes MCs, but high load of organic matter in source water, such as those in eutrophic lakes, can diminishes the effectiveness of this method (Ho et al., 2006). As the world-wide demand for water increases, so does the threat of contamination of cyanobacteria toxins, which pushes for a less expensive, and more efficient clean water solution (Edwards et al., 2008; Manage et al., 2009).
Biodegradation is a less expensive, safer way to help render a solution to MC contamination (Edwards et al., 2008). And in fact, it is the most important mechanism for MC removal in natural environments (Christoffersen et al., 2002b; Lemes et al., 2008). Research focusing on microbial communities associated with this natural MC degradation could be used in future remediation efforts. However, research on MC-degrading bacterial community is limited. Available studies on MC-degrading bacteria are mostly culture-dependent. A small number of MC-degrading bacteria have been isolated and most of them belonging to Sphingomonos (Jones et al., 1994; Bourne et al., 1996; Park et al., 2001; Saito et al., 2003; Ishii et al., 2004; Ho et al., 2006; Edwards et al., 2008). Rapala et al. (2005) reported 13 strains of a new novel genus and species, Paucibacter toxinivorans, which have the capabilities to degrade MCs and nodularins. Using the BioLog MT2 assay, Manage et al. (2009) has isolated 10 strains of MC-LR degrading Actinobacteria. Using the isolates as model systems, genes that are associated with MC degradation have been identified (Bourne et al., 1996). A gene cluster of four genes (mlrABCD) is involved with the MC degradation pathway. The gene, mlrA, encodes for a transporter protein that enables the uptake of MCs into the cell where three enzymes are sent to degrade the MCs into secondary metabolites (Bourne et al., 1996, 2001). The mlr genes have been detected in most of the available MC-degrading bacterial isolates, however, in strains of MC-degrading Actinobacteria, PCR amplification of mlr genes yielded negative results, suggesting diversity in MC degradation pathway (Manage et al., 2009).

Studies on bacterial communities’ natural ability in MC removal are limited. Indirect measurement of MC degradations by using ELISA kits and HPLC are effective along with the use of flow cytometry or MT2 BioLog plates are effective way to test whether or not MC-degrading bacteria communities have been grown in the lab (Rapala et al., 2005; Manage et al.,
Mou et al. (2013) found no homologs to the \textit{mlr} genes in their Lake Erie MC-amended methods, suggesting alternative MC degrading genes and pathways. Additionally, the Mou et al. (2013) study found a highly diverse community of MC-degraders. Historical exposure to MCs is a significant driver in bacterioplankton communities (Giaramida et al., 2013).

\textbf{General Objectives and Hypothesis}

One objective of this thesis was to examine how bacterioplankton and zooplankton communities tracked environmental changes in Lake Erie. Generally, it is expected that bacterioplankton and zooplankton community structure and diversity will shift temporally due to seasonal fluctuations in physico-chemical parameters. It is also expected that these community structures and diversity will reflect trophic status of the three major habitats represented along this natural nutrient gradient.

A second objective was to examine the impacts of elevated MCs to the free-living bacterioplankton community structure and diversity during CyanoHAB events. It is expected that both lakes selected will experience free-living bacterioplankton community structure and diversity changes and evidence of MC-LR degradation upon increased supply of MCs, due to previous microcystin exposures from past CyanoHABS in both lakes.

\textbf{Study Area}

Two Ohio lakes stand out for their problems with CyanoHABs: one is a man-made reservoir, Grand Lake St. Mary’s, and one is a natural lake, Lake Erie. Anthropogenic effects, such as agricultural runoff and fertilizers, are thought to be major contributors to these CyanoHABs (Rinta-Kanto \textit{et al.} 2005). However, cyanobacterial blooms of these two lakes have distinct features: typical Grand Lake St. Mary’s CyanoHAB events are long and persistent
occurring in a very shallow waters (2 m deep) over the course of the summer (Davenport and Drake, 2011), while Lake Erie’s CyanoHAB events are known to exhibit monthly shifts in cyanobacterial genera (Davis et al., 2015). Grand Lake St. Mary’s CyanoHAB species include *Aphanizomenon sp.* and *Microcystis sp.* (Davenport and Drake, 2011), while Lake Erie is known to have *Anabaena sp.*, *Aphanizomenon sp.*, *Planktothrix sp.* (Western Basin), and *Microcystis sp.* (Sandusky Bay) (Chaffin et al., 2013; Davis et al., 2015).

Lake Erie, the smallest and shallowest of the Laurentian Great Lakes, provides recreational, commercial and drinking water needs to 11.5 million people (Internation Joint Commission, 2014; Davis et al., 2015). Lake Erie is composed of three different basins: the Western Basin, Central Basin and Eastern Basin (Charlton et al., 1999; Rattan et al., 2012). In addition, due to unique nutrient concentrations, plankton abundances and circulation, the western part of the Central Basin is frequently denoted as Sandusky Sub-basin (León et al., 2005). Nutrient loading impacts Lake Erie extensively due to its size and depth (Davis et al., 2015). There is a strong nutrient gradient within Lake Erie running hyper-eutrophic to oligotrophic from West to East; highest nutrient concentrations and plankton abundances are found in the shallow Western Basin (mean depth 10 m) when compared with the deeper Central Basin (mean depth 20 m) and Eastern Basin (mean depth 47 m) (Charlton et al., 1999; León et al., 2005). Additionally, there is a decreasing phosphorus gradient from West to East within the lake (Petterson and Jansson, 1978). Lake Erie’s highest nutrient load is received in the Western Basin via the Detroit and Maumee Rivers (Charlton et al., 1999; Rattan et al., 2012). Implementation of the Great Lakes Water Quality Agreement introduced phosphorus reduction programs in 1972 and improved water quality in the 1970s and 1980s (Bertram, 1993). Nevertheless, increasing occurrences of CyanoHAB events have once again become an issue for Lake Erie since the
One cause that has been linked to these blooms is agricultural run-off (Stumpf et al., 2012), which leads to the debate about how managers should also take in account the short term effects nitrogen has on Lake Erie’s ecosystem (Davis et al., 2015). Additionally, factors involved with global climate change such as increased storm events, fluctuations in solar intensity and wind patterns also increase CyanoHAB occurrences.

Grand Lake St. Mary’s is Ohio’s largest inland lake and serves as a drinking water supply for the city of Celina, Ohio. The lake is 5,500 hectares in area and averages to 1.5-2 meters deep. Historically, Grand Lake St. Mary’s has an interesting land-use history. The reservoir was made from the years 1837-1845 to maintain the 1.5 meter depth of the Miami-Erie Canal (Davenport and Drake, 2011). Additionally, the surrounding area was drilled for oil and gas during an oil boom in western Ohio from mid 1880s to the early 1910s (Wells, 2007). Currently, the Ohio Environmental Protection Agency had deemed the reservoir “impaired” due to multiple surrounding land-use issues such as loss of floodplains and riparian vegetation, agricultural run off and sewage runoff from septic systems and the channelization of tributaries (Davenport and Drake, 2011).

**Brief Thesis Outline**

Within the following thesis, two separate studies were conducted to fill knowledge gaps about how nutrients affect bacterioplankton and zooplankton community structure and diversity spatially and temporally. The first study (Chapter 2) examined how the free-living bacterioplankton community structure and diversity track environmental changes along a natural nutrient gradient in Lake Erie for three months. Additionally, zooplankton communities (rotifers and microcrustaceans) along that transect (highlighting the three major basin habitats) were studied to understand how nutrients and the free-living bacterioplankton affect zooplankton
community structure and diversity. The second study (Chapter 3) delved deeper into the intricate roles nutrients and CyanoHABs have on free-living bacterioplankton community structure and diversity by testing the impacts of elevated MCs on the free-living bacterioplankton community structure and diversity in lakes with historical CyanoHAB exposure. These two studies will contribute to filling in the knowledge gaps of how free-living bacterioplankton and zooplankton community structure and diversity track environmental changes that are connected to CyanoHABS.
Figure 1: Microcystin-LR structure, modified after Lone et al. (2015).
References


Chapter 2: The Effects of Nutrient Variability on Microbial and Zooplankton Community Compositions Along a Transect in Lake Erie

Abstract

Through close interactions with biotic and abiotic environments, microbial communities in natural lakes mediate numerous biogeochemical processes that are essential in regional and global cycles of C, N and P. However, the relationship between bacterial community compositions and environmental conditions is still unclear. Lake Erie's natural gradient of nutrient supply and many other environmental parameters from the Sandusky Bay to the central basin provides an ideal experiment to examine how well bacterial community composition tracks environmental changes. In summer 2012, surface water samples were collected along a transect that ran from the Sandusky Bay (hypereutrophic) via Sandusky Sub-basin (mesoeutrophic) to the Central Basin (oligotrophic) for the months of June, July and August. Physico-chemical parameters including temperature, pH, conductivities and concentrations of ammonium, dissolved organic carbon, dissolved nitrogen, nitrate, nitrite and soluble reactive phosphorus were measured for each sample. Bacterioplankton and zooplankton community structures were examined by 16S rDNA-based terminal restriction fragment length polymorphism (T-RFLP) analysis and microscopic identification, respectively. Results showed that the free-living bacterioplankton structure differed significantly among sampling time, which was likely contributed by temporal variations in nutrient concentrations. As for the zooplankton community, Cyclopidae, Branchionidae and Synchaetidae were identified as major families
(>78.4% of total zooplankton) in all samples. Zooplankton family structure had no clear separation based on site location on site location or sampling time. In addition, no significant correlation was identified between zooplankton community structure and environmental parameters or with zooplankton community structure and bacterioplankton community structure. Zooplankton diversity tests revealed significant differences in zooplankton diversity among sites and months. This research contributes a better understanding of the zooplankton and bacteria community structure found in lake Erie.

**Introduction**

Freshwater microbial communities facilitate cycling and re-mineralization of nutrients through fitted relationships with biotic and abiotic environments on a regional and global scale. However, the relationship between the diversity and composition of microbial communities and environmental conditions is still unclear. A multitude of studies have indicated that spatial and temporal variations in abiotic factors influence bacterial community structure, compositions and diversity (Muylaert et al., 2002; Yannarell et al., 2003; Van der Gucht et al., 2007; Lymer et al., 2008; Wei et al., 2008; Mueller-Spitz et al., 2009; Tian et al., 2009). However, these environmental variations have not been well studied to reveal how zooplankton are affected and how bacterial community diversity and zooplankton community diversity interact and respond to environmental variations.

Lake Erie, the smallest and shallowest of the Laurentian Great Lakes is naturally divided into three basins: Western Basin, Central Basin and Eastern Basin (Davis et al., 2015). Lake Erie’s largest bay, Sandusky Bay (SB), connects the Sandusky river to the Western Basin of Lake Erie (Hwang and Heath, 1999). Due to this connection between the Sandusky River and Western Basin, nutrient loading (P and N) in the Western Basin, especially in the Sandusky Bay
areas lead to a hypereutrophic state characterized by harmful cyanobacterial blooms (Davis et al., 2015). Additionally water depth follows a natural gradient from the Western Basin to the Central Basin, where that the Western Basin (2 meters) is shallower than the Central Basin (40 meters), which would contribute to the presences of cyanobacterial blooms (Richards et al., 2001). Often, the western portion of the Central Basin is referred to as a sub-basin (Sandusky Sub-Basin) due to it’s distinctive features including nutrient concentrations, water circulation patterns, phytoplankton composition and depth (León et al., 2005). Due to heavy nutrient loading and unique physical aspects (circulation, size, depth) of Lake Erie, there is a strong nutrient gradient running hypereutrophic to oligotrophic in from West to East (Charlton et al., 1999; León et al., 2005; Davis et al., 2015).

Along with spatial variation of these environmental conditions, seasonal transition in temperate lakes has a great effect on changing abiotic conditions thus influencing microbial community structure and diversity (Lymer et al., 2008). Nutrient loading and mixing in the spring and drastic water temperature increase are typically observed in temperate lakes and have been found to affect the diversity and structure of bacterial community to a various extent (Crump and Hobbie, 2005; Lindström et al., 2005; Yannarell and Triplett, 2005; Boucher et al., 2006; de Figueiredo et al., 2010). Lake Erie’s Western Basin bacterioplankton community was found to be more diverse than Lake Erie’s Sandusky Bay bacterioplankton community, representing the sensitivity diversity measurements have when comparing two areas of the lake that had different in environmental parameters (Mou et al., 2013). Additionally, bacterioplankton community structure and diversity are linked to lake trophic status; bacterial community in eutrophic versus oligotrophic lakes typically have different structures and diversity and respond to temporal environmental changes differently. For example, bacterioplankton communities
associated with eutrophic states have high relative abundance of species that are adapted to live in environmental conditions that include increased water temperature, pH, conductivity, soluble reactive phosphorus (SRP) and chlorophyll \( a \) (Chl \( a \)) concentrations (de Figueiredo et al., 2010), such as \textit{Gammaproteobacteria}. Sandusky Bay’s hypereutrophic conditions have been indicative of inhibited bacterioplankton diversity, due to the loss of ecological niches (Mou et al., 2013), since it is thought that optimum bacterioplankton diversity is reached at an intermediate level of nutrient concentrations (Hewson and Fuhrman, 2004). In contrast, oligotrophic lakes generally experience a higher microbial diversity (Cotner and Biddanda, 2002).

A few general trends have been connected to temporal and spatial environmental fluctuations with zooplankton community structure and diversity. The microbial role as a carbon source (DOC and POC) available for higher trophic levels has been emphasized in the microbial loop (Azam et al., 1983). Seasonal variations of phytoplankton and zooplankton can cause a decrease in microbial diversity in lakes due to highly intense grazing pressure (Sommer et al., 1986). Decreased bacterial community diversity attributed to high grazing pressure was correlated with the following environmental parameters: light intensity, water temperature, wind and nutrient concentrations (Höfle et al., 1999). Overshadowed by their cyanobacteria counterparts, zooplankton diversity studies have not been well documented in Lake Erie. Only a few studies have focused on various aspects of zooplankton communities found in Lake Erie. One study by Therriault et al. (2002) examined the non-native \textit{Cercopagis pengoi}, which has expanded its range to Lake Erie. Additionally, another study by Kane et al. (2004) has documented the repopulation of the calanoid copepod, \textit{Limnocalanus marcrurus}, in the Western Basin of Lake Erie due to decline in phosphorus concentrations and higher DO concentrations. Reutter and Reutter (1975) found that top rotifer abundances were in June, decreased through
December and, additionally, found that cyclopoid copepods were unchanged over the summer months in the Western Basin of Lake Erie. Hwang and Heath (1999) conducted a study on zooplankton bactivory in Lake Erie and found that the dominant rotifer group found and all cladocerans sampled were bacterivorous, while all copepods were not.

Lake Erie's natural gradient of nutrient supply and many other environmental parameters from the Sandusky Bay (hypereutrophic) via Sandusky Sub-basin (mesoeutrophic) to the Central Basin (oligotrophic) provides an ideal experiment to examine how well bacterial community composition tracks environmental changes. The following study’s objective was to determine how well microbial community (bacterioplankton and zooplankton) structures and diversity track environmental changes spatially and temporally. In addition, this study also aimed to identify potential correlation between free-living bacterioplankton community structure and diversity and zooplankton community structure and diversity. We predicted that due to temporal and spatial variability, community structure and diversity of bacterioplankton and zooplankton would vary along the transect from Sandusky Bay to the Central Basin of Lake Erie.

**Methods**

*Study Site/Field Methods*

Surface water samples were collected at 0.5 m below the air-water interface in Lake Erie (LE) once a month in June, July and August 2012 (Figure 2). The five sampling sites were located along a transect which ran from Sandusky Bay (typically hypereutrophic) through the Sandusky Sub-basin (mesoeutrophic) to the Central Basin (oligotrophic) of Lake Erie (Charlton et al., 1999; León et al., 2005). Surface water was collected using a peristaltic pump and stored in 1 L bottles on ice in a cooler until brought back to the laboratory where water was stored at 4 °C.
Zooplankton samples also were collected from one site of each basin, including Site 1 of Sandusky Bay (SB-1), Site 3 of Sandusky Sub-basin (SSB-3) and Site 5 of the Central Basin (CB-5). Zooplankton were sampled using a zooplankton net (50 µm mesh size). Water (4.2 m³) was passed through the net as it was towed for 1 meter along the boat four times at 1 meter deep. Sample depth was selected to match the depth at which bacterioplankton communities were collected. Zooplankton samples were preserved using Lugol’s fixative immediately after sampling and stored out of sunlight at room temperature until further processing and identification.

At each sampling, physico-chemical variables were measured in situ using a Sonde (YSI, 600OMS Optical Monitoring Sonde, Yellow Springs, OH). These variables included dissolved oxygen (DO), temperature (Temp), conductivity (Cond) and pH. Photic zone depth was estimated by using a Secchi disk.

Water Filtration and Processing

For each sampling site, surface water (750 mL; 250 mL for each triplicate) was collected and filtered through glass fiber filters (GFF; Whatman, Sigma Aldrich, St. Louis, MO) on site to collect Chlorophyll a (Chl a). Another set of surface water samples (1L) were sequentially passed through 3.0µm filters and then 500 mL of the 3.0µm (Pall Life Sciences, Port Washington, NY) filtrate was then passed through 0.22µm filters (Pall Life Sciences, Port Washington, NY) to capture the free-living associated bacterioplankton. The 0.22µm filtrate was used for nutrient analysis, which include total dissolved organic carbon (DOC), total dissolved nitrogen (TDN), NO₃⁻ + NO₂⁻, NH₄⁺ and soluble reactive phosphorus (SRP). Ammonium and NO₃⁻ + NO₂⁻ were measured using microplate reader-based colorimetric methods (Ringuet and Johnson, 2011), SRP was measured spectrophotometrically (Rodriguez et al., 1994) and TDN
and DOC were measure using a Scientific Instruments TOC5000 Analyzer (Shimadzu, Columbia, MD). All GFF and membrane filters were immediately cooled on ice and stored at -80°C in the lab for molecular analysis. Triplicates were taken for each sample.

**Zooplankton Processing and Identification**

Using a Folsom plankton splitter, fixed zooplankton samples from each site were divided into 3 sub-samples, each of which contained 200-400 individuals. Each individual of every sub-sample was identified down to the family level using a compound microscope following the *Pannek’s Guide to Freshwater Invertebrates* (Smith, 2001) and “An-Image-based Key to the Zooplankton of North America” (Haney et al., 2013). After identification of sub-samples was completed, sub-samples were back calculated to find the number of each family per cubic meter of water for each sub sample. Zooplankton were back calculated using the calculations found in the EPA’s “Standard Operating Procedure for Zooplankton analysis” (Environmental Protection Agency, 2003).

**DNA Extraction and Terminal Restriction Fragment Length Polymorphism Analysis**

The 0.22μm filters were used to extract free-living bacterioplankton DNA using the MO BIO PowerSoil DNA Isolation Kit (Mobio Inc, Carlsbad, CA). Extracted DNAs were then processed for 16S rRNA gene-based terminal restriction fragment length polymorphism (T-RFLP) analysis (Liu et al., 1997). Briefly, polymerase chain reaction (PCR) was performed to amplify bacterial 16S rRNA genes. Each 25 μl-PCR reaction contained 12.5μl of Taqman Mastermix, 9.9μl deionized water, 0.8μl of fluorescently labeled FAM 27F (10 mM) primer (5’-FAM-AGAGTTTGATCCTGGCTCAG-3’), 0.8μl of 1522R (10 mM) primer (5’-AAGGAGGTGATCCANCCRCGA-3’) and 1.0 μl of DNA template. The PCR reaction program had the initialization temperature of 95°C for 3 minutes; each PCR reaction was cycled
for 30 times, with a denaturation temperature at 95 °C for 30 seconds, an annealing temperature at 58 °C for 1 minute and an elongation temperature of 72 °C for 1 minute. Once the PCR reactions went through the 30 cycles they were exposed to the final elongation step at 72°C for 10 minutes. Each sample’s PCR amplification was conducted in technical triplicates and then were pooled together and purified using a MO BIO UltraClean GelSpin DNA Purification kit (Mobio Inc, Carlsbad, CA) following the manufacturer’s instruction.

The purified PCR product (17.3 μl) was mixed with HaeIII (0.5μl), 10× buffer (2μl) and bovine serum albumin (BSA) (0.2μl) for each sample. Samples were digested at 37°C for three hours. To purify digested PCR amplicons, each DNA digest (18 μl) was added to deionized sterile water (70 μl), 7.5M ammonium acetate (10 μl), and dilute glycogen (1μl). Cold absolute ethanol (300 μl) was then added, and samples were stored in a -20 °C freezer overnight. DNA digests were then centrifuged at 15,000×g for 15 minutes at 4°C. Once DNA was cleaned with 70% ethanol twice, samples were left to dry out for 10 minutes at room temperature. Purified deionized water (13 μl) was then added to re-suspend DNA and sample were sent to the Plant-microbe Genomics Facility (PMGF) at The Ohio State University, Columbus, Ohio for further fragment analysis using Gene Mapper.

Statistical Analysis

R Script for T-RFLP analysis was conducted for the removal of noise signals, binning and aligning peak values with raw T-RFLP data set from PMGF Gene Mapper analysis (Abdo et al., 2006; Fetzer, 2009). Principal component analysis (PCA) was conducted using R based on nutrient concentration (NO₃⁻ + NO₂⁻, SRP, TDN, DOC and NH₄⁺) and physico-chemical variables (T, DO, conductivity and pH) data to produce a PCA biplot to interpret what environmental parameters were correlated for each site. Non-metric multidimensional scaling
(NMDS), an ordination method, was conducted to display what pattern separates the bacterioplankton T-RF community structure, zooplankton family community structure and zooplankton functional feeding group community structure for each site based spatial (basin) or temporal (month) factors and an analysis of similarities (ANOSIM) was used to determine whether that pattern was significant. ANOSIM yields a coefficient (R) that ranges from 0 to 1, where that ANOSIM coefficient value interpretation includes the following: $R_{\text{anosim}} > 0.75$ indicates a high separation between factors, $R_{\text{anosim}} > 0.5$ means some separation exists between factors and $R_{\text{anosim}} < 0.25$ indicates no separation of factors (Clarke and Warwick, 2001).

Analysis of variance test (ANOVA) was conducted to determine whether there was a significant difference in bacterial community and zooplankton community among samples. BIO-ENV analysis was used to find specific correlations between groups of abiotic and biotic factors, which explained patterns in bacterioplankton community structure and zooplankton community structure (Clarke and Warwick, 2001). Redundancy analysis (RDA) was performed to determine what environmental parameters were correlated for free-living bacterioplankton community structure and zooplankton community structure for each site. A Mantel test was used to test for correlation between zooplankton functional feeding group percent abundance with environmental factors measured.

Shannon-Weiner Diversity Index and Jaccard’s Index were used to measure diversity and evenness of zooplankton (based on zooplankton abundances calculated from sub-samples) and bacterioplankton (based on T-RFLP data). Multiple Mantel tests were conducted to test for correlation between free-living bacterioplankton community diversity with zooplankton community diversity, between free-living bacterioplankton percent abundance and zooplankton percent abundance (taxonomical unit) and between free-living bacterioplankton percent abundance.
abundance and zooplankton functional feeding group percent abundance. Bacterioplankton and zooplankton community structure profiles were compared to find a correlation between each other using Mantel tests comparing percent abundance, species diversity and zooplankton functional feeding groups percent abundance with bacterioplankton T-RF percent abundance, respectively. Environmental variable of samples were k means-clustered to form group sites where indicator species analysis (ISA) was used to determine if any zooplankton family or combinations of families were indicative of specific environmental parameters (Cáceres, 2013).

R statistics (Oksanen et al., 2015) was used to conduct PCA, RDA, Mantel Tests, regression analyses, Shannon-Weiner Indices, Jaccard Indices and Species Indicator Analysis. Primer5 (Clarke and Warwick, 2001) was used to conduct NMDS, ANOSIM and BIO-ENV analysis.

Results

Environmental Variable Analysis

Mean physico-chemical parameters, including NO$_3^-$ + NO$_2^-$, SRP, Chl a, TDN, NH$_4^+$, DOC, water temperature, conductivity, DO and pH, were used for principle component analysis (PCA). On the PCA plot, samples were separated based on month, but not by sampling location (Figure 3). PC1 axis alone explained 32% of the total variance among samples; and TDN, NO$_3^-$ + NO$_2^-$, DOC, pH and Chl a were most strongly loaded on this axis (regression analysis, $p < 0.05$). TDN, conductivity, NO$_3^-$ + NO$_2^-$ and DO were abiotic factors that grouped June samples, which had higher mean values of these variables (TDN= 1.8 mg L$^{-1}$; Conductivity= 0.3 $\mu$S; NO$_3^-$ + NO$_2^-$ = 1.7 mg L$^{-1}$; DO=114.1%) than samples from the other months. July samples were characterized with high mean pH (8.3) and mean DO concentrations (115.5 %). August samples had the highest mean pH (9.0) and SRP (0.02 mg L$^{-1}$).
Although, no significant difference was found among sampling sites based on overall environmental conditions, some individual variables did show significant spatial variations. Mean Chl \(a\) concentrations were the highest in Sandusky Bay (SB) for each sampling month (June: 10.2 \(\mu g\) L\(^{-1}\); July: 50.4 \(\mu g\) L\(^{-1}\); August: 45.4 \(\mu g\) L\(^{-1}\); \(p < 0.05\)). Sandusky Sub-basin (SSB) and Central basin (CB) both had relatively low Chl \(a\) concentrations for June (SSB: 1.4 \(\mu g\) L\(^{-1}\); CB: 1.0 \(\mu g\) L\(^{-1}\)) and July (SSB: 12.4 \(\mu g\) L\(^{-1}\); CB 13.0 \(\mu g\) L\(^{-1}\)). For August samples, Chl \(a\) concentrations were highest (45.4 \(\mu g\) L\(^{-1}\)) in Sandusky Bay and lowest in the Central Basin (3.5 \(\mu g\) L\(^{-1}\)) (Table 1). Two-way analysis of variance (ANOVA) showed that Chl \(a\) concentrations were significantly different when compared across sites (\(p < 0.001\)), compared across time (months) (\(p < 0.001\)) and the interaction of site and month (\(p < 0.01\)). Additionally, Secchi depth measurements showed that water turbidity was increasing from Sandusky Bay sites (0.7 m) to Central Basin sites (4.6 m) (T-Test, \(p < 0.05\)).

**Free-living Bacterioplankton Community Analysis**

Terminal restriction fragment (T-RF) generated by T-RFLP analysis was used as a taxonomic operational unit roughly at the species level (Liu et al., 1997). T-RF percent abundance for each sample was used to calculate diversity using the Shannon-Weiner Index and to calculate eveness using the Jaccard Index (Table 2). The Shannon index for the SB June sample was the lowest of all samples, while the Shannon Index for SSB in the August sample was the highest of all samples. One-way ANOVA found that bacterial community diversity in our samples was not significantly different based on either month or site (\(p > 0.05\)). Jaccard Indices for the sites were between 70-90% evenness, suggesting a large number of T-RFs were shared by samples of different sites, or months. One-way ANOVAs confirmed that community
evenness of Lake Erie’s free-living bacterioplankton among samples did not differ significantly ($p > 0.05$).

NMDS on free-living bacterioplankton T-RF percent abundance showed site community structure shifting with time (June, July, and August) (Figure 4). ANOSIM further confirmed the separation of bacterial community structures by sampling months, but also revealed that there was some overlap of taxa occurring (July and August) (Global $R_{\text{anosim}} = 0.5; p < 0.05$). Additional pairwise comparisons showed significant differences of bacterioplankton community structure only between June and July samples ($R_{\text{anosim}} = 0.7; p < 0.05$). BIO-ENV analysis was conducted to investigate potential correlations between biotic data and environmental variables; 70% of the biological data variability was explained by patterns with $\text{NO}_3^- + \text{NO}_2^-$, DOC, pH, conductivity, $\text{NH}_4^+$ and DO ($R_{\text{bioenv}} = 0.7$). RDA ordination (Figure 5) of physical chemical parameters in relation to free-living bacteria T-RF patterns showed consistent results on the correlation between bacterioplankton community structure and variations of DO, TDN, $\text{NO}_3^- + \text{NO}_2^-$ and Cond for July samples (regression analysis, $p < 0.05$). However, RDA showed that LE samples in July were correlated with $\text{NH}_4^+$, pH and Chl $a$; in August were associated with DOC and SRP. ANOVA showed a significant difference in free-living bacterial communities based on these physical chemical parameters among months ($p < 0.05$).

### Zooplankton Community Analysis

A total of 17 zooplankton families were identified (Table 3). The family with the greatest number of individuals found in one sample was *Synchaetidae* (4,228.4 individuals/m$^3$) in August’s SSB sample. The most rare families found within the lake were *Lynceidae* (5.0 individuals/m$^3$) and *Lecanidae* (10.0 individuals/m$^3$). June samples had a total of 17 zooplankton families identified, July samples had a total of 13 families identified, and for August samples, 15
families were identified, respectively. Compared among sampling sites, SB and CB both had 17 total families identified, while SSB had 15 families identified. August SSB sample had the highest number of total individuals found of in all samples (7,211.8 individuals/m$^3$). June CB sample has the lowest number of total individuals found in all samples (733.5 individuals/m$^3$).

In June, community structure of zooplankton families was dominated by two major families: *Branchionidae* and *Cyclopidae*. *Branchionidae* made up 47% of SB site community structure, and 24% of SSB site, respectively. *Cyclopidae*, made up 11% of the total abundance for SB site, 32% of total abundance for SSB site and 28% of the totally abundance for the CB site. Additionally there was a high total abundance of *Daphinidae*, in the SB at (31%). A clear dominating community structure of zooplankton (*Branchionidae*, *Cyclopidae* and *Synchaetidae*) occurred in Lake Erie during July and August. In July, *Cyclopidae* made up 31-42%. For July sites, *Branchionidae* made up 15-25%. For August sites, *Branchionidae* dominated the SB total abundance at (55%), while also being the second most abundant zooplankton family in SSB (26%) and CB sites (38%). In July, *Synchaetidae* contributed to 31% of total abundance of the SSB site community and 39% of total abundance to CB community. August *Synchaetidae* samples had high abundance at site SSB making up 59% of the community and at CB making up 39% of the community.

The Shannon index for SSB zooplankton families in August samples was the lowest of all samples (Table 4). The Shannon index for CB zooplankton families in June samples was the highest of all samples (1.6). Shannon indices revealed significant differences in zooplankton diversity among months (ANOVA, $p < 0.001$) with June as the highest (mean 1.5) and August as the lowest (mean 1.2), among sites with SB as the highest (mean 1.5) and SSB as the lowest (mean 1.3) (ANOVA, $p < 0.05$), and for the interaction of month and site (ANOVA, $p < 0.05$).
One-way ANOVAs further confirmed that there was no statistical significance in zooplankton family evenness over the three months sampled and among sites and the interaction between month and site (all $p > 0.05$).

Table 5 depicts zooplankton families divided into feeding function and food preference (Smith, 2001; Thorp and Covich, 2001). This connection to feeding function helps with understanding the dynamics of zooplankton family community structure over time and location on the study transect. There was a trend in feeding function/food type, where that the majority of the families collected belong to the filter feeder group that consumed bacteria, periphyton, algae and detritus. In June, Zooplankton composition transitioned from a community that was dominated by filter feeders (such as Branchionidae and Daphiniidae) of dead bacteria loose, periphyton and detritus in the SB sites to active predators (such as Cyclopidae) that feed on other microcrustaceans, metazoans and protists in the CB sites (Smith, 2001; Thorp and Covich, 2001).

Group sites were k means-clustered as the following for zooplankton indicator species analysis (ISA): Group Site 1 included all July site samples; Group Site 2 included June SSB and CB sites and Group Site 3 included June SB and all of August sites. ISA identified two families as indicators: Filinidae was an indicator species for Group Site 1 (all July sites) (IndVal = 0.9; $p < 0.05$). Additionally, three zooplankton family combinations were found to be indicators of Group Site 1 including: Hexarthridae and Filinidae (IndVal = 1.0; $p < 0.05$), Hexarthridae and Synchaetidae (IndVal = 1.0; $p < 0.05$) and Daphniidae and Synchaetidae (IndVal = 0.8; $p < 0.05$). No indicators were found for individual Group Site 2 or for individual Group Site 3, but Bosminidae was an indicator for the combination of Group Site 1 and 3 (IndVal = 0.9; $p < 0.05$).
NMDS analysis of zooplankton community distribution revealed that zooplankton community structures were similar among sites (SB, SSB, and CB) and among sampling times for any of a given site (Figure 6). ANOSIM found that statistically zooplankton community structure was similar among sites (Global R\textsubscript{anosim} = 0.2; \( p > 0.05 \)). Pairwise comparisons showed a significant difference between SB and SSB (R\textsubscript{anosim} = 0.9; \( p < 0.05 \)), but there were no other significant differences between any other sample locations (ANOSIM, Table 6). Additionally, there were no significant differences between zooplankton family community structures among months (R\textsubscript{anosim} = 0.1; \( p > 0.05 \)). Pairwise comparisons showed no significant differences between each month (ANOSIM, Table 6).

BIO-ENV analysis was conducted to best match biotic data with environmental variables using the Spearman rank correlation; 54% of the biological data variability can be explained by patterns with NO\textsubscript{3} + NO\textsubscript{2} and conductivity (R\textsubscript{bioenv} = 0.5). Additionally, 53% of the biological data variability can be explained by patterns with TDN and Conductivity (R\textsubscript{bioenv} = 0.5). RDA ordination (Figure 7) of physical chemical parameters in relation to zooplankton family percent abundances showed consistent results of physical parameters in relation to overall zooplankton community structure and variations with TDN, conductivity and NO\textsubscript{3} + NO\textsubscript{2}. However, RDA further showed that zooplankton community structures in the SSB samples collected in June, July and August and CB samples collected in July and August were correlated with DO and pH. The SB zooplankton community structures sampled in June and August were correlated with conductivity, TDN and SRP. While the zooplankton community structures samples in the CB sampled in June and the structures sampled in the SB in July were associated with NO\textsubscript{3} + NO\textsubscript{2}, NH\textsubscript{4}, and temperature. ANOVA showed no significant difference between zooplankton communities and physical chemical parameters (\( p > 0.05 \)). A Mantel test using dissimilarity
matrices showed that there was no correlation between zooplankton functional feeding group percent abundance with environmental parameters ($r = -0.03; p > 0.05$).

*Free-living Bacterioplankton Community and Zooplankton Community Correlation*

No correlation was found between each biotic community’s Shannon indices (Mantel test, $r = -0.2; p > 0.05$). Additionally, there was no correlation between percent abundances of each T-RF of the bacterioplankton community and percent abundances for each family of the zooplankton community (Mantel test, $r = 0.1; p > 0.05$). No correlation was found between zooplankton functional feeding group percent abundance and between bacterioplankton T-RF percent abundance (Mantel test, $r = 0.4; p > 0.05$).

**Discussion**

Overall, the results support our hypothesis that the free-living bacterioplankton community structure tracks temporal environmental changes (Figures 4 and 5). However, the hypotheses on temporal variation of free-living bacterioplankton diversity and on spatial variations of bacterioplankton community structure or diversity along a nutrient gradient were rejected. This is consistent with previous studies in Lake Taihu (China) and Fermentelos Lake (Portugal), which found that the major driving factors of bacterioplankton diversity in large temperate lakes were temporal variations of environmental factors (Tian et al., 2009; de Figueiredo et al., 2010).

The PCA analysis of environmental data (Figure 3) separated samples based on month, but not by sampling locations. Among the various environmental variables measured, strong correlations were found among NO$_3^-$ + NO$_2^-$, pH and DOC, which are supported by other research that recorded similar correlations with seasonal variations of NO$_3^-$ + NO$_2^-$, DOC (Lymer et al., 2008; Davis et al., 2015) and pH (Lindström et al., 2005; Yannarell and Triplett, 2005).
Chl $a$ concentrations are often used to estimate biomass of primary producers in natural environments (Hoilman et al., 2009). Chl $a$ concentrations measured along the study transect indicated a temporal nutrient shift, where the Chl $a$ concentrations gradually increased from June to August. In addition, the months of June and August exhibited a longitudinal gradient within the lake, with the highest Chl $a$ concentrations found in the Western portion of the lake (SB). The standard value for eutrophic lakes is $10^{-4} - 40 \mu g L^{-1}$ (Moore and Thornton, 1988), which is seen in June SB samples, all July site samples and August SB samples (Table 1).

The temporal shifts in abiotic factors and bacterioplankton structure in this study can be explained by the natural temporal nutrient variations found in Lake Erie during June, July and August (Figures 3-5). Lymer et al. (2008) found that bacterioplankton community composition varied over season with seasonal changes in water temperature and DOC concentration. Bacterioplankton community composition changes were highly correlated with $NO_3^- + NO_2^-$ concentrations across seasons (Shade et al., 2010). Additional research has also noted that bacterioplankton community composition potentially changes with lake trophic status (Lindström, 2000; Yannarell et al., 2003; Eiler and Bertilsson, 2004). Other research found that bacterioplankton diversity shifted temporally with environmental parameters that were correlated with trophic status of the lake including total phosphorus (TP), TDN, DO, and water temperature (Tian et al., 2009).

Bacterioplankton diversity is another way for researchers to understand the complexities of the study habitat at a minuscule measure, since microbial community structure often reflects environmental patterns (Pernthaler and Amann, 2005; Newton et al., 2011). Increased complexity of an environment promotes increased diversity, due to more niches available and more biotic interactions occurring. Additionally, diversity may promote functional redundancy.
within a microbial community, providing a buffer for vital ecological roles (Naeem and Li, 1997). Results from this study found that bacterioplankton T-RF diversity and eveness among sites and based on months were not significantly different \((p > 0.05)\) meaning the number of different bacterioplankton T-RFs did not change despite the temporal differences in the nutrient gradient.

Since, freshwater phytoplankton communities and bacterioplankton communities are influenced by nutrients (Vanni and Jun, 1987), another objective of this study was to see how well zooplankton community composition track temporal and spatial environmental changes along the Lake Erie’s natural nutrient gradient. The second part of this study’s hypothesis i.e., zooplankton community structure tracks environmental changes temporally and spatially, is rejected (Figures 6 and 7; \(p > 0.05\)), although zooplankton family diversity was significantly different when compared among site \((p < 0.05)\), based on month \((p < 0.001)\) and the interaction of site and month \((p < 0.05)\), this could be due to intermediate disturbances caused by biotic interactions. Most likely feeding relationships among zooplankton families are driving these structures (Brendelberger, 1991; Hwang and Heath, 1999; Brandl, 2005). The microbial loop concept (Azam et al., 1983) suggests that its these close biogeochemical processes with bacteria that allow for carbon and other nutrients to be available for protozoa and zooplankton. Three major groups make up the zooplankton community structure in lakes: rotifers, cladocerans and copepods (Brandl, 2005), which aligns with the zooplankton structures revealed by this study. Nano-flagellates and ciliates were not included since they typically are not considered to be major groups of zooplankton in lakes (Brandl, 2005).

Table 5 depicts zooplankton families divided into feeding function and food preference (Smith, 2001; Thorp and Covich, 2001). Although taxonomic structures did not show clear
spatial transitions, their functional trait did show a clear spatial trend. The high percentage of *Daphinidae* found in Sandusky Bay (depth < 2 m) aligns with research that state they play a big role in shallow water bodies (Brendelberger, 1991). *Cyclopidae* prey on *Daphinidae*, which may suggest the lower total abundance in other sites and sampling periods, since *Cyclopidae* numbers increase in the sample sites with low *Daphinidae* numbers. High rotifer (*Branchionidae*) abundance suggests a high bacterial biomass, since they are the top bacterivores in lake systems besides heterotrophic nano-flagellates (Wallace, 2002). July and August zooplankton communities share similarities in composition where samples were dominated by a rotifer filter feeder (*Branchionidae*) and a rotifer predator (*Synchaetidae*) that preys on other rotifers and metazoans (Smith 2001 and Thorp and Covich 2001). This dominance in late summer might be explained by high bacterial turnover at this time of the year, which would serve as a food source (Millie et al., 2009). This high abundance of rotifer predators could explain why *Branchionidae* is less abundant at the SSB sites due to predation.

The family, *Filinidae*, was found to be an indicator for all July sites sampled (Group Site 1). Table 3 shows that no individuals of the *Filinidae* family were found at any of the sites in July (Group Site 1), thus the absence of *Filinidae* indicates the environmental parameters that comprise July sites as a whole. De Cáceres et al. (2010) suggests that combining group sites when conducting indicator species analysis (ISA) allows for the recognition of niche breadth for a select taxa or group of taxa, allowing it to indicate conditions for more than one Group Site at a time. *Bosminidae* is an indicator for the combination of Group Site 1 (all July sites) and Group Site 3 (June SB and all August sites). When comparing Group Site 2 to the combination of Group Sites 1 and 3, Group site 2 has a higher number of individuals found (more than 1000 individuals found at one site) than the combination of Group Sites 1 and 3 (no more than 360
individuals found at each site) (Table 3). Combinations of taxa have an increased predictive power than individual taxa in ISA (De Cáceres et al., 2012). The combination of Filinidae and Hexarthridae were found to be indicators of the combined Group Site 1 (all July sites). There was low representation of the Filinidae and Hexarthridae families (Filinidae: 5 individuals and Hexarthridae: 10 individuals) in Group Site 1 when compared to other Group Sites (2 and 3) and the combinations of these families, thus low numbers of these families indicate the set of environmental parameters observed in Group Site 1. Additionally, the combinations of Hexarthridae and Synchaetidae and Daphniidae and Synchaetidae were found to be indicators of Group Site 1 environmental conditions, which may indicate the combination of abundances that are influenced by characteristic environmental parameters of Group Site 1. No indicators were found for Group Site 2 or 3 or any combinations of those sites. This could be due to the identification of zooplankton down to the family level, which may mask any other indicators that would have been found at the genus or species level.

Generally speaking, bacterioplankton tracking temporal environmental changes has been heavily documented, but bottom-up factors controlling zooplankton community structure has not been well documented in aquatic environments. This study found that free-living bacterioplankton community percent abundances and zooplankton community percent abundances showed no correlation with each other. Additionally, bacterioplankton community diversity and zooplankton diversity and bacterioplankton community percent abundances and zooplankton functional feeding group percent abundances showed no correlation. Research suggests that bottom-up factors (zooplankton) are not governed by the seasonal changes in bacterioplankton diversity and composition (Muylaert et al., 2002). The Brandl (2005) study suggests that zooplankton community structure are governed by copepods feeding modes and
preferentially feed on rotifers. Studies on zooplankton community structure in Lake Erie are relatively limited. A study by Hwang and Heath (1999) observed two size classes of zooplankton bacterivores in Lake Erie and found the importance of bacterioplankton as a carbon source for zooplankton. The same study also found that bacterivorous rotifers dominated in Lake Erie; significantly contributing to zooplankton bactivity. This supports the results discussed in this chapter, with a high total abundance of rotifers (Synchaetidae) in the July and August samples.

There are a few potential limitations of our study. We did not measure microcystin-LR (MC-LR) concentration along the sampling transect, therefore whether or not these cyanotoxins influence free-living bacterioplankton and zooplankton community structure could not be addressed. However, studies have suggested that MC-LR may be important in shaping both bacterioplankton and zooplankton communities (Bouvy et al., 2001; Mitra and Flynn, 2006). Further research needs to be conducted examining the relationships with the particulate associated bacterioplankton communities for both the free-living bacterioplankton and zooplankton communities and the feeding relationships within the zooplankton community including competition and predator-prey interactions. This would also provide insight on zooplankton grazers and whether or not there is a relationship between specific families observed at sites with those phytoplankton. Additionally, it would be interesting to extend the transect to the Eastern Basin of Lake Erie and also have several sites in the Western Basin to capture the complete scope of the lake’s microbial and zooplankton communities spatially. Surveying zooplankton during CyanoHAB events would also provide insight on how zooplankton communities specifically respond to these events.

In conclusion, our results suggested that free-living bacterioplankton do track nutrient changes along the Lake Erie transect temporally, but not spatially. Free-living bacterioplankton
diversity was found to not be significantly different temporally or spatially. In addition, zooplankton community structure was found to not track nutrient variations among sampling sites or temporally. No correlation was found between zooplankton and free-living bacterioplankton community diversity and structure, although, zooplankton diversity was significantly different among months and sites. Potential top-down interactions creating intermediate disturbances could be used to help explain this pattern. This study provides new data on zooplankton community structure and diversity in Lake Erie, which has not been heavily documented.
Table 1: Mean Chl $\alpha$ Concentrations ($\mu$g L$^{-1}$) for Each Site Location (SB, SSB, CB) for June, July and August.

<table>
<thead>
<tr>
<th>Month</th>
<th>Site</th>
<th>Mean Concentration ($\mu$g L$^{-1}$)</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>SB</td>
<td>10.2</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>SSB</td>
<td>1.4</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>1.0</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>SB</td>
<td>50.4</td>
<td>10.47</td>
</tr>
<tr>
<td>July</td>
<td>SSB</td>
<td>12.4</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>13.0</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>SB</td>
<td>45.4</td>
<td>10.84</td>
</tr>
<tr>
<td>August</td>
<td>SSB</td>
<td>4.8</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>3.6</td>
<td>0.86</td>
</tr>
</tbody>
</table>
Table 2: Free-living Bacterioplankton Diversity. Mean number of T-RFs for each site was calculated. Shannon-Weiner Index was used to calculate species richness for each sample and Jaccard Index was used to calculate species evenness for each sample.

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean # TRFs</th>
<th>Shannon Index</th>
<th>Jaccard Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB June</td>
<td>10.0</td>
<td>1.63</td>
<td>0.71</td>
</tr>
<tr>
<td>SSB June</td>
<td>13.5</td>
<td>1.94</td>
<td>0.76</td>
</tr>
<tr>
<td>CB June</td>
<td>12.0</td>
<td>1.99</td>
<td>0.80</td>
</tr>
<tr>
<td>SB July</td>
<td>16.0</td>
<td>2.49</td>
<td>0.90</td>
</tr>
<tr>
<td>SSB July</td>
<td>13.5</td>
<td>2.30</td>
<td>0.81</td>
</tr>
<tr>
<td>CB July</td>
<td>14.5</td>
<td>2.34</td>
<td>0.81</td>
</tr>
<tr>
<td>SB August</td>
<td>14.0</td>
<td>1.90</td>
<td>0.72</td>
</tr>
<tr>
<td>SSB August</td>
<td>15.0</td>
<td>2.55</td>
<td>0.81</td>
</tr>
<tr>
<td>CB August</td>
<td>14.5</td>
<td>2.35</td>
<td>0.81</td>
</tr>
</tbody>
</table>
Table 3: Mean Zooplankton Absolute Abundance (Individuals/m³). Zooplankton subsamples taken at three locations of the study transect were identified to family level and back calculated to figure out the mean number of individuals per cubic meter of water. *Ostracoda individuals could not be identified down to family due to preservation issues.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>SB June</th>
<th>SSB June</th>
<th>CB June</th>
<th>SB July</th>
<th>SSB July</th>
<th>CB July</th>
<th>SB August</th>
<th>SSB August</th>
<th>CB August</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthropoda (Subphylum: Crustacea)</td>
<td>Branchiopoda</td>
<td>Cladocera</td>
<td>Bosminidae</td>
<td>80.52</td>
<td>1006.53</td>
<td>16.36</td>
<td>161.04</td>
<td>125.82</td>
<td>196.27</td>
<td>357.32</td>
<td>35.23</td>
<td>27.68</td>
</tr>
<tr>
<td></td>
<td>Maxillopoda (Subclass: Copepoda)</td>
<td>Laevicaudata</td>
<td>Daphniidae</td>
<td>1071.95</td>
<td>201.31</td>
<td>48.44</td>
<td>583.79</td>
<td>95.62</td>
<td>20.13</td>
<td>357.32</td>
<td>15.10</td>
<td>12.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leptodoridae</td>
<td>25.16</td>
<td>20.13</td>
<td>2.52</td>
<td>0.00</td>
<td>10.07</td>
<td>0.00</td>
<td>20.13</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sidae</td>
<td>0.00</td>
<td>0.00</td>
<td>1.89</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>211.37</td>
<td>5.03</td>
<td>7.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lynceidae</td>
<td>5.03</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Centropagida</td>
<td>30.20</td>
<td>0.00</td>
<td>5.66</td>
<td>0.00</td>
<td>20.13</td>
<td>0.00</td>
<td>161.04</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diaptomidae</td>
<td>0.00</td>
<td>0.00</td>
<td>168.59</td>
<td>120.78</td>
<td>0.00</td>
<td>0.00</td>
<td>478.10</td>
<td>5.03</td>
<td>2.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Temoridae</td>
<td>60.39</td>
<td>60.39</td>
<td>175.51</td>
<td>1539.99</td>
<td>65.42</td>
<td>15.10</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cyclopidae</td>
<td>367.38</td>
<td>1972.79</td>
<td>201.93</td>
<td>1922.47</td>
<td>2611.94</td>
<td>1414.17</td>
<td>644.18</td>
<td>1001.49</td>
<td>515.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cyclopoidea</td>
<td>75.49</td>
<td>0.00</td>
<td>0.00</td>
<td>10.07</td>
<td>0.00</td>
<td>25.16</td>
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<td>5.03</td>
<td>12.58</td>
</tr>
<tr>
<td></td>
<td>Ostracoda</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotifera</td>
<td>Monogononta</td>
<td></td>
<td>Collothecacea</td>
<td>0.00</td>
<td>0.00</td>
<td>4.40</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Filinidae</td>
<td>0.00</td>
<td>10.07</td>
<td>0.63</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>5.03</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hexarthridae</td>
<td>25.16</td>
<td>271.76</td>
<td>3.77</td>
<td>0.00</td>
<td>10.07</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Branchionidae</td>
<td>1615.48</td>
<td>1519.86</td>
<td>64.80</td>
<td>1529.92</td>
<td>956.20</td>
<td>865.61</td>
<td>2898.80</td>
<td>1907.37</td>
<td>961.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gastropoda</td>
<td>10.07</td>
<td>20.13</td>
<td>0.63</td>
<td>221.44</td>
<td>10.07</td>
<td>55.36</td>
<td>5.03</td>
<td>5.03</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lecanidae</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>10.07</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Synchaetida</td>
<td>0.00</td>
<td>1016.59</td>
<td>34.60</td>
<td>60.39</td>
<td>1942.60</td>
<td>1655.74</td>
<td>4222.38</td>
<td>986.40</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trichocerida</td>
<td>40.26</td>
<td>130.85</td>
<td>3.77</td>
<td>0.00</td>
<td>342.22</td>
<td>35.23</td>
<td>10.07</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
<td></td>
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<td></td>
<td>Mean Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td>3407.09</td>
<td>6230.40</td>
<td>733.51</td>
<td>6149.88</td>
<td>6190.14</td>
<td>4282.77</td>
<td>5294.33</td>
<td>7211.76</td>
<td>2526.38</td>
</tr>
</tbody>
</table>

*Ostracoda individuals could not be identified down to family due to preservation issues.
Table 4: Zooplankton Diversity. Mean number of zooplankton families from each site was calculated. Shannon-Weiner Index was used to calculate species richness for each sample and Jaccard Index was used to calculate species eveness for each sample.

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean # Families</th>
<th>Mean Shannon Index</th>
<th>Mean Jaccard Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB June</td>
<td>9.0</td>
<td>1.35</td>
<td>0.61</td>
</tr>
<tr>
<td>SSB June</td>
<td>8.7</td>
<td>1.59</td>
<td>0.75</td>
</tr>
<tr>
<td>CB June</td>
<td>10.7</td>
<td>1.64</td>
<td>0.69</td>
</tr>
<tr>
<td>SB July</td>
<td>7.7</td>
<td>1.57</td>
<td>0.74</td>
</tr>
<tr>
<td>SSB July</td>
<td>7.7</td>
<td>1.37</td>
<td>0.70</td>
</tr>
<tr>
<td>CB July</td>
<td>7.7</td>
<td>1.35</td>
<td>0.65</td>
</tr>
<tr>
<td>SB August</td>
<td>10.7</td>
<td>1.51</td>
<td>0.59</td>
</tr>
<tr>
<td>SSB August</td>
<td>6.0</td>
<td>1.00</td>
<td>0.62</td>
</tr>
<tr>
<td>CB August</td>
<td>6.3</td>
<td>1.16</td>
<td>0.65</td>
</tr>
</tbody>
</table>
**Table 5: Zooplankton Family Feeding Ecology.** Zooplankton samples from three sites Sandusky Bay (SB), Sandusky Sub-basin (SSB) and Central Basin (CB) sampled during three different months (June, July and August) were collected and identified down to family level. Families were then further divided by feeding function and food preference to depict their roles in the microbial loop and classic food web. *Ostracoda individuals could not be identified down to family due to preservation issues. Information gathered using Smith, 2001 and Thorp and Covich, 2001.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Feeding Function</th>
<th>Food Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthropoda</td>
<td>Branchiopoda</td>
<td></td>
<td>Bosminidae</td>
<td>Filter feeder</td>
<td>Algae, protists, detritus, bacteria</td>
</tr>
<tr>
<td>Subphylum: Crustacea</td>
<td>Diplostraca</td>
<td></td>
<td>Daphniidae</td>
<td>Filter feeder</td>
<td>Algae, protists, detritus, bacteria</td>
</tr>
<tr>
<td></td>
<td>Laevicaudata</td>
<td></td>
<td>Leptodoridae</td>
<td>Predator</td>
<td>Microcrustacea, rotifers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sidae</td>
<td>Filter feeder</td>
<td>Algae, protists, detritus, bacteria</td>
</tr>
<tr>
<td></td>
<td>Maxilloboda</td>
<td></td>
<td>Centropagida</td>
<td>Filter feeder/ ambush predator</td>
<td>Detritus</td>
</tr>
<tr>
<td></td>
<td>(Subclass: Copepoda)</td>
<td></td>
<td>Diaptomidae</td>
<td>Filter feeder/ ambush predator</td>
<td>Detritus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Temoridae</td>
<td>Filter feeder/ ambush predator</td>
<td>Detritus</td>
</tr>
<tr>
<td></td>
<td>Cyclopoidea</td>
<td></td>
<td>Cyclopidae</td>
<td>* Filter feeder</td>
<td>Protists, metazoans, microcrustaceans, detritus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td>Loose periphyton, dead bacteria, detritus</td>
</tr>
<tr>
<td>Rotifera</td>
<td>Monogononta</td>
<td></td>
<td>Collothecacea</td>
<td>Ambush predator</td>
<td>Other rotifers, metazoans</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Collothecida</td>
<td>Filter feeder</td>
<td>Loose periphyton, dead bacteria, detritus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Filinidae</td>
<td>Filter feeder</td>
<td>Loose periphyton, dead bacteria, detritus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hexarthriida</td>
<td>Filter feeder</td>
<td>Loose periphyton, dead bacteria, detritus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Branchionida</td>
<td>Filter feeder</td>
<td>Loose periphyton, dead bacteria, detritus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gastropodida</td>
<td>Predator</td>
<td>Dinoflagellates, other rotifers, metazoans</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
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<td>Ploima</td>
<td></td>
<td>Synchaetida</td>
<td>Predator</td>
<td>Other rotifers, metazoans</td>
</tr>
<tr>
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<td>Trichocercida</td>
<td>Predator</td>
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Table 6: Zooplankton Community Structure ANOSIM Results.

<table>
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<td>SB, SSB, CB</td>
<td>0.2</td>
<td>$p &gt; 0.05$</td>
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<tr>
<td>Between Sites</td>
<td>SB and SSB</td>
<td>0.9</td>
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<td></td>
<td>SSB and CB</td>
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<tr>
<td></td>
<td>SB and CB</td>
<td>0.1</td>
<td>$p &gt; 0.05$</td>
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<tr>
<td>Among Months</td>
<td>June, July, August</td>
<td>0.1</td>
<td>$p &gt; 0.05$</td>
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<tr>
<td>Between Months</td>
<td>June and July</td>
<td>0.1</td>
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<td>July and August</td>
<td>0.1</td>
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<td></td>
<td>June and August</td>
<td>0.1</td>
<td>$p &gt; 0.05$</td>
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Figure 2: Lake Erie Sample Sites. The five sites were selected based on the natural trophic gradient of the Sandusky Bay, Sandusky Sub-basin and Central Basin. The Sandusky Bay (SB-1) is known for its hyper-eutrophication status in the summer months, while Sandusky Sub-basin (SSB-2 and SSB-3) is mesotrophic and the Central Basin (CB-4 and CB-5) is oligotrophic.
Figure 3: Principle Component Analysis (PCA) Environmental Data. PCA using environmental data based on site and time depicts how samples are different based on physico-chemical parameters (TDN, NO$_3^-$ + NO$_2^-$, DOC, SRP, Chl a, pH, NH$_4^+$, DO, temperature (Temp) and conductivity (Cond)).
**Figure 4:** Free-living Bacterioplankton Community Non-metric Multi Dimensional Scaling (NMDS). Bacterioplankton T-RFs percent abundances were used to examine bacterioplankton community structure using NMDS. Sites along the transect are labeled within the plot while color represents month of site was sampled.
Figure 5: Redundancy Analysis (RDA) Ordination of Free-living Bacterioplankton Community. RDA ordination of physicochemical parameters ($\text{NO}_3^- + \text{NO}_2^-$, DOC, TDN, SRP, $\text{NH}_4^+$, Chl $a$, pH, DO, temperature (Temp) and conductivity (Cond) in relation to free-living bacteria T-RFs percent abundance display how bacterioplankton community structure for each sample (site and month) is separating out based on physicochemical parameters.
Figure 6: Zooplankton Community Non-metric Dimensional Scaling (NMDS). Zooplankton family percent abundances were used in NMDS to depict how community structures were different based on site location and time sampled. Time sampled is denoted within the plot while color represents site sampled along the transect.
Figure 7: Redundancy Analysis (RDA) Ordination of Zooplankton Community. RDA ordination of physicochemical parameters ($\text{NO}_3^- + \text{NO}_2^-$, DOC, TDN, SRP, $\text{NH}_4^+$, Chl $a$, pH, DO, temperature (Temp) and conductivity (Cond)) in relation to zooplankton family percent abundances depicts how physicochemical parameters influence zooplankton community structure based on site location along the transect and time sampled.
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Cáceres, M. De (2013) How to use the indicspecies package (ver. 1.7.1). *R Proj.* **29**.


Chapter 3: Culture-independent Identification of Microcystin-degrading Bacterial Communities in a Natural Lake and a Man-made Reservoir

Abstract

Harmful cyanobacteria blooms (CyanoHABs) produce cyanotoxins, such as microcystins that lead to skin irritation, illness and liver tumors. Therefore, they cause a serious water quality issue that affects wildlife, human health, recreation and local economics. Research efforts have focused on using culture-dependent techniques to obtain microcystin-degrading isolates that transform and detoxify these harmful toxins to less harmful compounds that can be further metabolized. However, very few studies have examined the potential impacts of microcystins on diversity and structures of bacteria in CyanoHAB impacted freshwaters. This study aimed to address this knowledge gap. Microcystin-enriched microcosms were established using natural lake water taken from two CyanoHAB impacted lakes, i.e., Lake Erie (natural lake) and Grand Lake St. Mary’s (reservoir) and the changes of bacterial communities were tracked using 16S rRNA gene based T-RFLP. Significant differences between start and end MC-LR concentrations ($p < 0.05$) measured in the incubation experiment indicated MC-LR degradation. Shannon diversity indices for bacterioplankton T-RF percent abundances were not significantly different between treatments for both lakes (ANOVA, $p > 0.05$). T-RFLP results showed that bacterioplankton community structures were significantly different between microcystin amended and original free-living bacterioplankton communities for Grand Lake St. Mary samples, but there was no significant difference between community structure for MC-amended treatments and non amended controls. In contrast, Lake Erie’s MC-amended communities
experienced no shift in community structure. Non-amended controls had natural occurring MC-concentration, which suggests that there is a large subset of bacterioplankton that could degrade MC-LR before the treatments were administered. The extensive CyanoHAB history found in both lakes can explain these results. There are two dominating cyanobacterial species in Grand Lake St. Mary’s and four in Lake Erie, which suggests that these differences may affect the differences in MC degraders found in both lakes and the overall bacterioplankton community structure. Evidence of MC-degradation could be explained by bacterioplankton using MC-LR as an energy source.

**Introduction**

Harmful cyanobacterial blooms (CyanoHABs) are a worldwide ecological and human health issue, largely due to their production of cyanotoxins such as microcystins. Microcystins (MCs) are hepatotoxins produced by a number of bloom-forming cyanobacterial species such as *Microcystis, Anabaena, Nostoc* and *Planktothrix* (Jochimsen et al., 1998; Kuiper-Goodman and Fitzgerald, 1999). MCs have stable cyclic heptapeptide structures (Figure 1), which make them resistant to physical chemical breakdowns at normal ranges of temperatures and pH (Tsuji et al., 1994; Lone et al., 2015). MCs in natural environments are mainly decomposed by microbial-mediated processes (Jones and Orr, 1994; Tsuji et al., 1994; Nybom et al., 2012). More than 90 different MC variants have been isolated from natural blooms and laboratory cyanobacterial cultures; and microcystin-LR (MC-LR) is the most common and toxic variant (Cousins et al., 1996; Zurawell et al., 2005; Bourne et al., 2006; Ho et al., 2006; Manage et al., 2009; Pearson et al., 2010). The ubiquity of MC producing blooms across the globe and the challenge to render this issue has invoked a search for bioremediation methods, which would first start with identifying and characterizing MC-degrading bacteria (Carmichael, 1994).
Both culture-dependent and independent studies have been conducted to identify MC-degrading bacteria in CyanoHAB impacted environments. It has been found that environments with recurrent contact to cyanobacterial toxins are more likely to have a natural bacterial community that can degrade MCs. In addition, the rate of MC degradation is related to MC exposure history, where that lakes with natural high exposure history to MCs degrade MCs faster than lakes with no exposure histories (Jones and Orr, 1994; Rapala et al., 1994; Christoffersen et al., 2002a; Edwards et al., 2008; Giaramida et al., 2013). Giaramida et al. (2013) found that previous exposure of MCs significantly impacted the whole entire structure and physiology of lake bacterial communities based on correlations with MC-LR half-life, community structure changes and past exposure history suggesting that MC-LR exposure history can influences the entire bacterioplankton community structure not just the abundance of MC-degraders. Bacterial isolation studies have reported over twenty members of alphaproteobacteria, including the family, Sphingomonadaceae, with the capability of MC-degradation (Jones and Orr, 1994; Bourne et al., 1996, 2001; Park et al., 2001; Saito et al., 2003). Some of the isolates, such as Sphingosincella microcystinvorans, can degrade multiple MC variants, including MC-LR, MC-RR and MC-YR (Maruyama, 2006). Besides genera found within Sphingomonadaceae, a few other genera have been identified as MC degraders. These include Pseudomonas (Gammaproteobacteria) (Takenaka and Watanabe, 1997), Burkhoderia (betaproteobacteria) (Lemes et al., 2008), Paucibacter (betaproteobacteria) (Rapala et al., 2005), and Bacillus (Bacilli) (Zhang et al., 2015).

Although much of the knowledge on MC degradation is from culture dependent studies, these studies are limited on their ability to reflect in situ conditions, since less than 1% of bacteria can be readily cultured in the lab. However, there are few studies that have examined
MC-degradation bacteria in natural environments. One such study has been performed in Lake Erie recently and suggested that a diverse array of bacterial taxa were responsive to MC additions (Mou et al. 2013). Unlike the culture-dependent studies, which suggested the dominance of *Sphingomodales* of the Alphaproteobacteria in MC degradation, the above study suggested that *Methylophilales* and *Burkholderiales* of betaproteobacteria were the most abundant MC degraders in Lake Erie. Another study revealed that bacterial community diversity decreased with MC-LR amendments and degradation (Christoffersen et al., 2002). Further more, different lakes may have different indigenous bacteria and history of microcystin exposure due to spatial variation (Jones and Orr, 1994; Christoffersen et al., 2002; Edwards et al., 2008; Giaramida et al., 2013) and therefore may harbor different MC degraders. More studies are needed to examine the potential lake-specific features of MC-degraders and the impacts of elevated MCs to the structure and diversity of bacterioplankton community during CyanoHABs.

This study aimed to address this important knowledge gap. Two CyanoHAB impacted freshwater lakes were selected as sampling sites, Lake Erie and Grand Lake St. Mary’s. Within Lake Erie, the Western Basin and Sandusky Bay areas experience the most intense CyanoHAB events. Grand Lake St. Mary’s is a smaller, shallower reservoir, but is Ohio’s largest inland lake. Both lakes share a eutrophic status due to similar surrounding agricultural land-use practices (high nutrient loading) and both lakes have similar past histories documenting CyanoHABs. The following study hypothesized that both lakes selected will experience free-living bacterioplankton community structure and diversity changes and evidence of MC-LR degradation upon increased supply of MCs, due to previous microcystin exposures from past CyanoHABS. We predicted that the free-living bacterial community diversity will be different after MC-LR treatment within each lake’s treatments (original, control and MC-LR treatment
It is also predicted that MC-LR concentrations will be lower at the end of incubation study due to natural occurring MC-degraders present in the lake water.

**Methods**

*Study Site/Field Methods*

Surface water (0.5 m below air-water interface) was collected at 6 sites in Lake Erie (LE) (3 in early (E) June and 3 in late (L) June) (Figure 8) and 3 sites in Grand Lake St. Mary’s (GLSM) (Figure 9) in June 2012. Sample sites were chosen for LE based on their potential differences in trophic status and geographic locations. Sites WB-1-E, SB-2-E, and SB-3-L represent Lake Erie’s Western Basin and Sandusky Bay; sites SSB-4-E and SSB-5-L represent Lake Erie’s Sandusky Sub-basin and Site CB-6-L represents Lake Erie’s Central Basin. GLSM sites were selected based on recreational use. GLSM-1 represents a swimming area, GLSM-2 represents a fishing area and GLSM-3 represents a boating area.

Surface water (750 mL; 250mL triplicates) was collected and filtered through glass fiber filters (GFF; Whatman, Sigma-Aldrich, St. Louis, Missouri) on site to collect Chlorophyll-α (Chl a) using a hand pump. GFF filters were stored in whirl packs, covered in aluminum foil and stored on ice until brought back to the laboratory, where they were stored at -80°C before analysis. Additional surface water (0.5 m below air-water interface; 6 L) was collected for free-living bacterioplankton analysis and nutrient analysis. Immediately after surface water was collected, samples were stored in a cooler until transported back to the laboratory.

*Water Processing and Filtration*

Sequential filtration was performed in lab to pass whole water samples (6 L) through 3.0-μm-pore-size and 0.2 -μm-pore-size membrane filters (1 L) (Pall Life Sciences, Port
Filtrates (3 L) that passed 3.0-\(\mu\)m-pore-size membrane filters were stored on ice until incubation started. Filtrates (1 L) that passed 0.2-\(\mu\)m-pore-size membrane filters were cooled on ice and stored at -20°C before measurements for a number of nutrients, which include total dissolved organic carbon (DOC), total dissolved nitrogen (TDN), \(\text{NO}_3^-\), + \(\text{NO}_2^-\), \(\text{NH}_4^+\) and soluble reactive phosphorus (SRP). Ammonium and \(\text{NO}_3^-\), + \(\text{NO}_2^-\) were measured using microplate reader-based colorimetric methods (Ringuet et al., 2011), SRP was measured spectrophotometrically (Rodriguez et al., 1994) and TDN and DOC were measured using a Scientific Instruments TOC5000 Analyzer (Shimadzu, Columbia, MD). All membrane filters were immediately stored at -80°C for molecular analysis. Triplicates of unprocessed water (3 ml) and filtrates (3 ml) that passed through the 3.0-\(\mu\)m-pore-size filter for each site, were preserved with 1% PFA for bacterial cell counting for each site. Bacterial cell counts were determined using flow cytometry (Mou et al., 2005).

Microcosm Establishment and Incubation

For each sample site, 5 liters of the 3\(\mu\)m filtrates were filled in a 10 L carboy, then amended with a mixture of inorganic nitrogen and phosphorous (5 \(\mu\)M \(\text{NH}_4\text{Cl}\), 5 \(\mu\)M \(\text{NaNO}_3\) and 1 \(\mu\)M \(\text{NaH}_2\text{PO}_4\), final concentrations) and incubated in the dark for one week at room temperature. Water samples in carboys were mixed twice a day manually every day during the incubation. After a week of incubation, 750 mL of each sample was divided into six 1L conical flasks. Three of these microcosms were treated with a final concentration of 10 \(\mu\)g L\(^{-1}\) of MC-LR. The remaining three microcosms were not treated and used as controls. Microcosms were incubated in the dark at 25°C at 180 RPM for a total of 48 hours. At 48 hours, the water in each microcosm (MC treatments and controls) was filtered through 0.22\(\mu\)m filters to capture the bacterioplankton community.
Cell counts were taken at 24 hour intervals starting at 0 hours (start) and ending at 48 hours (end) for each site’s 6 microcosms. At the 0 hour and 48 hour interval, 10 mL of microcosm water from each triplicate was filtered through 0.22μm filters for MC-LR concentrations. Filtrates were stored at -80°C until measurements could be conducted.

**Microcystin Concentration Measurement**

Samples of 0.2μm filtrates taken during the incubation experiment were used to measure MC-LR concentration. Microcystins/Nodularins (ADDA) ELISA microtiter plates (Abraxis BioScience, Warminster, PA) were used to measure microcystin concentrations using the manufacturer’s protocol. Technical triplicates for each sample were used.

**Bacterioplankton Enumeration**

Using the same protocol as Mou et al. (2013), flow-cytometric analysis (FCM) conducted with a FACSIAria (BD, Franklin Lakes, NJ) was used to count cells for the original particulate associated cell abundance, the original free-living associated bacterial community and the experimental intervals taken from incubation microcosms. Cell counts were, also, quantified during the incubation to help determine if MC-LR was being degraded; increase in free-living bacterioplankton cell numbers at the end of the experiment would indicate that cells were using MC-LR as a carbon source, since all microcosms were starved of carbon.

**Genetic Analysis**

The 0.22μm filters were used to extract free-living bacterioplankton DNA using the MOBIOL PowerSoil DNA Isolation Kit (Mobio Inc, Carlsbad, CA). Extracted DNAs were then processed for 16S rRNA gene-based terminal restriction fragment length polymorphism (T-RFLP) analysis (Liu et al., 1997). Briefly, polymerase chain reactions (PCR) were performed to amplify bacterial 16S rRNA genes. Each 25 μl-PCR reaction contained 12.5μl of Taqman
Mastermix, 9.9μl deionized water, 0.8μl of fluorescently labeled FAM 27F (10 mM) primer (5’-FAM-AGAGTTTGATCCTGGCTCAG-3’) and 0.8μl 1522R (10 mM) primer (5’-AAGGAAGGTGATCCANCCRCA-3’) were combined with 1.0 μl of DNA template. The PCR reaction program had the initialization temperature of 95°C for 3 minutes; each PCR reaction was cycled for 30 times, with a denaturation temperature at 95 °C for 30 seconds, an annealing temperature at 58 °C for 1 minute and an elongation temperature of 72 °C for 1 minute. Once the PCR reactions went through the 30 cycles, they were exposed to the final elongation step at 72°C for 10 minutes. Each sample’s PCR amplification was conducted in technical triplicates and then they were pooled together and purified using a MO BIO UltraClean GelSpin DNA Purification kit (Mobio Inc, Carlsbad, CA) following the manufacturer’s instruction.

The purified PCR product (17.3 μl) was mixed with HaeIII (0.5μl), 10× buffer (2μl) and bovine serum albumin (BSA) (0.2μl) for each sample. Samples were digested at 37°C for three hours. To purify digested PCR amplicons, each DNA digest (18 μl) was added to deionized sterile water (70 μl), 7.5M ammonium acetate (10 μl), and dilute glycogen (1 μl). Cold absolute ethanol (300 μl) was then added, and samples were stored in -20 °C freezer overnight. DNA digests were then centrifuged at 15,000×g for 15 minutes at 4°C. Once DNA was cleaned with 70% ethanol twice, samples were left to dry out for 10 minutes at room temperature. Purified deionized water (13 μl) was then added to re-suspend DNA and sample were sent to the Plant-microbe Genomics Facility (PMGF) at The Ohio State University, Columbus, Ohio for further fragment analysis using Gene Mapper.
**Statistical Analysis**

T-RFLP data analysis, including removal of noise signals, binning and aligning peak values was conducted using R statistics program and the R Script for T-RFLP analysis designed by Abdo et al. (2006) and implemented into R by Fetzer (2009). T-RF percent abundance was calculated and used for the following statistics. Shannon-Weiner Index and Jaccard’s evenness index were used to obtain abundance and evenness data for each sample.

Principal component analysis (PCA) was conducted to identify the how environmental factors differentiate the two study sites. Redundancy Analysis (RDA) was conducted to identify correlated factors on the original water bacterial community composition. Cluster and heat map analyses were conducted to determine and depict free-living bacterioplankton community via percent abundance of T-RFs of each site’s original community, MC-LR treatment community and control community. Non-metric multidimensional scaling (NMDS) was conducted to determine whether bacterioplankton communities were separating out by treatment for each lake. An analysis of similarities (ANOSIM) was conducted to statistically confirm any separation depicted in the NMDS. ANOSIM yields a coefficient (R) that ranges from 0 to 1, where that ANOSIM coefficient value interpretation includes the following: \( R_{\text{anosim}} > 0.75 \) indicates a high separation between factors, \( R_{\text{anosim}} > 0.5 \) means some separation exists between factors and \( R_{\text{anosim}} < 0.25 \) indicates no separation of factors (Clarke and Warwick, 2001). BIO-ENV were used to figure out how much of the bacterioplankton structure could be explained by environmental factors (Clarke and Warwick, 2001). Two-way analysis of variance (ANOVA) was conducted to determine whether MC concentrations significantly decreased between start (0 hour) and end time (48 hour) of the incubation experiment.
Results

Nutrient Analysis of Initial Samples

Mean nutrient concentrations (NO$_3^-$, + NO$_2^-$, SRP, Chl $a$, TDN, NH$_4^+$ and DOC) were used for principle component analysis (PCA) for original water samples from the study sites. On the PCA biplot (Figure 10), samples were separated out based on which lake they were collected from reflecting the fact that nutrient conditions were different between the two lakes (LE and GLSM). GLSM samples and LE samples formed two distinct clusters and separated mainly along the PC1 axis, which explained 93% of the total variance. SRP, Chl $a$ and DOC were the variables that most strongly loaded on this axis. GLSM samples generally had higher Chl $a$ and DOC and lower NO$_3^-$, + NO$_2^-$. Lake Erie’s Western basin and Sandusky Bay sites generally had higher SRP than GLSM samples. Lake Erie’s Sandusky sub-basin (SSB-4-E and SSB-5-L) and central basin samples generally have higher NO$_3^-$, + NO$_2^-$.

Free-living Bacterioplankton Original Community Analysis

RDA ordination (Figure 11) of nutrient measurements in relation to free-living bacteria T-RFs showed that that GLSM sites and LE sites grouped separately. Although, GLSM Sites 1 and 3 and Lake Erie site WB-1-E, had similar community structures, which had a correlation with SRP and NO$_3^-$, + NO$_2^-$. The GLSM-2 original community was correlated with TDN load. Sites SB-2-E, SB-3-L, SSB-4-E and SSB-5-L shared similar community structures and were heavily driven by DOC and Chl $a$. The CB-6-L community was correlated with NO$_3^-$, + NO$_2^-$ and DOC concentrations. LE mean original particulate associated cell abundance was 7.9 x 10$^6$ cells mL$^{-1}$ and mean free-living cell abundance was 2.0 x 10$^6$ cells mL$^{-1}$. GLSM had a mean of 8.2 x 10$^5$ cells mL$^{-1}$ for particulate associated cell abundance and a mean of 6.6 x 10$^7$ cells mL$^{-1}$ for free-living cell abundance.
Treatment Analysis

Microcystin-LR Analysis

MC-LR concentrations were measured at the start of the LE sample incubation (0 hours) and at the end of the incubation (48 hours) for control microcosms and MC-LR addition microcosms (Figure 14). A two-way ANOVA confirmed that there was a significant decrease in MC-LR concentrations from start (Control: 2.9 µg L⁻¹; Treatment: 12.5 µg L⁻¹) to end (Control: 1.1 µg L⁻¹; Treatment: 4.5 µg L⁻¹) of the incubation experiment for both the MC-LR addition treatments and controls ($p < 0.05$). MC treatments had an overall degradation rate of 4.0 µg L⁻¹ day⁻¹, while controls had a significantly lower rate of 1.2 µg L⁻¹ day⁻¹ (T-Test, $p < 0.01$). Filter-sterilized controls without the presence of bacteria were not included in the experiment. But, previous studies have repeatedly confirmed that spontaneous degradation of MC-LR without involving bacterial activities is negligible (Christoffersen et al., 2002; Edwards et al., 2008; Mou et al., 2013).

MC-LR concentrations were measured at the start of the GLSM incubation (0 hours) and at the end of the incubation (48 hours) for control microcosms and MC-LR addition microcosms (Figure 18). A two-way ANOVA confirmed that there was a significant difference in MC-LR concentrations from start (Control: 9.5 µg L⁻¹; Treatment: 15.8 µg L⁻¹) to end (Control: 4.8 µg L⁻¹; Treatment: 4.2 µg L⁻¹ ) of the incubation experiment for both the MC-LR addition treatments and controls ($p < 0.05$). MC-LR treatments had an overall degradation rate of 4.6 µg L⁻¹ day⁻¹, while controls had a significantly lower rate of 2.0 µg L⁻¹ day⁻¹ (T-Test, $p < 0.05$).

Free-living Cell Enumeration

Free-living bacterioplankton cell counts were measured at the start (0 hours), during (24 hours) and at the end (48 hours) of the incubation experiment for both control and MC-LR
addition microcosms for LE samples (Figure 15). Number of cells were highest at the start of the experiment for both the control (4.7 x 10^6 cells mL^\textsuperscript{-1}) and the MC-LR addition microcosms (3.6 x 10^6 cells mL^\textsuperscript{-1}). Cell concentration declined by the 24\textsuperscript{th} hour (Control: 4.5 x 10^6 cells mL\textsuperscript{-1} and Treatment: 2.3 x 10^6 cells mL\textsuperscript{-1}), but then increased for the MC-LR addition (2.3 x 10^6 cells mL\textsuperscript{-1}) at the end and continued to decline for the control (8.3 x 10^5 cells mL\textsuperscript{-1}) microcosm until the end. There were no significant differences in cell concentrations between Lake Erie treatment (control versus MC-LR) end times or between start and end times for both types of treatment (T-Test; all \(p > 0.05\)).

Free-living bacterioplankton cell counts were measured at the start (0 hours), during (24 hours) and at the end (48 hours) of the incubation experiment for both control and MC-LR addition microcosm for Grand Lake St. Mary\’s samples (Figure 19). Cell counts were highest at the start for MC-LR addition microcosm (1.4 x 10^6 cells mL\textsuperscript{-1}). By mid incubation (24 hours) cell concentrations dropped for the MC-LR addition microcosm (7.0 x 10^5 cells mL\textsuperscript{-1}), but increased at the end of the incubation period (5.5 x 10^6 cells mL\textsuperscript{-1}). Control microcosms showed an increase in cell concentration from the start (2.6 x 10^6 cells mL\textsuperscript{-1}) and did not exhibit as much of a dramatic change in the incubation like the MC-LR addition microcosm did (24 hours: 2.2 x 10^6 cells mL\textsuperscript{-1}; 48 hours: 4.4 x 10^6 cells mL\textsuperscript{-1}). There were no significant differences in cell concentrations between Grand Lake St. Mary\’s treatment (control versus MC-LR) end times or between start and end times for both types of treatment (T-Test; all \(p > 0.05\)).

*Free-living Bacterioplankton Structure and Diversity*

*Lake Erie Treatment*

Shannon-Weiner Index was used to calculate biodiversity for each sample using T-RF percent abundance and Jaccard Index was used to calculate evenness for each sample (Table 7).
Free-living bacterioplankton communities in some MC-LR treatments maintained a similar diversity to their original free-living bacterioplankton communities (SB-2-E, SSB-3-E and SSB-5-L). In other MC-LR treatments, free-living bacterioplankton communities experienced a decreased diversity from their original free-living bacterioplankton communities (WB-1-E, SB-3-L and CB-6-L). One-way analysis of variance (ANOVA) indicated that diversity based on treatment was not significantly different among LE sites ($p > 0.05$). Evenness value for each free-living bacterioplankton community ranges from 66-84 percent.

Cluster and heat map analyses, based on free-living bacterioplankton T-RF percent abundance, showed separation of original free-living bacterioplankton communities from their corresponding treatment samples (Figure 12). MC-LR treated communities were overlapping with the control communities, but SB-2 MC-LR and SSB-5 MC-LR were more similar to each other, while their controls shared similarities, too. The SB-3 MC-LR community was most similar to it’s control community. The SSB-4 MC-LR community was most similar to the CB-6 Control and the CB-6 MC-LR was most closely related to the SSB-4 Control community.

Additionally, NMDS showed that LE free-living bacterioplankton were separating by treatment (MC-LR addition, Control and Original) (Figure 13), but ANOSIM analysis, which gives a statistical power, indicated that there was some overlap within the communities. Original free-living bacterioplankton had a tight cluster, while some MC-LR free-living bacterioplankton communities (SB-2-E and SSB-5-L) were among the Controls and Originals, but the majority of the communities of the MC-LR additions were separated. Analysis of similarities (ANOSIM) confirms that there is minimal separation within the treatment communities (Global $R_{anosim} = 0.3; p > 0.05$). Pairwise comparisons reveal that there were significant differences between MC-LR treatment and original bacterioplankton community structures ($R_{anosim} = 0.5; p < 0.01$) and there
were significant differences between control treatment and original bacterioplankton community structures ($R_{anosim}= 0.5; p < 0.01$). There were no significant differences between MC-LR treatment and control treatment bacterioplankton community structures $R_{anosim}= -0.1; p > 0.05$.

Grand Lake St. Mary’s Treatment

GLSM-1 MC-LR treatment free-living bacterioplankton communities had higher diversity than its original free-living bacterioplankton community (Table 8). GLSM-2 MC-LR had similar diversity to its original and control counterparts. Overall diversity was very similar among treatments. One-way analysis of variance (ANOVA) confirmed that diversity based on treatment was not significantly different for GLSM sites ($p > 0.05$). Evenness value for each free-living bacterioplankton community ranged from 75-91 percent.

Cluster and heat map analyses based on GLSM free-living bacterioplankton T-RF percent abundance (Figure 16) showed separation between original, MC-LR amended and non-amended control bacterioplankton community structures. Original free-living bacterioplankton communities for each GLSM site sampled were more closely similar to each other than to the treatment free-living bacterioplankton communities. MC-LR communities were slightly mixed in with the control communities. GLSM-1 MC-LR and GLSM-2 MC-LR were more closely grouped together, while GLSM-1 and GLSM-2 controls were grouped together.

Additionally, NMDS depicts the separation of treatments (MC-LR addition, Control and Original) (Figure 17) observed in GLSM free-living bacterioplankton community structures. ANOSIM confirmed that there was separation among the communities (Global $R_{anosim}= 0.6735; p < 0.01$). Pairwise comparisons found that there were no significant differences between MC-LR treatment and control treatment bacterioplankton communities ($R_{anosim}= 0.75; p > 0.05$ and between control treatment and original bacterioplankton community structure ($R_{anosim}= 0.593; p$
Pairwise comparisons did find that there was a significant difference between MC-LR treatment and original bacterioplankton community structure ($R_{\text{anosim}} = 1.00; p < 0.05$). GLSM-3 MC-LR could not be used due to PCR error and did not have enough peaks to be considered a reputable community.

**Discussion**

Our study found that MC amendments did not induce the bacterioplankton community structure or diversity to change, rejecting our hypothesis. Although, Figure 17 suggests that there was separation between treatments in Grand Lake St. Mary’s, there was some overlap among treatments in Lake Erie (Figure 13). This result indicates a difference between treatment communities, but is not enough to confirm that the treatment communities are significantly different. Both lakes control communities are similar to their respective original community and MC-LR addition community, since these communities possess the original community, but starved of carbon. Bacterial cell concentrations were found to increase after 48 hours of incubation with MC-LR (Figure 15 and Figure 19), but not to a degree where cell concentrations were found to be significantly different from each other for both lakes. Even after removing the Central Basin site from the data set (to see if this site was skewing the data due to its different trophic status) there was no significant difference between cell concentrations.

Simultaneously, MC-LR concentrations decreased in both treatments for both lakes from start of incubation to end of incubation, with degradation rates of 4.6 $\mu$g L$^{-1}$ day$^{-1}$ and 4.0 $\mu$g L$^{-1}$ day$^{-1}$ (Figure 14 and Figure 18). Different degradation rates have been found by various research ranging from: MC-LR was not detected after 13 days of initial degradation (Cousins et al., 1996), 80% of MC-LR was degraded within 9 days of initial degradation (Zhang et al., 2015), MC-LR degraded 5.4 mg L$^{-1}$ day$^{-1}$ (Park et al., 2001) and 4-16 $\mu$g L$^{-1}$ hour$^{-1}$ (Rapala et al., 2005).
degradation rates for MC-amended microcosms for both lakes do fall within the range of those studies. Treatment microcosm degradation rates for both lakes degraded MCs quicker than controls. This demonstrates that the free-living bacterioplankton community is degrading MC-LR, since no other carbon source was available to use (Rapala et al., 2005; Zhang et al., 2015). Control samples had naturally occurring MC-LR, which can explain why the MC-amended treatment bacterioplankton community structure did not change as we hypothesized. Grand Lake St. Mary’s CyanoHAB species include Planktothrix sp. and Microcystis sp. (Davenport and Drake, 2011), and Lake Erie is known to have Anabaena sp., Aphanizomenon sp., Planktothrix sp., and Microcystis sp. (Chaffin et al., 2013; Davis et al., 2015), which supports presence of naturally occurring MC-LR in our controls. An alternative option that could be used in the future to control for MC-LR concentrations would include testing an oligotrophic lake with no MC-LR concentrations present for MC-degraders. No significant difference among community structure and diversity suggest along with evidence that MC-LR was degraded during the incubation suggest that a large amount of bacteria are responding to the MC amendment. Although, there were no significant changes to free-living bacterioplankton community structure and no significant differences in change to cell numbers, significant changes in MC-LR degradation indicated that bacterioplankton could have used it as energy sources or respired them (Jones et al., 1994). This could also, be due to a T-RFLP limitation such as different species generating the same peak.

Principle component analysis (PCA) on physical chemical parameters showed that lakes were separating out based on their unique environmental conditions (Figure 10). LE Western Basin and Sandusky Bay sites (WB-1-E, SB-2-E, SB-3-L) differed significantly by SRP. Lake Erie’s Western Basin has been heavily documented on having heavy annual SRP concentrations.
from the Maumee and Sandusky Rivers (Watson and Carpenter, 2013). High SRP concentrations along with high nitrogen concentrations lead to CyanoHABs (Davis et al., 2015). Presence of CyanoHABS in lakes that have had previous exposures to MCs are more likely to have bacterial communities with more MC-degraders (Giaramida et al., 2013). GLSM sites were plotted close to each other on the biplot and are correlated with Chl $a$, DOC and TDN. Chl $a$ concentrations are often used as an indicator for algal biomass (Hoilman et al., 2009), which was predominant in GLSM at this time of year. GLSM is considered a nutrient rich, shallow lake, our finding supports it’s hyper-eutrophic state (Davenport and Drake, 2011).

Lake Erie’s WB and SB original (before treatment) bacterioplankton communities and Grand Lake St. Mary’s original bacterioplankton communities shared correlations with the same nutrients. GLSM-1 and GLSM-3 and Lake Erie site, WB-1-E, are associated with SRP, and $\text{NO}_3^- + \text{NO}_2^-$ and had similar free-living bacterioplankton communities; GLSM-2 free-living bacterioplankton community was correlated with a high TDN load. High TDN load could be related to high nutrient loading due to land use surrounding GLSM, which is predominantly agricultural (Davenport and Drake, 2011). The Sandusky Bay and GLSM have similar shallow, eutrophic environments along with similar heavily agricultural surrounding areas (Davenport and Drake, 2011; Stumpf et al., 2012; Davis et al., 2015). Agricultural run-off provide lakes with fixed organic forms of nutrients, responding with high algal and bacterial grow which allows for immeasurable concentrations of N and P(Guildford and Hecky, 2000). This supports that these areas have experienced nutrient loading that has caused past CyanoHABs, which would be the exposure that is important for being able to grow MC-degrading bacteria (Christoffersen et al., 2002; Edwards et al., 2008; Giaramida et al., 2013).
Cluster and heat map analysis depicted the similarities and differences of T-RF percent abundance among treatments. Control and MC-LR treated communities were very similar for both lakes (Figures 12 and 16). Free-living bacterioplankton diversity values for LE and GLSM had no significant difference between original, control and MC-LR treatments ($p > 0.05$). Slight changes in community structure and diversity indicated that a large subset of bacteria are MC degraders. Lack of diversity changes do not necessarily indicate that the free-living bacterioplankton communities do not have MC-degrading bacteria, but it could be that due to past exposure, bacterioplankton commonly found within these sites have the ability to degrade MC-LR or that previous exposure to MC-producing CyanoHABs have changed the overall structure of the bacterioplankton community.

This study could be extended with future directions. More sites at each lake is statistically desirable, especially when running multivariate statistics on community structure, because more sites would serve as more statistical replicates for each lake. GLSM had a clear separation of communities, but only three sites were sampled, overall. Repeating this study at a different time point, perhaps during July or August to see whether temporal variations has any affect on how original communities are able to provide the bacterioplankton that are able to degrade MC-LR. Another study should examine the optimal length of time for MC-LR degradation by the natural lake community. Additionally, 16S rRNA gene tag pyrosequencing would be interesting to identify the actual free-living bacterioplankton taxa in each treatment. Isolation studies could be conducted from this treatment study as an extension of this study—if multiple MC-degrading taxa can be cultivated then bioremediation efforts could very much be a future solution to CyanoHABS.
In conclusion, it was found that Lake Erie and Grand Lake St. Mary’s free-living bacterioplankton diversity did not change significantly upon receiving high levels of microcystins, but there was evidence of MC-LR degradation suggesting that a large subset of free-living bacterioplankton are using MC-LR. Grand Lake St. Mary’s free-living bacterioplankton community structure showed a strong separation based on microcosm treatment (MC-LR amended communities, control communities and original communities), while Lake Erie had a weak separation based on microcosm treatments. Our controls did naturally have some MC-LR in them, so perhaps the reason that MC treated and control communities did not differ significantly was that they already had a pre-existing MC-degrading bacterioplankton community that was dominating.
Table 7: Lake Erie Free-living Bacterioplankton Diversity for Original Communities and Treatment Communities. Mean number of bacterioplankton TRFs were taken for each Lake Erie site. Shannon-Weiner Index was used to calculate biodiversity for each sample using TRF percent abundance and Jaccard Index was used to calculate eveness for each sample.

<table>
<thead>
<tr>
<th>Site</th>
<th>TRFs</th>
<th>Shannon Index</th>
<th>Jaccard Index</th>
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</thead>
<tbody>
<tr>
<td>WB-1-E Original</td>
<td>15</td>
<td>2.28</td>
<td>0.84</td>
</tr>
<tr>
<td>WB-1-E MC-LR</td>
<td>9</td>
<td>1.51</td>
<td>0.73</td>
</tr>
<tr>
<td>WB-1-E Control</td>
<td>8</td>
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</tr>
<tr>
<td>SB-2-E Original</td>
<td>14</td>
<td>1.99</td>
<td>0.75</td>
</tr>
<tr>
<td>SB-2-E MC-LR</td>
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<td>2.14</td>
<td>0.79</td>
</tr>
<tr>
<td>SB-2-E Control</td>
<td>13</td>
<td>2.14</td>
<td>0.84</td>
</tr>
<tr>
<td>SB-3-L Original</td>
<td>17</td>
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<td>0.81</td>
</tr>
<tr>
<td>SB-3-L MC-LR</td>
<td>10</td>
<td>1.37</td>
<td>0.59</td>
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<tr>
<td>SB-3-L Control</td>
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<tr>
<td>SSB-4-E Original</td>
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<td>0.68</td>
</tr>
<tr>
<td>SSB-4-E MC-LR</td>
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<td>0.74</td>
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<td>SSB-5-L Original</td>
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<tr>
<td>SSB-5-L Control</td>
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<td>2.11</td>
<td>0.85</td>
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<tr>
<td>CB-6-L Original</td>
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<td>0.80</td>
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<tr>
<td>CB-6-L MC-LR</td>
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<td>1.64</td>
<td>0.66</td>
</tr>
<tr>
<td>CB-6-L Control</td>
<td>11</td>
<td>1.75</td>
<td>0.73</td>
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Table 8: Grand Lake St. Mary’s Free-living Bacterioplankton Diversity for Original Communities and Treatment Communities. Mean number of bacterioplankton TRFs were taken for each site. Shannon-Weiner Index was used to calculate biodiversity for each sample using TRF percent abundance and Jaccard Index was used to calculate eveness for each sample.

<table>
<thead>
<tr>
<th>Site</th>
<th>TRFs</th>
<th>Shannon Index</th>
<th>Jaccard Index</th>
</tr>
</thead>
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<td>GLSM-1 Original</td>
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</tr>
<tr>
<td>GLSM-1 MC-LR</td>
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<td>GLSM-1 Control</td>
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<td>2.22</td>
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<td>GLSM-2 Original</td>
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<tr>
<td>GLSM-2 MC-LR</td>
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<td>2.15</td>
<td>0.81</td>
</tr>
<tr>
<td>GLSM-2 Control</td>
<td>15</td>
<td>2.15</td>
<td>0.79</td>
</tr>
<tr>
<td>GLSM-3 Original</td>
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<td>2.33</td>
<td>0.91</td>
</tr>
<tr>
<td>GLSM-3 Control</td>
<td>19</td>
<td>2.63</td>
<td>0.89</td>
</tr>
</tbody>
</table>
Figure 8: Lake Erie Sample Sites. The six sites were selected based on the natural trophic gradient of the Western basin, Sandusky Bay, Sandusky Sub-basin and Central Basin and geographic location of those sites. Site 1 represents the Western Basin site in early June (WB-1-E), Site 2 represents a Sandusky Bay site sampled in early June (SB-2-E), Site 3 represents Sandusky Bay Site sampled in late June (SB-3-L), Site 4 is a Sandusky Sub-basin site sampled in early June (SSB-4-E), Site 5 is a Sandusky Sub-basin site sampled in late June (SSB-5-L) and Site 6 represents the Central
Figure 9: Grand Lake St. Mary’s Sample Sites. The three sites were selected based on the eutrophic status of the lake and the lake’s recreational uses such as a swimming area (GLSM-1), fishing area (GLSM-2) and boating area (GLSM-3).
Figure 10: Principle Component Analysis (PCA) Nutrient Data. PCA using nutrient data based on site location depicts how sites are different based on TDN, NO$_3^-$ + NO$_2^-$, DOC, SRP, Chl $a$, and NH$_4^+$. 
Figure 11: Redundancy Analysis (RDA) Ordination of Original Free-living Bacterioplankton Community in Lake Erie and Grand Lake St. Mary’s. RDA ordination of nutrient concentrations (NO$_3^-$+NO$_2^-$, DOC, TDN, SRP, NH$_4^+$, and Chl a) in relation to free-living bacteria T-RFs percent abundance display how original (before treatment) bacterioplankton community structure for each site are separating out based on physicochemical parameters.
Figure 12: Lake Erie Cluster Analysis and Heat Map. Heat map gradient shows the most abundant T-RF as black and white represents absence of that T-RF in a site’s free-living bacterioplankton community. The bottom cluster represents how each T-RF is related. The side cluster exhibits how similar each site is. Original (blue text) free-living bacterioplankton communities for each Lake Erie site sampled are more closely similar than the treatment free-living bacterioplankton communities. MC-LR (green text) communities are mixed in with the control (black text) communities.
Figure 13: Non-metric Multidimensional Scaling (NMDS) of LE Original and Treatment Free-living Bacterioplankton Communities. Bacterioplankton T-RFs percent abundances were used to examine Lake Erie original and treatment bacterioplankton community structure using NMDS. Sites are labeled within the plot while color represents treatment type.
Figure 14: Control and Treatment Microcystin-LR Concentrations Over Time for Lake Erie. MC-LR concentrations were measured at the start of the incubation (0 hours) and at the end of the incubation (48 hours) for control microcosms (light gray) and MC-LR addition microcosms (dark gray).
Figure 15: Number of Cells per Milliliter for LE Control and MC-LR Addition Microcosms for 0, 24 and 48 Hours of Incubation Period. Free-living bacterioplankton cell counts were measured at the start (0 hours), during (24 hours) and at the end (48 hours) of the incubation experiment for both control and MC-LR addition microcosms.
Figure 16: Grand Lake St. Mary’s Cluster Analysis and Heat Map. Heat map gradient shows the most abundant T-RF (percent abundance) as black and white represents absence of that T-RF in a site’s free-living bacterioplankton community. The bottom cluster represents how each T-RF is related. The side cluster exhibits how similar each site is. Original (blue text) free-living bacterioplankton communities for each Grand Lake St. Mary’s site sampled are more closely similar than the treatment free-living bacterioplankton communities. MC-LR (green text) communities are mixed in with the control (black text) communities. GLSM-1-MC-LR is more different from GLSM-1 and GLSM-2 controls than GLSM-2-MC-LR is.
Figure 17: Non-metric Multidimensional Scaling (NMDS) of GLSM Original and Treatment Free-living Bacterioplankton Communities. Bacterioplankton T-RFs percent abundances were used to examine Lake Erie original and treatment bacterioplankton community structure using NMDS. Sites are labeled within the plot while color represents treatment type (MC-LR addition*, Control and Original). (*MC-LR addition for GLSM-3 was unable to be used due to error and not enough peaks to be considered a reputable community).
Figure 18: Control and Treatment Microcystin-LR Concentrations Over Time for Grand Lake St. Mary’s.
Microcystin-LR concentrations were measured at the start of the incubation (0 hours) and at the end of the incubation (48 hours) for control microcosms (light gray) and MC-LR addition microcosms (dark gray).
Figure 19: Number of Cells per Milliliter for GLSM Control and MC-LR Addition Microcosms for 0, 24 and 48 Hours of Incubation Period. Free-living bacterioplankton cell counts were measured at the start (0 hours), during (24 hours) and at the end (48 hours) of the incubation experiment for both control and MC-LR addition microcosms.
References


Chapter 4: Conclusions

Microbial communities in lakes referee biogeochemical processes that contribute to regional and global cycles of carbon, nitrogen and phosphorous. They achieve these important tasks through their tight relationships with their biotic and abiotic environment. How free-living bacterioplankton track spatial and temporal environmental changes in lakes provide snapshots of these complexities. Connecting the microbial loop to the classic food chain provides insights to how bacterioplankton and abiotic factors influence transition trophic levels such as bacterivores zooplankton, connecting microorganisms and macro-organisms.

CyanoHABs are a worldwide issue that impact freshwater systems. CyanoHABs ecological impacts include 1) changes in biodiversity, 2) increased water turbidity which limiting light availability for other primary producers (diatoms), 3) toxin bioaccumulation in organisms in higher trophic levels, 4) decreased oxygen concentrations, 5) Competition for limiting nutrients, 6) predation failure due to toxin levels, 7) selective grazing, and more (Fiedler, 1982; Bouvy et al., 2001; Eiler and Bertilsson, 2004; Bláha et al., 2009). CyanoHABs are regularly seen in many Ohio lakes, including the two, i.e., Lake Erie (LE) and Grand Lake St. Mary’s (GLSM) that were selected as study sites for this thesis. Although different in location and size, these lakes are similarly affected by extensive anthropogenic effects, such as receiving large amount of agricultural runoff. CyanoHABs in LE and GLSM are dominated by different cyanobacterial species. GLSM CyanoHAB species include Planktothrix sp. and Microcystis sp. (Davenport and Drake, 2011), while Lake Erie is known to have Anabaena sp., Aphanizomenon sp., Planktothrix sp., and Microcystis sp. (Chaffin et al., 2013; Davis et al., 2015). LE CyanoHAB events have
been well documented, but bacterioplankton and zooplankton community structure in response to temporal and spatial variations of abiotic factors have not, while GLSM is an understudied lake.

The two main objectives of this thesis included the following: 1) to examine how the structure and diversity of bacterioplankton and zooplankton community tracked environmental changes along a natural nutrient in Lake Erie under the context of temporal and spatial variations (Chapter 2) and 2) to understand the impact of amended MC-LR concentrations on free-living bacterioplankton community structure and diversity in two lakes with past histories of CyanoHAB exposure (Chapter 3).

Chapter 2 results identified significant differences in free-living bacterioplankton structures based on temporal variation, but not on spatial differences. The temporal separation of the bacterioplankton communities was due to temporal changes in NO\textsubscript{X}, DOC, pH, conductivity and ammonium and DO. Bacterioplankton diversity had no significant differences among sites or months. Environmental changes and bacterioplankton diversity variation were not significant across the lake transect for each month, probably because the sites are located in a connected water body. Moderate diversity levels within the lake suggest an array environmental parameters are present allowing a diverse group of bacterioplankton to exist.

Zooplankton family structure did not show a significant separation by site location or sample time. Significant differences in zooplankton diversity were also identified among sites and months ($p < 0.05$). These structural and diversity differences cannot be contributed to difference in environmental conditions, since no significant relationship was identified between zooplankton community structure and environmental parameters that we measured. Biotic relationships such a predator-prey interaction and competition may be major factors influencing zooplankton structure and diversity. Bottom-up effects on zooplankton community may also play
a big role on zooplankton community structure, but further research needs to be conducted to make a solidified argument.

Chapter 3 results found that diversity of bacterioplankton did not change significantly upon receiving high levels of microcystins. Bacterioplankton community structures for GLSM showed a strong separation based on microcosm treatment (MC-LR amended communities, control communities and original communities), while LE had a weak separation based on microcosm treatments. MC-LR concentrations did decrease at the end of the MC-LR incubation experiment, but cell concentrations were not significantly different from start to end of the incubation experiment. These results indicate that a larger subset of bacterioplankton could be using MC-LR as something other than a carbon source such as an energy source. Historically, there are two dominating cyanobacterial species in GLSM and four in LE, which suggests that past exposure to toxin producing CyanoHABs maybe influencing the overall free-living bacterioplankton community structure found within and between both lakes.

Bacterial community compositions are highly variable in comparison from lake to lake around the world. Recent research points to seasonal fluctuations in physicochemical parameters as main drivers bacterial community composition, structure and diversity in lakes (Yannarell et al., 2003; Xing et al., 2007). Chapter 2 and Chapter 3 results are consistent with this general consensus. When spatial differences was tested among sites within a lake, many studies, including ours identified no spatial bacterial community variations (Van Der Gucht et al., 2005; Mueller-Spitz et al., 2009; Tian et al., 2009). One reason contributing to the similarity among LE samples from different sites is that physicochemical parameters did not differ greatly from site to site. This would imply that seasonal variations of physicochemical measurements are greater from month to month that site to site. This could be due to land use and nutrient loading
contributing to the eutrophication of lakes during the spring and summer, increasing the occurrence of CyanoHABs, which is supported by our Chl a data. Research has recorded decreased biodiversity in phytoplankton communities and free-living bacterioplankton communities during blooms (Xing et al., 2007). Past exposure to microcystins (extensive bloom history in lake) and the presence of microcystins can influence free-living community structure and drivers. Previous exposures to microcystins result in a higher frequency of natural occurring microcystin degrading free-living bacterioplankton communities and an overall change in bacterioplankton community structure (Manage et al., 2009; Giaramida et al., 2013).

Other lake communities including phytoplankton and zooplankton also reflect these seasonal variations (Sommer et al., 1986). Chapter 2 found no correlation between zooplankton community diversity with free-living bacterioplankton community diversity. Muylaert et al. (2002) study found that bottom-up affects have a stronger control on bacterioplankton communities. Further research is needed, because our results have high bacterivorous rotifer abundance. Hwang and Heath (1999) found that Lake Erie had high bacterivorous rotifers and that bacterioplankton are an important source of carbon to zooplankton. CyanoHABs cause ecological issues including disturbance in food webs and toxins bioaccumulating in higher trophic levels.

The interplay between temporal environmental changes and bacterioplankton and zooplankton communities is complex. Significant differences in bacterioplankton community structure based on temporal variation in environmental parameters, found in Chapter 2, suggest that bacterioplankton communities are influenced by bottom-up control. High abundances of bacterivorous zooplankton suggests that top-down control in the bacterioplankton may influence community bacterioplankton structure, but to a lesser extent nutrients availability. Based on
reviewing feeding relationships for the families found in this study, trophic interactions maybe driving these changes in structure and diversity.

One important temporal factor in the study lakes is the presence of CyanoHABs. Bacterioplankton community structures may change largely due to the growth of bacterioplankton that can use bloom produced nutrients (Chapter 2) and MCs (Chapter 3) as a carbon source or energy sources. Although there are an agreed set of levels of MCs for drinking water, human fish consumption and recreational water use, there is no concentration standard determined for ecological health risk. This would be difficult to employ, since setting limits on smaller organisms (zooplankton) may not necessarily be a large enough impact to prevent bioaccumulation in higher trophic levels. Future directions may include testing whether zooplankton community diversity correlates with particulate-associated bacterioplankton (cyanobacterial) community diversity. Another interesting study that could be done is to test the effects of zooplankton grazing pressure on bacterioplankton community (particulate associated and free-living) with the presence of MC-producing cyanobacteria. Chapter 3 examined the change in bacterial community structure and diversity with the addition MC-LR. Future studies would include identification of MC-degraders via 16S rRNA gene pyrotag sequencing. Additionally, future research can specifically test to see if MC-degraders have a novel degradation pathway and what other nutrients (besides MC-LR) specifically increase MC-degrader diversity.
References


