A HOLISTIC APPROACH TO CYANOBACTERIAL HARMFUL ALGAL BLOOMS IN SHALLOW, EUTROPHIC UTAH LAKE

by

Anwar Alsanea

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

Department of Civil and Environmental Engineering

The University of Utah

August 2018

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The thesis of **Anwar Alsanea**

has been approved by the following supervisory committee members:

Ramesh K. Goel	, Chair	**May 3, 2018** Date Approved
Michael E. Barber	, Member	**May 3, 2018** Date Approved
P. K. Andy Hong	, Member	Date Approved

and by **Michael E. Barber**, Chair/Dean of

the Department/College/School of **Civil and Environmental Engineering**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

Utah Lake is the largest freshwater lake in the western United States located near Provo, Utah. It receives discharges from point and nonpoint sources and has only one outlet North of it in the form of Jordan River. In recent years, the lake has been experiencing algal blooms that have caught attention of many state agencies including the Environmental Protection Agency (EPA). To understand the dynamics of harmful algal blooms, Utah Lake was sampled at five sites during the summer of 2016, which included the largest algal bloom in the lake. To determine the relationship between harmful cyanobacterial blooms (CyanoHABs) to environmental factors, water samples were collected from May to August in the summer of 2016. Sediment core samples were also collected one time in August. The average temperature increased from 15.69 ^{o} C in May to 22.85 ^{o} C in July and then reached 23.72 ^{o} C in August. Chlorophyll a concentrations and pH were the highest during the bloom in July. High through-put amplicon sequencing results show that *Synechocuccos sp*. dominated the cyanobacterial community before and after the bloom, while *Aphanizomenon flos-aquae* dominated during the bloom. The toxic *Microcystis aeruginosa* appeared at several sites during and after the bloom. As for sediments, *Aphanizomenon flos-aquae* dominated most sites. Based on Principle Component Analysis, the growth of *Aphanizomenon flos-aquae* depended on pH, temperature, phosphate, and chlorophyll a. *Synechocuccos sp's* growth had a negative correlation with *Aphanizomenon flos-aquae's* growth.

TABLE OF CONTENTS

ABSTRACT iii

LIST OF TABLES vi

LIST OF FIGURES vii

ACKNOWLEDGMENTS viii

Chapters

1. INTRODUCTION 1

2. LITERATURE REVIEW 6

- 2.1 Water Resources and Water Quality 6
- 2.2 Trophic Status 8
- 2.3 Nutrients 9
  - 2.3.1 Nitrogen 9
  - 2.3.2 Phosphorus 11
  - 2.3.3 Redfield Ratio 12
- 2.4 Chlorophyll a 13
- 2.5 Bottom Lake Sediments 13
- 2.6 Harmful Algal Blooms (HABs) 14
  - 2.6.1 Identifying Cyanobacteria 16
- 2.7 Water Quality Laws and Regulations 16
- 2.8 Utah Lake 17

3. METHODS AND MATERIALS 19

- 3.1 Site Description and Sampling Sites 19
- 3.2 Surface Water Samples 19
- 3.3 Chlorophyll a 21
- 3.4 Nutrient Analysis 21
- 3.5 Sediment Samples 21
- 3.6 Sediment Total Phosphorus and Phosphorus Speciation 22
- 3.7 Sediment X-Ray Diffraction 23
- 3.8 Cyanobacteria Identification Using High Throughput DNA Amplicon Sequencing 23
  - 3.8.1 Sequencing 24
  - 3.8.2 Denoising and Diversity Analysis 24

3.9 Data Analysis 25

4. RESULTS AND DISCUSSION 26

4.1 Overall Water Chemistry 26
4.3 Bacterial Identification Using High Throughput DNA Amplicon Sequencing 27
4.2 Sediment Samples 36
4.4 PCA 40

5. CONCLUSION 43

REFERENCES 44

LIST OF TABLES

Tables

1. Lake trophic status average characteristics 9

2. Cyanotoxins 15

3. Water quality parameters 27

4. Shannon Index (measurement of richness and diversity) of water and sediment sample cyanobacterial speciation shown for each site 36

5. Total phosphorus and phosphorus speciation concentrations in mg of phosphorus per kg of sediment is shown for each sediment core collected at each site in August of 2016..... 38

6. XRD results for top 5 cm of sediment samples. 39

7. Correlations between sediment Total Phosphorus (TP), Loosely bound P (L-P), Clay bound P (C-P), Calcium bound P (Ca-P), and Residual P(R-P) to the most dominant cyanobacterial species found in sediments for August 2016 samples. 40

8. Sediment P speciation and minerology correlations. 40

LIST OF FIGURES

Figures

1. Nitrogen cycle schematic 10

2. Utah Lake location. 18

3. A GIS map of Utah Lake's sampled sites 20

4. Relative abundancies for bacteria (phylum level) of sampled sites at Utah Lake. 29

5. Relative abundancies for cyanobacterial species of sampled sites at Utah Lake 32

6. Principle Component Analysis for water samples plot for components (dimensions) 1 and 2 representing 39.9% and 23.1%, respectively 42

ACKNOWLEDGMENTS

I would like to first thank my advisor Professor Ramesh Goel for his guidance and support during my master's research. I would also like to thank each and every research lab member for their continuous help and friendship. My family has been a great support during this time; a special thank you goes to my mother who has been my biggest supporter. I would also like to give special thanks to the Department of Water Quality for their help. Thank you to my committee members for their suggestions and guidance.

CHAPTER 1

INTRODUCTION

Surface water quality is a major concern due to increased anthropogenic activities leading to excess input of nutrient to surface waters (Qin et al., 2013; Smith et al., 1999; Smith & Schindler, 2009). Excess nutrients added with other environmental conditions, such as elevated temperatures, support the growth of phytoplankton (Paerl, 2017; Smith et al., 1999). Phytoplankton, an aquatic group of microorganisms, are primary producers that use photosynthesis to convert inorganic carbon to oxygen that provide support to the food web (Paerl et al., 2001). Phytoplankton group includes eukaryotic organisms such as micro-algae and prokaryotic organisms such as cyanobacteria (Reynolds et al., 2002). The growth of phytoplankton depends on many different factors that include physical, chemical, and biological factors (Paerl et al., 2001). Physical factors account for temperature and availability of light for photosynthesis. Chemical factors refer to available nutrients for their growth and reproduction, while biological factors depend on surrounding biological community and their effect on phytoplankton species (Winder & Sommer, 2012). Excess growth phytoplankton causes oxygen to decrease in the water after the decay of their biomass. This increase of phytoplankton growth is known as eutrophication (Qin et al., 2013). Because of this, the characterization of water quality of aquatic systems, especially freshwater bodies, is mainly described in terms of limiting growth nutrients.

Nitrogen (N) and Phosphorus (P) are two vital nutrients that limit the growth of terrestrial, aquatic plants, and phytoplankton (Paerl, 2017; Smith et al., 1999). Freshwater bodies with increased nutrient concentrations beyond the required amounts are characterized as eutrophic, while bodies with insufficient nutrients are characterized as oligotrophic (Qin et al., 2013; Smith et al., 1999). A mesotrophic status is the intermediate between oligotrophic and eutrophic status. N and P loads in freshwater bodies can come from known sources such as wastewater effluent inputs (point source pollution) and unknown sources such as runoff (nonpoint source pollution) (Smith et al., 1999 ; Smith & Schindler, 2009). N and P loads also depend on the freshwater body's morphology and hydrology as well as biological and chemical attributes (Paerl, 2017). For example, shallow lakes are more susceptible to water column mixing with bottom sediments, which causes N and P dynamics to change from other freshwater bodies (Sondergaard et al., 2003).

Eutrophic freshwater bodies are prone to have extremely harmful algal bloom (HABs) events when the appropriate biological, chemical, and physical attributes are present (Qin et al., 2013). Cyanobacteria (e.g., blue-green algae) are prokaryotic oxygenic cells that often form an integral part of HABs often known as CyanoHABs (Paerl et al., 2001). Although there are several environmental problems associated with CyanoHABs, species capable of producing toxic secondary metabolites, such as the hepatotoxin microcystin, are of a particular concern as they have direct negative health implications to humans, pets, livestock, and aquatic food webs (Paerl et al., 2001). Moreover, CyanoHABs can also cause serious economic losses by reducing water quality that effect multiple water uses (Dodds et al., 2009). In the United States alone, CyanoHABs result in losses of recreational, drinking, and agricultural water resources that are worth ~$2 billion annually

(Dodds et al., 2009). Furthermore, HABs also disturb food chain dynamics and can cause unwanted changes in the ecosystem processes (Rigosi et al., 2014). This emphasizes the importance of understanding the occurrence of cyanoHAB. Utah Lake has been experiencing algal blooms that include cyanoHABs according to Utah's Department of Water Quality (DWQ), which makes the lake a great study location.

Utah lake is the largest freshwater lake in the state of Utah. It is located in the Utah valley near the city of Provo with a surface area of 145 square miles and average depth of around 10 feet (PSOMAS & SWCA, 2007). Utah Lake has been listed as an impaired water body by Utah's Department of Environmental Quality (DEQ). The Total Maximum Daily Load (TMDL) report for Utah Lake by Utah's DWQ indicates extremely high concentrations of Total Phosphorus (TP). Due to the large surface area of the lake, generalizing the water quality to a specific attribute is a challenge.

According to Utah's DWQ, Utah Lake experienced a heavy algal bloom around mid-July of 2016. Utah DWQ has identified that the cyanobacterial cell counts were the highest compared to the historical data. Utah Lake was closed to the public on July 15th, 2016, to avoid human health risk, as cyanobacterial concentrations reached 36 million cells per mL. The Utah Department of Agriculture and Food (UDAF) concurrently issued a strong advisory to farmers and ranchers, urging them to avoid using water from Utah Lake for crop irrigation and livestock watering (UDAF, 2016). Despite a high concentration of cyanobacteria belonging to the genus *Aphanizomenon*, no alarming toxin concentrations were measured by the Utah DWQ when samples were analyzed by a U.S. EPA certified laboratory (UDWQ, 2016). However, several episodes of diarrhea, vomiting, and animal sickness were reported by local residents.

Despite the fact that high number of the genus *Aphanizomenon* were recorded in Utah Lake and no alarming concentrations of toxins were detected, episodes of diarrhea, vomiting, and animal sickness were reported. This gives rise to three possibilities: (1) Either the standard methods employed by Utah DWQ to identify CyanoHABs were not adequate. (2) Toxin quantification was not satisfactory. And/or (3) The wrong toxins were targeted. Identifying and characterizing the cyanobacterial community present during blooms will help regulatory agencies in understanding potential harms that these cyanoHAB can cause. By understanding their growth dynamics and their capabilities of releasing toxins, a better evaluation of the HAB can be assessed to address water quality. Mostly microscopic techniques were used to quantify and characterize cyanoHABs in Utah Lake. One of the main challenges where practitioners and managers are unable to forecast CyanoHABs is the vast genetic diversity among cyanobacteria (Paerl et al., 2001). CyanoHABs diversity is sometimes difficult to catch using the established microscopic and/or phycocyanin-based quantification and identification techniques. Microscopic techniques are still used to identify and enumerate many planktonic species (Utermohl, 1958). Although these methods are somewhat specific, they are time consuming and require specialized training (Whitton, 2012). Furthermore, microscope-based identification is more difficult for small-sized picocyanobacteria (e.g., *Synechococcus*), which are abundant in many cases (Callieri, 2008).

It is not simply a matter of which and when cyanobacterial species are present, but the interactions among those species and other bacteria (biological), as well as their interactions (chemical and physical) with their surroundings (Paerl, 2017). Cyanobacteria coexist with many other prokaryotes at the time of CyanoHABs, but the role of these other

prokaryotes has been generally ignored (Eiler & Bertilsson, 2014). Genetic identification of cyanobacteria using DNA-based high throughput sequencing has the potential to not only reveal the holistic identity of cyanoHAB but also inform about other flanking microbial community present in situ. An appreciation for the tight interrelationship between microbes and their microscale physical and chemical environments is particularly important to determine the ecosystem tipping point (Lenton, 2013; Paerl, 2017).

The primary objective was to analyze the interaction between cyanoHABs and environmental factors by employing high throughput DNA sequencing to decipher the temporal and spatial diversity of cyanobacteria and other bacteria community members in Utah Lake. In order to meet the primary objective, the following approaches were carried out:

1. Surface water quality parameters were monitored at five sites across Utah Lake over the months May - August of 2016.
2. Surface water and sediment samples were collected for high throughput DNA sequencing to attain bacterial community identification.
3. Sediments were analyzed for Total Phosphorus and Phosphorus speciation to determine any relationships with sediment minerology and sediment bacterial community identification.

CHAPTER 2

LITERATURE REVIEW

2.1 Water Resources and Water Quality

Water is vital for the survival of humans and all living organisms (Oki & Kanae, 2006). Water also keeps environmental productivity to retain food sources necessary for all organisms (Pimentel et al., 2004). Although the Earth consists of 97% water, around only 3% is freshwater (Oki & Kanae, 2006). Only 0.3% of that freshwater is in rivers, lakes, and reservoirs, and the remaining is stored in deep groundwater or glaciers (Pimentel et al, 2004). Fresh surface water has many purposes such as drinking, public-supply, irrigation, and industrial use (Kenny et al., 2009). With the increase of population, urbanization and the demand on water, the water quality of fresh water bodies is degrading due to pollution (Carpenter et al., 1998). In the United States, around 40% of freshwater is contaminated by harmful microorganisms and pesticides, which makes it unfit for many of its purposes (Pimentel et al., 2004). Water quality degradation and pollution impact human health; around 4 million people die of waterborne diseases annually worldwide (Kreamer et al., 2001).

Pollution in waters not only impacts human health, it impacts the health of those water bodies (Kreamer et al., 2001). Biodiversity is declining in fresh waters and that decline is greater than terrestrial ecosystems making freshwater ecosystems the most

endangered ecosystems in the world (Dudgeon et al., 2006). Another major concern with pollution in waters is ecosystem dysfunction (Kreamer et al., 2001). It disrupts nutrient cycling and changes biogeochemical cycles that control nitrogen, phosphorus, oxygen, and carbon cycles in water.

Sources for pollution in fresh water bodies are characterized into point source pollution and nonpoint source pollution (Paerl, 2017; Smith et al., 1999; Smith & Schindler, 2009). Point source pollution, for example, can come from direct wastewater effluents that were not treated properly, and nonpoint pollution such as urban and agricultural runoff refers to any indirect sources of pollution (Smith et al., 1999). Types of pollutants coming into water bodies can be chemical. for example toxic contaminants from anthropogenic sources and/or biological, for example pathogens and viruses (Kreamer et al., 2001; Paerl, 2017). Nutrients such as phosphorus and nitrogen required for photosynthetic organisms' growth are also considered pollutants to freshwater bodies when their concentrations are too high for the freshwater system needs (Smith et al., 1999; Qin et al., 2013). Excess nutrients coming in from point and nonpoint source pollution lead up to eutrophication. Eutrophication is the excess of nutrient inputs into a water body that will lead to algal bloom events. According to the US Environmental Protection Agency (EPA), water bodies can be classified as impaired for nutrients. Around 50% of impairment of freshwater lakes in the United States are due to eutrophication (Carpenter et al., 1998). Eutrophication is the increase of primary production rates, which increase with the increase of nutrients (Qin et al., 2013). Lakes are characterized in terms of their ability to provide sufficient nutrients for primary production growth.

2.2 Trophic Status

Lake productivity is defined as the ability of the lake to support primary producers such as plants and photosynthetic microorganisms to provide food for secondary and tertiary consumers to support the ecological food chain (Winder & Sommer, 2012). Freshwater lakes' trophic status refers to its productivity (Smith & Schindler, 2009). An oligotrophic lake contains low nutrients that are insufficient for primary production. On the other hand, a lake with high productivity (high nutrients) is a eutrophic lake. A mesotrophic lake is the intermediate of both trophic statuses (Smith et al., 1999; Smith and Schindler, 2009). A lake with very high productivity that experiences frequent algal blooms is considered a hypertrophic lake (Paerl, 2008). Eutrophication in lakes promotes the growth of primary producers such as algae and phytoplankton. Table 1 displays lake characterization depending on nutrients that promote lake productivity. Although primary producers are essential to sustain life and the food web, an overabundance of them is harmful. The overabundance of primary producers is also known as algal blooms. Algal blooms cause oxygen depletion in freshwater bodies when they decay. As a result, aquatic biodiversity is lost (Carpenter et al., 1998; Paerl, 2008). Bloom forming phytoplankton such as some species of cyanobacteria can produce cyanotoxins that are both harmful to humans and living organisms (Carmichael et al., 2001). For freshwater bodies impaired by eutrophication, nutrient inputs are a major concern. Nitrogen (N) and Phosphorus (P) are principal nutrients required for the growth of algae and phytoplankton (Paerl, 2017; Smith et al., 1999; Smith & Schindler, 2009).

Table 1: Lake trophic status average characteristics. TP is Total Phosphorus concentration, TN is Total Nitrogen concentration, and Chl a is chlorophyll a pigment concentrations. Values were obtained from Smith et al, 1999.

Trophic Status	TP (mg/L)	TN (mg/L)	Chl a (mg/L)
Oligotrophic	< 350	< 10	< 3.5
Mesotrophic	350 - 650	10 - 30	3.5 - 9
Eutrophic	650 - 1200	30 - 100	9 - 25
Hypereutrophic	> 1200	> 100	> 25

2.3 Nutrients

2.3.1 Nitrogen

Nitrogen (N) is an essential element for life. The availability of N in any system depends on the N cycle (Jetten, 2008). N transformations in the ecosystem are generally dictated by microorganisms. N is available in gaseous form (N_2 gas) and inorganic and organic forms. The major forms of N for cyanobacteria to uptake are nitrate, ammonium, and nitrogen gas (Herrero et al., 2001). Major N transformations in the N-cycle as shown in Figure 1 include the processes of nitrification, denitrification, and N-fixation. N_2 gas represents a major source of N in the environment, which is also the most stable forms of N (Jetten, 2008). Only a few microorganisms can uptake N_2 gas in the process of N-fixation. N-fixation converts N_2 gas into ammonia (NH_3). After N-fixation, ammonia is now available for other microorganisms to uptake and convert to nitrite and nitrate through the process of nitrification in oxic conditions. Under anaerobic conditions, nitrates are converted back to N_2 gas through the process of denitrification (Jetten, 2008).

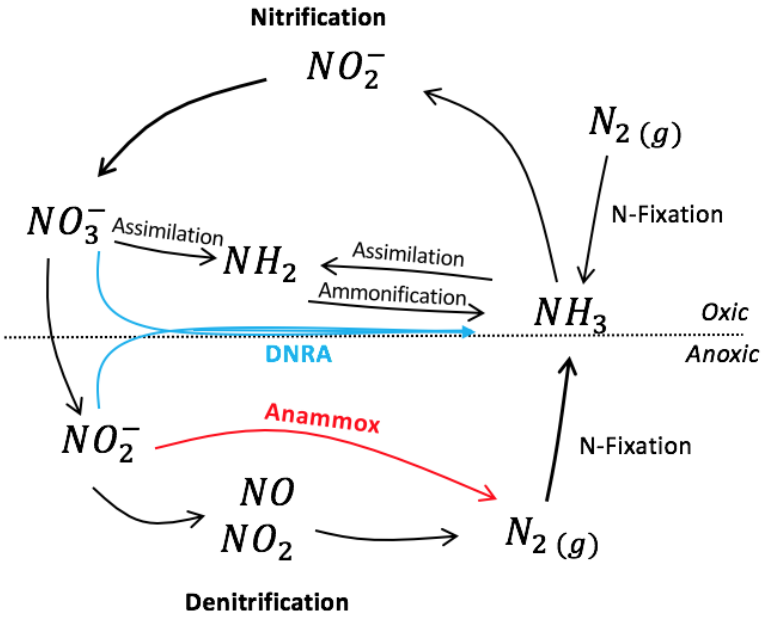

Figure 1: Nitrogen cycle schematic adapted from (Jetten, 2008).

Some cyanobacteria, such as *Aphanizomenon flos-aquae,* are able to fix N_2 gas. N-fixing cyanobacteria can dominate the water systems when other forms of N are limited, given that P is also available. These blooms are harmful in that they cause a change in the food web. Also, some species of cyanobacteria, such as *Aphanizomenon flos-aquae*, can produce cyanotoxins, which are harmful to humans and living organisms (Conley et al., 2009). In freshwater bodies, the N cycle is altered due to point source and nonpoint source of pollution (Jetten, 2008). Sources of N that come in into freshwater bodies can come in from agricultural run-off, urban run-off, and wastewater discharges. Another concern for a disrupted N-cycle is that high levels of nitrate concentrations are considered toxic to humans and livestock (Carpenter et al., 1998).

2.3.2 Phosphorus

Phosphorus (P), along with oxygen, carbon, and nitrogen, is considered one of the most important elements needed for ecosystems. P is an essential component for all living organisms. It is involved in a number of important processes such as photosynthesis in plants for energy transformation. It is also important for plant growth, and sufficient amounts can help with early root and seed formation. Thus, agricultural fertilizers are enriched with P (Mullins, 2009). P can play two roles in the environment. It can be a limiting nutrient by being in an unavailable form for plant uptake, or it can act as a pollutant in lakes (Smith et al., 1999). This complicated behavior of P depends on the inputs and outputs of the aquatic system along with other factors such as water and soil chemistry.

Unlike the N cycle, P enters and leaves the system in dissolved or solid form. The cycle starts with P in soil and rocks making its way to aquatic systems such as lakes. Inputs of P into the system come from weathering of rocks, plant residue, animal waste, and biosolids (Correll, 1998). With the erosion and weathering in the soil, the inputted organic – P is mineralized into inorganic soluble – P that can be absorbed by plants, which can then get back into the system from plant residue and animal waste. Inorganic soluble – P can also be adsorbed into mineral surfaces and get precipitated with other compounds available in the system (Correll, 1998). This causes phosphorus to be unavailable in the soluble form, which is the only form plants can uptake, given that P becomes a limiting nutrient. When P is low, it reduces plant growth and slows down soil microbial growth. On the other hand, when excess P is available it can cause eutrophication. Excess P can cause overgrowth of algae and as a result will decrease the

amount of oxygen needed when algae decays. Low oxygen levels free more P from the soil, consequently further increasing the amounts of P (Hyland et al., 2005).

In the case of excess P, since the P cycle does not contain a gaseous phase, P does not exit the system easily (Correll, 1998). It is usually deposited in sediments surrounding the lake. It is crucial to quantify the amount of phosphorus available in sediments as well as differentiating species of P available. As mentioned in the P cycle, P can be available in the system as organic and inorganic forms. P is dominant in organic form in soils, and the main source for excess P is agricultural fertilizers and waste water discharge (Lukkari et al., 2007). Organic material is broken down by soil microorganisms releasing the inorganic P. Inorganic – P is divided into multiple categories. The first is soluble – P, which is in the form of phosphates and orthophosphates. Soluble – P can precipitate with compounds such as Ca, Fe, Mn, and Al to make inorganic insoluble – P (Lukkari et al., 2007). Soluble – P can also get adsorbed back into the soil by iron oxides, aluminum oxides, and carbonates available. Adsorption is a quick process while desorption is a much slower process, and again this makes P become unavailable in the soluble form for plants (Hyland et al., 2005).

2.3.3 Redfield Ratio

Nutrients N and P are often limiting to the growth of phytoplankton and algae (Downing & McCauley, 1992; Paerl, 2017; Smith & Schindler, 2009). The Redfield ratio represents a relationship between the elemental composition of an phytoplankton in terms of inorganic Carbon:Nitrogen:Phosphorus (C:N:P ratio) and the surrounding water chemistry (Geider et al., 2002). This ratio identifies the microorganisms needs in terms of

C, N, and P. Cyanobacteria and algae have different C:N:P ratios. Some algae and cyanobacteria species will dominate based on which nutrient is limited (Downing & McCauley, 1992).

2.4 Chlorophyll a

Chlorophyll a (chl a) is a pigment found in photosynthetic organisms. Chl a constitutes around 1-2% of the dry weight of algae (APHA, 1999). Chl a is used to estimate phytoplankton and algae biomass as well as an indicator to trophic status for freshwater lakes (Boyer et al., 2009). Chl a accounts for the biomass of both eukaryotic algae and prokaryotic cyanobacteria (Gregor & Marsalek, 2004). Chl a is a pigment found in all photosynthetic organisms and will count for both biomasses.

2.5 Bottom Lake Sediments

Water-sediment interactions are important to consider for nutrient loadings especially in shallow lakes. Shallow lakes are more susceptible to water column mixing with bottom sediments, which causes N and P dynamics to change from other freshwater bodies (Sondergaard et al., 2003). Multiple studies indicated that nutrient loading, especially P is one of the main factors for algal bloom events (Rigosi et al., 2014). Bottom lake sediment plays an important role in P loading especially in shallow lakes where the water column is well mixed (Soondergard et al., 2003). Sediments can act as a source and/or sink to P loads. The release of P is affected by sediment characteristics, such as pH, soil texture, and phosphorus-bounded compounds (Huang et al., 2005; Li, et al., 2013). The implementation of sediment P availability is not widely studied in

regards to algal blooms. Compared to surface water, the importance of algae in bottom lake sediments is less studied. Cyanobacteria have a buoyancy that enables them to migrate among surface and benthic environment, making them a driving force of phosphorus release from sediments (Cao et al., 2016; Cottingham et al., 2014). Benthic phytoplankton communities are greatly influenced by nutrient availability and transformation. For example, benthic freshwater habitats with low nitrogen availability and/or low N:P supply ratios may favor N2- fixing cyanobacteria and have high nitrogen-fixing rates (Douterelo et al. 2004; Marcarelli et al. 2008). However, few field studies have yet studied benthic algae with sediment phosphorus availability during harmful algal bloom events.

2.6 Harmful Algal Blooms (HABs)

Harmful Algal Blooms are the excess growth of algae, phytoplankton, and cyanobacteria (commonly known as blue-green algae). These organisms are either macroscopic (visible to the eye) or microscopic (Carmichael et al., 2001). HABs are harmful in that they cause hypoxia in the aquatic environment when the HABs decay. Also, some species of HABs produce harmful toxins. The toxins are associated with cyanobacterial blooms (cyano-blooms), which are also known as cyanotoxins. Cyanotoxins include neurotoxins such as anatoxin-a and hepatoxins such as microcystins, nodularins, and Cylindrospermopsin (Carmichael et al., 2001). A list of current known cyanotoxins is shown in Table 2. Toxins are categorized according to the affected organ it targets (Merel et al., 2013). Human exposures to these toxins through drinking water and/or recreational activities will cause symptoms such as skin rashes,

Table 2: Cyanotoxins. List of different types of toxins based on the targeted affected organ that cyanobacteria may produce along with their associated main cyanobacteria. (Merel et al., 2013).

Type	Toxin	Associated Cyanobacteria
Hepatotoxins	Microcystins	*Microcystis, Oscillatoria, Anabaena,*
	Nodularins	*Nodularia spumigena*
	Cylindrospermopsin	*Cylindrospermopsis raciborskii, Aphanizomenon ovalisporum,*
Neurotoxins	Anatoxin-a	*Anabaena, Aphanizomenon*
	Saxitoxins	*Anabaena circinalis, Aphanizomenon flos-aquae*
	β-N-methylamino-L-alanine	may be produced by all 16 known groups of cyanobacteria
Dermatotoxins	aplysiatoxins lynbyatoxins	*Lyngbya majuscula*

respiratory distress, and gastrointestinal distress (Hudnell, 2008; NSTC, 2016). Many cases have been reported regarding animal mortality and morbidity related to cyanobacterial blooms as well as human illness reports in 11 states in the year 2007 (Backer et al., 2015). Biological monitoring is needed when water quality issues involve HABs. Monitoring will include identifying algae and cyanobacteria as well as monitoring their response to other environmental factors (Chapman & WHO, 1996).

2.6.1 Identifying Cyanobacteria

Traditionally, cyanobacteria identification is performed by microscopic techniques. Microscopic analysis is based on morphological characteristics, which are problematic when it comes to smaller sized bacteria. For example, microscopic techniques have been found to overlook picocyanobacteria, which cell sizes range from 0.2 - 2 μm (Ye et al., 2011). Compared with microscopic counting and phycocyanin (pigment found in cyanobacteria) measurements techniques to quantify cyanobacteria biodiversity (Johnson & Martiny, 2015; Kasinak et al., 2014), genomic techniques are more reliable to further detect cyanobacteria and the primer targeting cyanobacteria 16s rRNA is widely used (Nu¨bel et al., 1997).

2.7 Water Quality Laws and Regulations

In order to ensure water quality, research and monitoring is being applied. Laws and regulations in the United States have also been created towards water quality. The Clean Water Act was established to regulate and control pollutions coming into water bodies (EPA, 2017). Under the Clean Water Act, section 303(d) requires states to identify water bodies where pollution control technologies are insufficient to meet water quality standards. A Total Maximum Daily Loads (TMDL) study is required for each impaired water body. In this study, TMDL amounts are calculated for each pollutant that is causing impairment. The amounts calculated would represent the maximum amount (load) of that pollutant the water body can receive without affecting the specified water body's beneficial uses. TMDL studies require intensive monitoring efforts that include field sampling and laboratory experiments along with modeling analysis. Once TMDL is

implemented to a water body, permits are then required for any treated waste discharges. TMDL studies also target nonpoint source pollution by understanding possible causes that lead to nonpoint pollution (EPA, 2017).

2.8 Utah Lake

Utah Lake is the largest freshwater lake in the state of Utah. It is also the largest natural occurring freshwater lake in the western United States (PSOMAS & SWCA, 2007). It is located in the Utah valley near the city of Provo (shown in Figure 2) with a surface area of 145 square miles and average depth of around 10 feet (PSOMAS & SWCA, 2007). According to trophic status, Utah Lake is classified as hypereutrophic that experiences extreme HABs in late summer (Stackelberg, 2016). Utah Lake's beneficial uses include secondary contact recreation, warm water fishery, and agricultural water supply (PSOMAS & SWCA, 2007). Major source of water to Utah Lake are from snow melt from the Wasatch mountains. Major tributaries into Utah Lake are Provo river, American Fork river, Spanish Fork river and several creeks, such as Millrace creek, Hobble creek, and Currant creek (PSOMAS & SWCA, 2007). The lake has only one outlet located north of the lake, the Jordan river. The outlet runs downstream to the Great Salt Lake (PSOMAS and SWCA, 2007). Utah Lake's TMDL indicates Total Phosphorus (TP) and Total Dissolved Solids (TDS) as water quality impairments. TP refers to all different forms of P available in the water column. TDS is the concentration of mineral salts in water. TP impairment in Utah Lake is affecting the warm water fishery use, while TDS impairment is affecting agricultural water use (PSOMAS and SWCA, 2007).

Utah Lake experienced an extreme HAB in summer of 2016. The lake was closed

Figure 2: Utah Lake location. A Google Earth map showing the location of Utah Lake.

to the public during July for health concerns. Advisories were established with warning to not recreate at the lake. An agricultural advisory was also established. According to Utah's DWQ, cyanobacterial cell counts for this algal bloom has reached the highest compared for previous years' data. Efforts lead by the DWQ have identified *Aphanizomenon flos aquae* as the abundant cyanobacteria species using microscopic methods. Genetic studies, such as high amplicon sequencing, were not applied to identify cyanobacteria in Utah Lake before.

CHAPTER 3

METHODS AND MATERIALS

3.1 Site Description and Sampling Sites

A total of five sites were sampled in Utah Lake in the summer of 2016. The locations of all sites are shown in Figure 3. The sites were sampled each month and were distributed across the lake. “Saratoga Springs” site is located northwest of the lake near Jordan River outlet and Dry Creek tributary. “Geneva Discharge” is located northeast of the lake near Timpanogos wastewater treatment plant and American Fork river discharges. “Pelican Point” represents the middle of the lake. “Lincoln Beach” represents south of the lake. “Provo Bay” is a site located at the entrance of Provo Bay that receives wastewater treatment discharges from multiple wastewater treatment plants (WWTPs).

3.2 Surface Water Samples

In order to meet our objectives, surface water samples from Utah Lake were collected following the Standard Operating Procedure for collection of lake water samples by Utah’s Division of Water Quality (UDWQ, 2014). An on-field environmental monitoring system (YSI SONDE 600 XL) was used to measure on-field parameters pH, temperature, and Total Dissolved Solids (TDS).

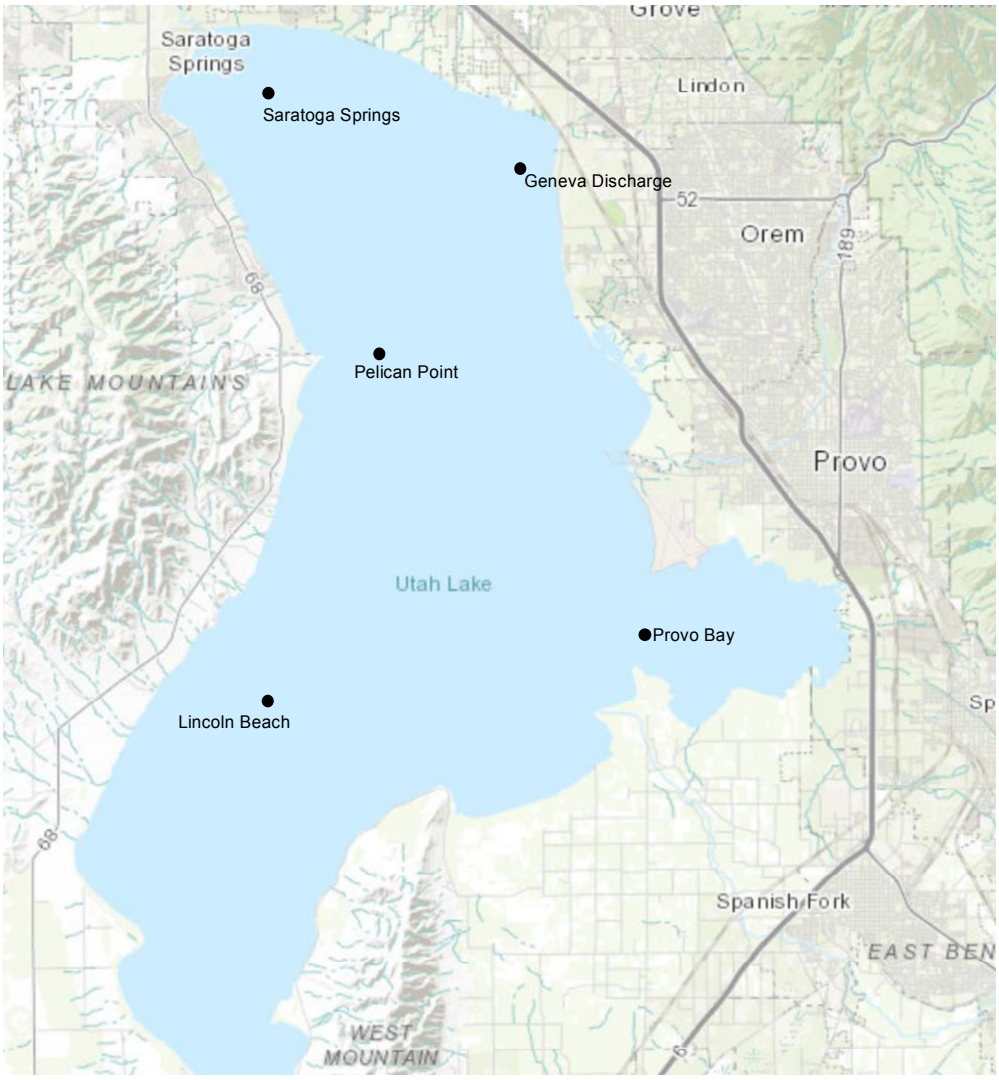

Figure 3: A GIS map of Utah Lake's sampled sites. Five sites monitored are shown. Saratoga Springs and Geneva Discharge represent north of the lake, Pelican Point in the middle, Provo Bay is located at the entrance of the bay, and Lincoln Beach represents south of the lake. *Sources:* Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoB.

3.3 Chlorophyll a

Chlorophyll a measurements were determined spectrophotometrically in the laboratory following the standard methods of water and wastewater (Chlorophyll) by American Public Health Association (APHS, 1999). Water samples were collected in aluminum covered bottles to prevent pigment degradation due to the sun and were filtered immediately after collection using a 0.45 μm pore-size Whatman filter. A mortar and pestle was used instead of a mechanical tissue grinder. Ninety percent acetone solution was added gradually without exceeding the 10 mL limit while grinding the filters. Filters were ground by hand for around 1 min for each sample. Samples were then incubated in 4^{o} C for at least 2 hrs and were centrifuged after. The supernatant was then used, and the chlorophyll pigment was measured using a spectrophotometer following the standard methods.

3.4 Nutrient Analysis

Nitrate-N, nitrite-N, and phosphate-P were measured each month using Ion Chromatography [Chromatograph model: Metrohm 883 Basic IC plus]. EPA method 300 for determination of inorganic anions by ion chromatography was followed. The Ion Sample preparation and preservation was according to EPA method 300 (Pfaff, 1993).

3.5 Sediment Samples

Sediment samples were collected using a 30-cm long and 10-cm diameter transparent plexiglass core sampler. Samples were collected by diving with scuba gear and pushing the core into the bottom of the lake. A rubber stopper was used to seal the

top of the core while pulling out the core from the bottom of the lake, and another rubber stopper was used in the bottom. Cores were labelled appropriately and placed in a cooler while transferring them to the laboratory. Each core was divided into sub cores of 0-5 cm, 5-15 cm, and 15-30 cm length. Each sub core was subjected to total phosphorus and phosphorus speciation.

3.6 Sediment Total Phosphorus and Phosphorus Speciation

For total phosphorus, oven dried (108^{o}C) homogenized sediment samples were digested with sulfuric acid and ammonium peroxydisulfate. Total phosphorus was determined using the acidic molybate-ascorbic acid method (Murphy & Riley, 1962). As for P speciation, oven dried (108^{o}C) homogenized sediment samples (1 g in weight) were placed in centrifuge tubes. Sequential extraction of all species is as follows: Loosely bound P (L-P) was first extracted by adding 25 mL of sodium chloride solution to a centrifuge tube while shaking for 2 hours. Supernatant was evaluated for orthophosphate-P using HACH's PhosVer® 3 pillows (PhosVer 3 Phosphate Reagent Powder Pillows). Remaining sediment was saved for the next step. Clay bound P (C-P) was extracted from remaining sediment from L-P step by adding 25 mL of sodium hydroxide solution while shaking for 18 hours. Supernatant was evaluated for orthophosphate-P. Finally, calcium bound P (Ca-P) was extracted from the sediment sample remaining after the loosely bound step and clay bound step by adding hydrochloric acid solution while shaking for 24 hours. Supernatant was evaluated for orthophosphate-P. (Paludan & Jensen, 1995 ; Psenner et al., 1988). Residual P was calculated by subtracting C-P, L-P, and Ca-P from the TP measurements. All total P and P speciation measurements were determined in

triplicates.

3.7 Sediment X-Ray Diffraction

Whole-rock and clay X-ray diffraction (XRD) analyses were performed on the first 5 cm of each collected sediment core in the XRD laboratory at the Energy & Geoscience Institute at the University of Utah, using a Bruker D8 Advance X-ray diffactometer. The Rietveld method was performed for phase quantification using TOPAS software, developed by Bruker AXS.

3.8 Cyanobacteria Identification Using High Throughput DNA Amplicon Sequencing

To detect cyanobacteria community structures, DNA was extracted from surface water samples and top 5 cm of the sediment samples using PowerWater DNA isolation kit and Power Soil DNA isolation kit, respectively, according to manufacturer's instructions. Genomic DNA concentrations were measured using a Thermo NanoDrop 2000c at 260/280, and the samples with 260/280 ratio higher than 1.80 were used for further analysis. The extracted DNA samples were further sent to RTLGenomics (Lubbock,TX) for high-throughput amplicon sequencing using universal bacterial primers 515F (*GTGCCAGCMGCCGCGGTAA)* - 806R (*GGACTACHVGGGTWTCTAAT**)* amplifying hypervariable region V4 of 16S rRNA gene.

3.8.1 Sequencing

Amplification was performed in a two-step process. Forward primer was constructed with Illumina i5 sequencing primer (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG) and the reverse primer was constructed with Illumina i7 sequencing primer (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG). Reactions were performed on ABI Veriti thermocyclers (Applied Biosytems, Carlsbad, California). Products were added to a second PCR to determine concentrations. Primers for the second PCR were designed based on the Illumina Nextera PCR primers: Forward - AATGATACGGCGACCACCGAGATCTACAC[i5index]TCGTCGGCAGCGTC and Reverse - CAAGCAGAAGACGGCATACGAGAT[i7index]GTCTCGTGGGCTCGG. Amplification products were visualized with eGels (Life Technologies, Grand Island, New York). They were then pooled equimolar. Each pool size was selected in two rounds using SPRIselect (BeckmanCoulter, Indianapolis, Indiana) in a 0.7 ratio. Size selected pools were then run on a Fragment Analyzer (Advanced Analytical) to assess the size distribution, quantified using the Qubit 2.0 fluorometer (Life Technologies), and loaded on an Illumina MiSeq (Illumina, Inc. San Diego, California) 2x300 flow cell at 10pM and sequenced at RTLGenomics.

3.8.2 Denoising and Diversity Analysis

PEAR Illumina paired-end read merger (Zhang et al., 2013) was used to merge the forward and reverse reads in FASTQ format. USEARCH algorithm (Edgar, 2010) was used to perform prefix dereplication. UPARSE OTU selection algorithm (Edgar,

2013) was used for OTU selection. Chimera checking was done on the selected OTU's using UCHIME chimera detection software executed in de novo mode (Edgar et al., 2011). After removing chimeric sequences, reads are mapped to their corresponding nonchimeric sequences via USEARCH global alignment algorithm (Edgar, 2010). Finally, the data is presented as percentage of sequences in the sample and are based on Kingdom, Phylum, Class, Order, Genus or Species. For our data, relative abundancies were normalized by phylum and then further normalized for cyanobacteria species.

3.9 Data Analysis

Principal Component Analysis (PCA) is a method that reduces the dimensionality of a set of data with multiple dimensions (parameters in our case) while still maintaining all relationships. Two dimensional plotting of PCA will give a generalized pattern for samples in terms of water quality parameters and cyanobacterial growth in the summer of 2016. PCA was performed for all sampled sites and months using the program R (R Development Core Team, 2008). Cyanobacterial relative abundancies were used to present cyanobacterial growth for comparisons between sampled months.

In order to understand the relationship between the growth of the abundant cyanobacteria in sediments and the different species of P found in sediment, a correlation analysis was applied using the same program R. Again, cyanobacterial relative abundancies were used to determine that relationship.

The Shannon Index is widely used to measure richness and diversity of an ecological community. The index was calculated using the software R [VEGAN package] for all sites in each month for all bacterial community and for cyanobacteria.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overall Water Chemistry

Utah Lake encountered an intense algal bloom event in July, 2016. A genetic identification approach to Utah Lake's bacterial and cyanobacterial community has not been implemented before. We have sampled the lake during that summer to observe the bacterial community present, specifically cyanobacteria. Our findings for water quality parameters pH, temperature, IC measured nutrients (nitrate-N, nitrite-N, and phosphate-P), and lab-measured chlorophyll a measurements for all sites at each month are shown in Table 3. Temperature increased from early summer to late summer, reaching the highest temperature in August. As for chlorophyll a, the highest measurements were observed during the bloom in the month of July. A similar pattern was observed for pH, which indicates high microbial activity during the bloom. Nitrogen species nitrate-N and nitrite-N had the highest concentrations during the bloom, which also indicates high microbial activity in addition to nitrification process occurring. As for phosphate-P, concentrations increased in the beginning of the bloom, decreased the next week, and then increased back up after the bloom. The release of phosphate into the water column occurs due to several processes. A possible source of P after the bloom would be the release of phosphate retained in algae after cells decay (de Montigny & Prairie, 1993).

Table 3: Water quality parameters. Temperature in Celsius degrees, pH, chlorophyll a in µg/L, and nutrients nitrate-N, nitrite-N, and orthophosphate-P concentrations in µg/L are shown for all 5 sites monitored monthly for Utah Lake in 2016.

		Parameters					
Month	**Site**	**Temp (C°)**	**pH**	**Chl a (µg/L)**	**Nitrate-N (µg/L)**	**Nitrite-N (µg/L)**	**Orthophosphate-P (µg/L)**
May	Provo Bay	16.47	8.3	6.41	0	0	2
	Lincoln Beach	15.58	7.87	1.07	158	0	0
	Geneva Discharge	15.55	7.96	13.88	92	0	0
	Saratoga Springs	15.2	7.76	18.16	5	0	2
	Pelican Point	15.62	8.16	9.61	113	0	0
June	Provo Bay	21.99	8.24	51.26	0	0	3
	Lincoln Beach	23.57	8.38	5.23	7	0	0
	Geneva Discharge	21.27	8	18.80	12	0	0
	Saratoga Springs	21.66	8.4	4.49	5	0	0
	Pelican Point	22.4	8.37	17.25	9	0	0
20-Jul	Provo Bay	21.5 [a]	9.06 [a]	103.6 [a]	127.5 ±0.57	511±108.1	15.75±2.3
	Lincoln Beach	23.25 [a]	9.27 [a]	223.6 [a]	6	0	3±1
	Geneva Discharge	22.53 [a]	8.65 [a]	188.8 [a]	13.25±0.5	65.3±10.4	37±3.2
	Saratoga Springs	21.46 [a]	8.49 [a]	76.4 [a]	109±0.57	133.3±37.8	15.75±2.3
	Pelican Point	22.42 [a]	8.98 [a]	226.4 [a]			
26-Jul	Provo Bay	22.96 [a]	8.95 [a]	84 [a]	50±2.82	929±45.2	0
	Lincoln Beach	24.27 [a]	9.07 [a]	22.6 [a]	0	1889.5±1160	3±1.7
	Geneva Discharge	23.45 [a]	8.81 [a]	61.3 [a]	65.3±10.4	0	0
	Saratoga Springs	23.16 [a]	8.7 [a]	50 [a]	132.5±31	108.5±0.57	0
	Pelican Point	22.83 [a]	8.65 [a]	108 [a]			
August	Provo Bay	23.28	8.71	26.3	1.5±0.7	0	4.5±0.57
	Lincoln Beach	24.26	8.69	10.69	2 ± 0	0	5 ± 1.41
	Geneva Discharge	22.54	8.67	24.06	66.5±0.57	0	15±3.8
	Saratoga Springs	23.33	8.77	34.75	9.5±3.7	0	5.5±1
	Pelican Point	25.19	8.87	48.11	7.25±0.5	0	6±1.4

[a] *is data provided by Utah's Department of Water Quality*

4.3 Bacterial Identification Using High Throughput DNA Amplicon Sequencing

Water samples 16S rRNA read count distributions in relative abundance on a bacterial phyla level are shown in Figure 4. Proteobacteria dominated all sites in all sampled months. Actinobacteria and Bacteriodetes phyla were second to dominate the sites. Proteobacteria dominated the water columns in all sampled sites throughout the summer. This large bacterial phylum contains six classes: *Alphaproteobacteria,*

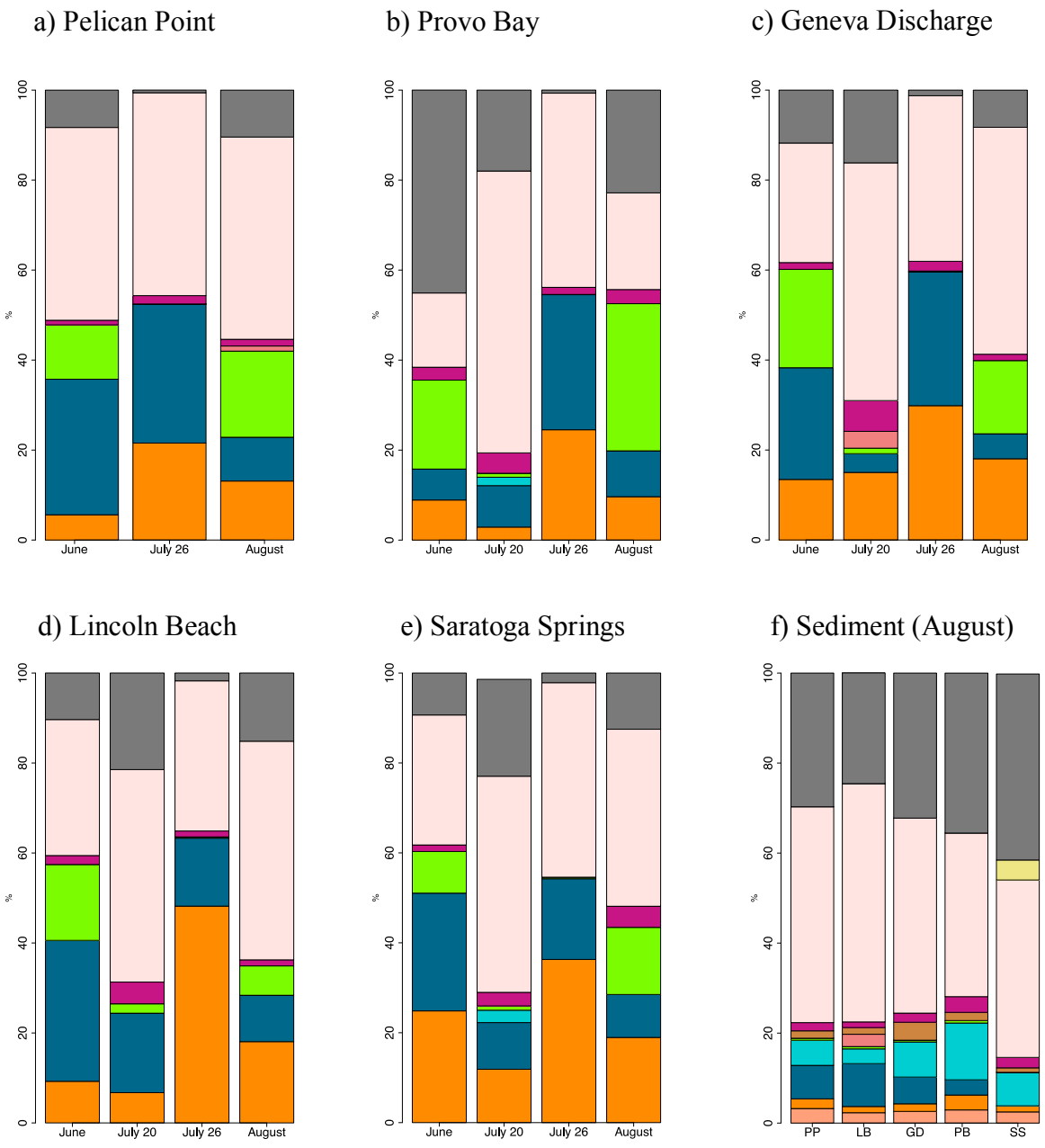

Figure 4: Relative abundancies for bacteria (phylum level) of sampled sites at Utah Lake. a) Pelican Point, b) Provo Bay, c) Geneva Discharge, d) Lincoln Beach, e) Saratoga Springs. Barplot f) shows sediment bacterial phyla relative abundancies for sediment collected in August 2016 for PP(Pelican Point), LB(Lincoln Beach), GD(Geneva Discharge), PB(Provo Bay), and SS(Saratoga Springs).

Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, and *Zetaproteobacteria* (Newton et al., 2011). Species under this phylum contain multiple metabolisms and traits that need to be studied specifically to the environment of shallow, eutrophic Utah Lake. Actinobacteria and Bacteriodetes were second to dominate the lake's water column. Research regarding freshwater lakes bacterial communities indicate that Proteobacteria, Actinobacteria, Bacteriodetes, and Cyanobacteria are prominent phylum (Eiler & Bertilsson, 2005; Newton et al., 2011). Our findings show that at a phylum level, fractions of bacteria throughout the lake were dominated by Proteobacteria, Bacteriodetes, Cyanobacteria, and Actinobacteria. Other minor phyla that were present were Planctomycetes and Firmicutes. Bacterial phyla dynamics shifted in the lake before, during, and after the HAB event. During the bloom (July 20th), cyanobacteria fractions dramatically decreased while Proteobacteria fractions increased. Bacteriodetes and Actinobacteria either decreased or remained the same on that first week of the bloom. During the second week (July 26th), however, it is apparent that Bacteriodetes and Actinobacteria fractions increased while Proteobacteria slightly decreased. Cyanobacteria remained in very small fractions. After the bloom in August, cyanobacteria fractions increased again while decreasing Bacteriodetes and Actinobacteria fractions.

Proteobacteria are gram-negative bacteria that are widely abundant in freshwater systems (Newton et al., 2011). Proteobacteria are divided into six classes: *alpha-, beta-, gamma-, zeta-, delta-, and epsilon-proteobacteria* (Newton et al., 2011). *Beta-proteobacteria* are abundant in freshwater systems; and they have the ability to degrade complex organic molecules (Eiler & Bertilsson, 2014). *Alpha-proteobacteria* can also degrade complex organic compounds and are competitive under low nutrient

conditions (Newton et al., 2011).

These characteristics allow Proteobacteria succession over other phyla. As for Bacteriodetes, studies show that there is no particular pattern as to their abundance in freshwater systems. However, they have been found to have high abundance after cyanobacterial blooms (Newton et al., 2011). This has shown to be true in our findings. Actinobacteria have been shown to have a negative correlation with cyanobacteria (Louati et al., 2015). Actinobacteria cell sizes are small and their cell walls are special in that they are resistant to flagellate grazing (Eiler, 2004). They also are UV stress resistant (Newton et al., 2011). These characteristics have allowed Actinobacteria to dominate in freshwater systems. However, their abundancies decrease with increased nutrient concentrations and decreased oxygen concentrations (Newton et al., 2011). Our findings show that Actinobacteria fractions grow during the second week of the bloom (July 27th). An explanation could be that nutrients have decreased, which allowed Actinobacteria's abundance to grow. Also, Actinobacteria's abundance decreases with increased Dissolved Organic Carbon (DOC) concentrations (Eiler, 2014). It is shown after the bloom that Actinobacteria fractions decrease, which can be explained by the decomposition of phytoplankton after decay of algal biomass.

As for the minor phylum Plactomycetes, it is understudied and underestimated when using 16S rRNA, since it is underestimated in the 16S rRNA gene clone libraries (Newton et al., 2011). Our results show Planctomycetes fractions grew in the first week of the bloom (July 20th), and at Saratoga Springs it had the largest fraction after the bloom. This phylum can perform ANaerobic AMMonium Oxidation (ANAMMOX) reactions; it also can degrade phytoplankton-derived carbohydrates (Newton et al., 2011). The phylum

Planctomycetes may have been underestimated in our findings; further studies are needed to identify their abundance in the future.

Water samples' 16S rRNA read count distributions in relative abundance for cyanobacterial species level is shown for each site and each sampling month in Figure 5. *Aphanizomenon flos-aquae* dominated most of the sites during (July) and after (August) the HAB. *Synechocuccos sp* dominated all sites in the month of June. However, it was also present during and after HAB events at some sites. *Microcystis aeruginosa* was present towards the end of the bloom around July 26 and August in some sites. Cyanobacteria phylum was more abundant before and after the bloom event. During the bloom, bacteria from the Planctomycetes phylum were more prominent than Cyanobacteria. Planctomycetes are a group of bacteria that contain unique bacterial structures and metabolism that play a critical part in the biochemical reactions and nutrient cycles (Fuerst & Sagulenko 2011; Newton et al., 2011). Planctomycetes contain ANAMMOX bacteria that oxidize ammonium to dinitrogen in anaerobic conditions, which is a significant step in the nitrogen cycle (Fuerst and Sagulenko 2011). Bacterial community identification and their interactions have not been studied in Utah Lake and need further investigations.

In this study, we are targeting cyanobacteria due to their toxicity. The lake had the lowest cyanobacterial abundancies during the bloom and increased after the bloom, which indicates that other algal species and bacteria dominated during the bloom. On a species level, *Aphanizomenon flos-aquae* and *Synechocuccos sp* were the dominant species during the summer of 2016. *Aphanizomenon flos-aquae* was dominant during and after the bloom in most sites.

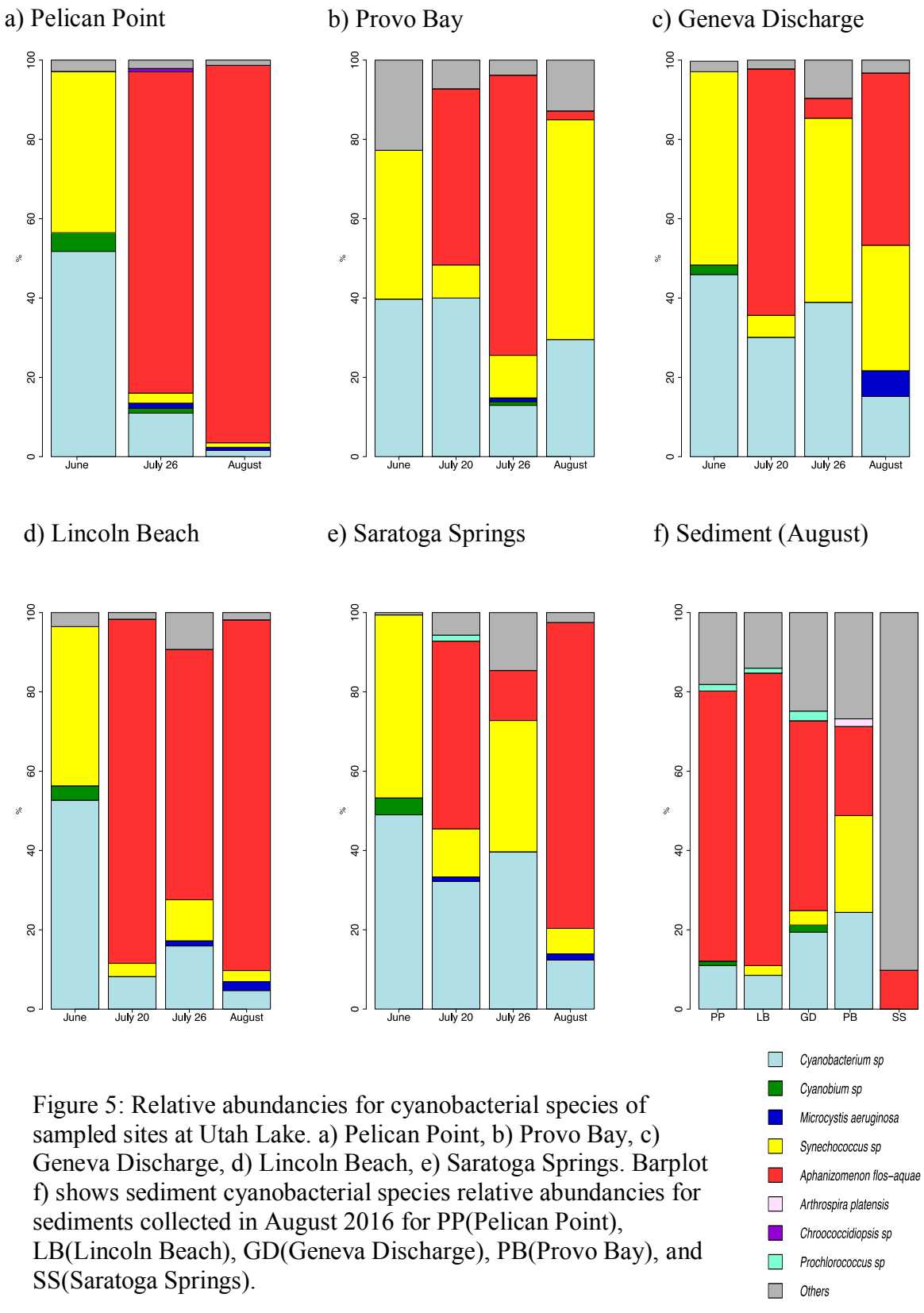

Figure 5: Relative abundancies for cyanobacterial species of sampled sites at Utah Lake. a) Pelican Point, b) Provo Bay, c) Geneva Discharge, d) Lincoln Beach, e) Saratoga Springs. Barplot f) shows sediment cyanobacterial species relative abundancies for sediments collected in August 2016 for PP(Pelican Point), LB(Lincoln Beach), GD(Geneva Discharge), PB(Provo Bay), and SS(Saratoga Springs).

Synechocuccos sp was dominant for all sites in June, in Geneva Discharge and Saratoga Springs in July 26, and Provo Bay in August. Both species correlated negatively in terms of growth. *Aphanizomenon flos-aquae* is a nitrogen-fixing bacteria that has the ability to produce toxins. However, to determine that ability, species must be isolated and cultured to identify the specific toxin-producing gene. (Cires & Ballot, 2016). *Synechocuccos sp* is an "autotrophic picoplankton (0.2-2 μm)" and is abundant in eutrophic freshwater lakes (Ye et al., 2011). Several strains of marine *Synechocuccos sp* have been found to be toxin-producing. However, freshwater *Synechocuccos* is poorly studied (Jakubowska & Szeląg-Wasielewska, 2015). *Microcystis aeruginosa* appeared in July 26 and August. Higher abundance was found in Geneva Discharge in August. Many strains of *Microcystis aeruginosa* are toxin-producing. However, genetic studies are required for identification (Scherer et al., 2016). Water column cyanobacterial species diversity pattern differed at sites.

Another minor species present was *Cyanobium sp.* The particular species that were present in *Synechocuccos sp* and *Cyanobacterium sp* were not identified. However, they belonged to the genera *Synechocuccos* and *Cyanobacterium*. Initially, species of *Cyanobacterium* genus were included in *Synechococcus* genus by NAG in 1849 due to their similarities. However, *Cyanobacterium* genus was later separated and established by Rippka and Cohen-Bazire in 1983 due to different mean DNA base composition (Moro et al., 2007). Both genera are considered a pico-cyanobacteria. Most of the phytoplankton production biomass belongs to pico-cyanobacteira, thus making them greatly influence the rate and amount of matter in the system (Jakubowska & Szeląg-Wasielewska, 2015). Efforts led by Utah's DWQ have indicated that *Aphanizomenon flos-aquae* was dominant

throughout the lake using microscopic methods and counting. Although our results also show that *Aphanizomenon flos-aquae* was the most dominant species during the bloom, Geneva Discharge and Saratoga Springs were shown to have *Synechocuccos sp* and *Cyanobacterium sp* dominant during July 27th. Before the bloom, both pico-cyanobacteria dominated the lake in all sites. The first week of the bloom (July 20th), *A. flos-aquae* dominated the water column in all sites. Fractions of *A. flos-aquae* correlated negatively with fractions of *Synechocuccos sp* and *Cyanobacterium sp*.

The change in cyanobacteria dynamics depends on multiple factors. Factors that influence the competition and dominance of cyanobacteria in a system include nutrients such as nitrogen and phosphorus, water temperature, light intensity, grazing, competition with other bacteria, etc. (Wu et al., 2016). During the bloom, our findings show *Cyanobacterium sp* to dominate second after *A. flos-aquae*. This is different at other times where it seems that *Synechocuccos sp* and *Cyanobacterium sp* have similar abundance fractions. Cyanobacteria sometimes dominate in the system either individually or synergistically (Wu et al., 2016). Our data show that *Synechocuccos sp* and *Cyanobacterium sp* may be aiding each other's growth; however, this relationship is different during July 20th. Pico-cyanobacteria can also produce cyanotoxins such as microcyctin toxins, which were found in the genus *Synechococcus* (*Synechococcus nidulans*) and in the genus *Cyanobacterium* (*Cyanobacterium cedrorum*) (Jakubowska & Szeląg-Wasielewska, 2015).

While nutrients control phytoplankton biomass in aquatic systems, temperature, light availability, and water residence time are key factors that promote the growth of specific cyanobacteria genera at different times (Paerl & Otten, 2016). *A. flos aquae's*

optimal growth occurs at temperatures ranging from 23-29 ^{o}C (Wu et al., 2016). Temperatures in the lake have reached > 23^{o}C in July 20th, which explains *A. flos aquae's* sudden growth during that time. During the warmer months, *A. flos aquae's* akinetes that were formed during colder months that rest at bottom sediments will germinate and move into the water column; and with higher temperatures, pH and sunlight *A.flos-aquae* will grow and form blooms (Yamamoto et al., 2009). Also, a lower N:P ratio (N:P <16) stimulates the growth of N-fixing cyanobacteria (Teubner et al., 1999). Nitrogen fixation requires bioavailable iron and trace metals that are essential for enzymatic reaction and their absence inhibits nitrogenase activity (Paerl & Otten, 2016). Multiple factors come into play regarding cyanobacteria succession among cyanobacteria phyla and against other bacterial phyla present. The growth of *Microcystis aeruginosa* is severely limited by low temperatures and its growth greatly declines below 15^{o}C (Wu et al, 2016). Indeed, our findings show that with higher temperatures, *Microcystis aeruginosa* fractions grew.

The Shannon Index (the index of diversity) was highest in July for Saratoga Springs but lowest for Pelican Point (shown in Table 4). A high Shannon Index refers to higher community diversity. Pelican Point and Lincoln Beach had a lower diversity index in August. The change in cyanobacterial diversity before, during, and after the bloom is different at each site.

Sediment samples 16S rRNA read count distributions in relative abundance on a bacterial phyla level are shown in Figure 4(f). Proteobacteria dominated all sites. A higher phylum variation was present in the sediment column in comparison to August's surface water phylum results. Read distributions of cyanobacterial species level is shown for each site in Figure 4(f).

Table 4: Shannon Index (measurement of richness and diversity) of water and sediment sample cyanobacterial speciation shown for each site. SI was calculated using the VEGAN package in R.

Shannon Index (Species)					
	June	20-Jul	26-Jul	August	August Sediment
Pelican Point	0.95	NA	0.74	0.26	0.93
Lincoln Beach	0.94	0.51	1.09	0.51	0.85
Geneva Discharge	0.89	0.90	1.09	1.30	1.30
Provo Bay	1.07	1.12	0.96	1.03	1.45
Saratoga Springs	0.87	1.25	1.27	0.79	0.32

Aphanizomenon flos-aquae dominated most sites. Shannon Index results for water and sediment samples are shown in Table 4. Diversity index ranged from 0.2 to 1.3. Shannon index also indicates that August's sediment diversity was higher than August's water samples diversity. Sediment samples were collected after the bloom in August. Compared to water column bacterial phylum, sediment samples had higher phylum diversity. Cyanobacteria phylum in sediment was present at a very low relative abundance compared to August water samples. On a cyanobacterial species level, *Aphanizomenon flos-aquae* dominated most sites. For most freshwater lakes, P is a limiting nutrient for algae growth. In shallow lakes, sediment plays a crucial part in P dynamics due to well mixing between water-sediment columns (Soondergaard et al., 2003). Many cyanobacteria have eco-physiological traits that allows them to uptake available P from sediments (Cottingham et al., 2015).

4.2 Sediment Samples

Sediment samples were collected in August after the bloom to observe P in sediments. Total phosphorus (TP) and P speciation measurements for each site in August

are shown in Table 5. Measurements were determined in triplicates along with quality control samples. Lake sediments were mostly dominated by calcium-bound phosphorus (Ca-P). TP measurements were highest at Pelican Point, with high residual-P (organic bound P) concentrations. Saratoga Springs and Lincoln Beach had the lowest residual-P concentrations, and the lowest TP concentrations between the sites. Our results for TP for the top 5 cm of cores collected ranged between 316 to 1105 mg-P/kg. Loosely bound P (L-P) results ranged from 4 to 28 mg-P/kg, clay-bound including iron and aluminum bound P (C-P) ranged from 4 to 27 mg-P/kg, and calcium bound P (Ca-P) ranged from 120 to 348 mg-P/kg. Results for TP in a study done by Chen et al. in 2014 on eutrophic Dongping Lake, China ranged from 425.9 to 729.6 mg-P/kg, Ca-P ranging from 230.7–417.3 mg-P/kg, and C-P ranging from 23.3–107.4 mg-P/kg. Similar to our results, top sediments from Dongping Lake were dominated by Ca-P (Chen et al., 2014). The range for C-P at Dongping Lake was much higher than our observed C-P concentrations. As for TP, our range for TP was much higher, reaching to 1105 mg-P/kg at Pelican Point. C-P and Ca-P concentrations have a negative correlation in sediments from Dongping Lake; this relationship was not observed in our data. P speciation in surface sediments of shallow, eutrophic Lake Dianchi has TP ranging from 1330 to 3711 mg-P/kg (Zhang et al., 2015), which is much higher than TP observed in our results. As for P fractions in surface sediments of Lake Dianchi, sediments in the Waihai region of the lake were dominated by different fractions. HCl-P dominated south of the region, residual-P dominated the center, and NaOH-P dominated the north. As for the Caohai region of Lake Dianchi, NH_4Cl-P, which is loosely-bound P, ranged from 23.4 to 51.5 mg-P/kg, while HCl-P ranged from 538.4 to 552.8 mg-P/kg (Zhang et al., 2015). These fraction ranges are considerably higher

Table 5: Total phosphorus and phosphorus speciation concentrations in mg of phosphorus per kg of sediment is shown for each sediment core collected at each site in August of 2016. Measurements are taken at three depths in each core, starting from 0-5 cm, then 5-15 cm, and finally 15-30 cm to cover up the entire sediment core. Standard deviation is calculated from the three measurements taken.

Sample	TP mg-P/kg sediment	L-P mg-P/kg sediment	C-P mg-P/kg sediment	Ca-P mg-P/kg sediment	R-P mg-P/kg sediment
Geneva Discharge (0-5cm)	536.03 ± 61.45	14.51 ± 3.23	8.87 ± 0.09	320.14 ± 21.74	192.48
Geneva Discharge (5-15cm)	576.65 ± 13.19	18.31 ± 6.25	5.58 ± 0.26	292.65 ± 7.24	260.09
Geneva Discharge (15-30cm)	515.84 ± 17.97	28.12 ± 3.15	4.49 ± 0.19	291.02 ± 19.17	192.20
Saratoga Springs (0-5cm)	316.55 ± 53.37	8.79 ± 0.76	27.77± 0.05	277.78 ± 0.51	2.19
Saratoga Springs (5-15cm)	371.87 ± 37.33	11.41 ± 0.622	27.76± 0.03	277.69 ± 0.32	55.01
Saratoga Springs (15-30cm)	455.37 ± 16.49	14.65 ± 1.12	27.74± 0.06	277.46 ± 0.65	135.51
Lincoln Beach (0-5cm)	356.66 ± 42.62	12.15 ± 0.92	8.47 ± 0.93	278.26 ± 18.03	57.76
Lincoln Beach (5-15cm)	411.85 ± 32.91	10.23 ± 0.74	6.77 ± 0.68	348.13 ± 19.02	46.7
Lincoln Beach (15-30cm)	428.18 ± 58.25	8.7 ± 0.04	5.9 ± 0.4	326.95 ± 13.02	86.63
Pelican Point (0-5cm)	1105.9 ± 268.45	13.6 ± 1.41	7.13 ± 0.34	186.55 ± 19.54	726.25
Pelican Point (5-15cm)	656.06 ± 19.40	11.86 ± 0.07	7.14 ± 0.26	173.34 ± 22.09	463.71
Pelican Point (15-30cm)	617.29 ± 116.41	7.76 ± 0.36	5.54 ± 0.19	142.93 ± 61.63	461.05
Provo Bay (0-5cm)	377.66 ±13.38	11.67 ± 1.05	5.73 ± 0.17	119.88 ± 40.85	240.37
Provo Bay (5-15cm)	374.86 ± 18.98	9.34 ± 0.38	5.16 ± 0.24	168.41 ± 31.31	191.93
Provo Bay (15-30cm)	411.61 ± 16.32	4.27 ± 0.38	3.7 ± 0.23	124.44 ± 21.92	279.2

Note: TP = Total Phosphorus (P), L-P = Loosely bound P, C-P = Clay bound P, Ca-P = Calcium bound P, and R-P = residual P

than our results, but this is also due to higher TP concentrations found at Lake Dianchi. Bottom lake sediment phosphorus fractions vary from lake to lake and depend on multiple factors such as sediments' minerology, biological activity, trophic status, and physiochemical and biogeochemical processes (Chen et al., 2014).

Looking at sediment minerology, XRD results are shown in Table 6. Results determined that calcite dominated the lake's sediments mineral composition. Calcite represented around 70% of the mineral composition in lake sediments at all sites. Pelican Point and Provo Bay had a higher quartz composition than other sites. During the summer, calcite precipitates during uptake of CO_2 by phototrophic organisms (algae), which can then enable coprecipitation of phosphate along with calcite at a high pH (~9), and in return

Table 6: XRD results for top 5 cm of sediment samples.

Utah Lake Mineral Composition by % mass (top 1 - 5cm)									
	Carbonate Minerals		Clay Minerals			Silica Oxides	Feldspars	Iron-rich Silicate Minerals	
Sample	Calcite	Dolomite	Smectite	Illite	Kaolinite	Quartz	Plagioclase	K-Feldspar	Amphibole
Geneva Discharge	71	4	tr	10			2	2	
Saratoga Springs	71	3	tr	9		10			
Lincoln Beach	75	3	tr	12		10			
Pelican Point	59	4	tr	4	2	20	6	4	
Provo Bay	10	4	tr	tr	tr	62	18	3	4
tr (trace) indicate that mineral is present, but that its abundance calculated from the Rietveld refinement was less than one weight percent, and/or it was observed in the clay-sized fraction, but not in the bulk sample.									

causes P limitation in the lake (Hamilton et al., 2009). High calcite content in Utah Lake's sediments is an indication to a high photosynthetic productivity.

Correlations for sediment phosphorus speciation in to relative abundancies of *Aphanizomenon flos-aquae* and *Synechocuccos sp* in sediments is shown in Table 7. Loosely-bound P had a high positive correlation with *Aphanizomenon flos-aquae.* Following that, TP had the next highest correlation. As for *Synechocuccos sp.*, there was no positive correlation to any of the P species in sediments. For *Cyanobacterium sp.,* however, it correlated positively to both C-P and Ca-P. Correlations for sediment TP and P-speciation results correlations with sediment's minerology is shown in Table 8. TP positively correlated to calcite and illite presence in the sediment samples. As for the highest P-species found in most of Utah Lake's sediments, Ca-P correlated positively to high calcite concentrations found in the sediments.

Table 7: Correlations between sediment Total Phosphorus (TP), Loosely bound P (L-P), Clay bound P (C-P), Calcium bound P (Ca-P), and Residual P(R-P) to the most dominant cyanobacterial species found in sediments for August 2016 samples.

	TP	L-P	C-P	Ca-P	R-P
Aphanizomenon flos-aquae	**0.53**	**0.69**	-0.62	**0.16**	**0.44**
Synechocuccos sp.	-0.29	-0.02	-0.43	-0.73	-0.03
Cyanobacterium sp.	-0.68	-0.49	**0.72**	**0.54**	-0.77

Table 8: Sediment P speciation and minerology correlations.

	TP	L-P	C-P	Ca-P	R-P
Calcite	0.424	0.317	0.147	0.728	0.162
Dolomite	-0.828	-0.797	0.359	0.188	-0.686
Illite	0.645	0.592	-0.249	0.461	0.445
Kaolinite	-0.412	-0.854	0.991	0.283	-0.471
Quartz	-0.282	-0.274	-0.126	-0.766	-0.026
Plagioclase	-0.481	-0.433	-0.053	-0.717	-0.217
K-Feldspar	-0.783	-0.946	0.625	-0.174	-0.657
Amphibole	-0.278	-0.122	-0.359	-0.801	-0.006

4.4 PCA

For a holistic approach to Utah Lake's HABs, Principle Component Analysis was performed for water samples for all sites in each month (shown in Figure 6). Sampled sites had dimensions of 39.9% and 23.1% of the dataset's variance, respectively.

A clear pattern distinction is shown between samples collected in June (before the bloom) and during and after the bloom. The growth of *Aphanizomenon flos-aquae* correlated highly to pH, chlorophyll a, dissolved phosphate (PO_4^{-2}-P), and water temperature. It is apparent that *Synechocuccos sp.* negatively correlated with *Aphanizomenon flos-aquae's* growth. Trends for Provo Bay samples in August related closely to trend for June's sampled sites in the PCA analysis. June samples overall had a different trend than the other sampled months. *Synechocuccos sp* dominated June sampled

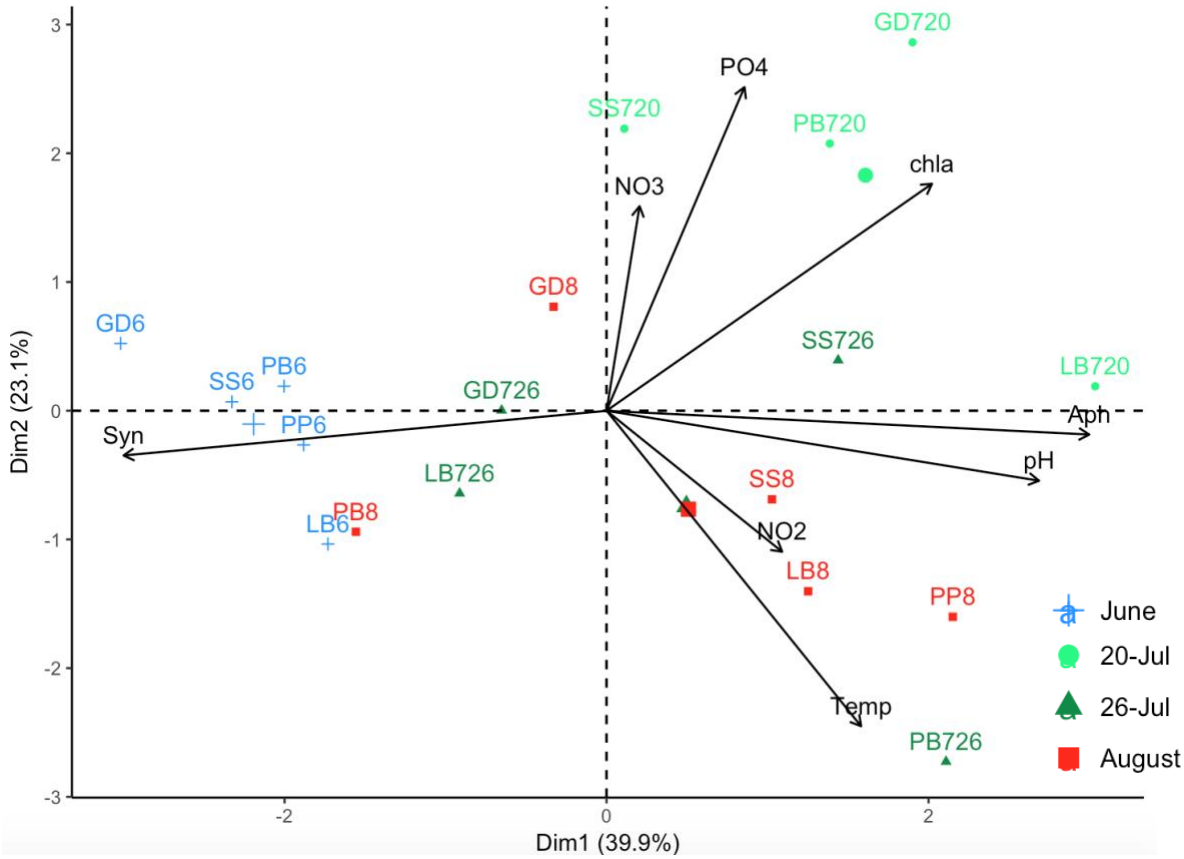

Figure 6: Principle Component Analysis for water samples plot for components (dimensions) 1 and 2 representing 39.9% and 23.1%, respectively. Variables for the PCA were pH, temperature (Temp), chlorophyll a (chla), nitrate-N (NO3), nitrite-N (NO2), orthophosphate-P (PO4), *Aphanizomenon flos-aquae* correlated relative abundance (Aph), and *Synechocuccos sp*. correlated relative abundance (Syn). Individuals represent the sampled five sites in the months of June-August of summer 2016.

sites as well as Provo Bay in August. July 20th and July 26th samples are closely related as *Aphanizomenon flos-aquae* dominated the sites with the exception of Provo Bay in July 26th. This analysis supports our observations and builds a holistic view and relationship to all water quality parameters.

CHAPTER 5

CONCLUSION

High throughput amplicon sequencing was able to identify an abundant picocyanobacteria *Synechococcus sp.* that was overlooked when using microscopic methods for cyanobacteria identifications. The two most abundant cyanobacterial species were *Aphanizomenon flos-aquae* and *Synechocuccos sp,* which can have toxic strains that would be harmful to both humans and other living organisms. Toxic *Microcystis aeruginosa* was also detected in our study for summer 2016. We conclude that it is important to consider water quality parameters when understanding eutrophication of freshwater lakes and harmful cyanobacterial blooms. Our findings also show a shift in the bacterial community before, during, and after the bloom. It is important to consider other bacterial influences on cyanobacteria.

Our conclusions lay a foundation to the importance of incorporating genetic analysis when studying harmful algal blooms in Utah Lake. Moving forward on Utah Lake's research on harmful cyanobacterial blooms, further studies are needed to determine toxic strains and cyanobacterial growth triggers. Further studies are also needed to determine relationships between other bacteria and cyanobacteria.

REFERENCES

American Public Health Association (1999). "Section 10200 Chlorophyll." *Standard Methods for the Examination of Water and Wastewater*, 20th Ed., Clescerl, Lenore S., Greenberg, Arnold E., and Eaton, Andrew D.

Backer, L. C., Manassaram-Baptiste, D., LePrell, R., and Bolton, B. (2015). "Cyanobacteria and Algae Blooms: Review of Health and Environmental Data from the Harmful Algal Bloom-Related Illness Surveillance System (HABISS) 2007-2011." *Toxins*, 7(4), 1048-1064.

Boyer, J. N., Kelble, C. R., Ortner, P. B., and Rudnick, D. T. (2009). "Phytoplankton Bloom Status: Chlorophyll a Biomass as an Indicator of Water Quality Condition in The Southern Estuaries of Florida, USA." *Ecological Indicators*, 9(6), S56-S67.

Callieri, C. (2008). "Picophytoplankton in Freshwater Ecosystems: The Importance of Small-Sized Phototrophs." *Freshwater Reviews*, 1(1), 1-28.

Cao X., Wang Y., He J., Luo X., and Zheng Z. (2016). "Phosphorus Mobility among Sediments, Water and Cyanobacteria Enhanced by Cyanobacteria Blooms in Eutrophic Lake Dianchi." *Environmental Pollution*. 219, 580-587.

Carmichael, W. W. (2001). "Health Effects of Toxin-Producing Cyanobacteria:"the CyanoHABs"." *Human and Ecological Risk Assessment: An International Journal*, 7(5), 1393-1407.

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., and Smith, V. H. (1998). "Nonpoint Pollution of Surface Waters with Phosphorus and Nitrogen." *Ecological Applications*, 8(3), 559-568.

Cires, Samuel and Ballot, Andreas (2016). "A Review of the Phylogeny, Ecology and Toxin Production of Bloom-Forming Aphanizomenon spp. and Related Species within the Nostocales (Cyanobacteria)." *Harmful Algae*, 54, pp 21-43

World Health Organization. (1996). "Chapter 3 - Selection of Water Quality Variables." Water Quality Assessments: A Guide to the use of Biota, Sediments and Water in Environmental Monitoring, Second Edition. Chapman, D. V. pp.74-133

Chen, Y., Chen, S., Yu, S., Zhang, Z., Yang, L., and Yao, M. (2014). "Distribution and Speciation of Phosphorus in Sediments of Dongping Lake, North China." *Environmental Earth Sciences*, 72(8), 3173-3182.

Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., and Likens, G. E. (2009). "Controlling Eutrophication: Nitrogen and Phosphorus." *Science*, 323(5917), 1014-1015.

Correll, D. L. (1998). "The Role of Phosphorus in the Eutrophication of Receiving Waters: a Review." *Journal of Environmental Quality*, 27(2), 261-266.

Cottingham, K. L., Ewing H. A., Greer M. L., Carey C. C., and Weathers K. C. (2015). "Cyanobacteria as Biological Drivers of Lake Nitrogen and Phosphorus Cycling." *Ecosphere*, 6(1), 1-19

Delwiche, C. C. (1970). "The Nitrogen Cycle." *Scientific American*, 223(3), 136-147.

Douterelo I., Perona E., and Mateo P. (2004). "Use of Cyanobacteria to Assess Water Quality in Running Waters." *Environmental Pollution,* 127, 377–384

Downing, J. A. and McCauley, E. (1992). "The Nitrogen: Phosphorus Relationship in Lakes." *Limnology and Oceanography*, 37(5), 936-945.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., and Sullivan, C. A. (2006). "Freshwater Biodiversity: Importance, Threats, Status and Conservation Challenges." *Biological Reviews*, 81(2), 163-182.

Eiler, Alexander and Bertilsson, Stefan (2004). "Composition of Freshwater Bacterial Communities Associated with Cyanobacterial Blooms in Four Swedish Lakes." *Environmental Microbiology*, 6(12), 1228-1243.

EPA (2017). "Summary of the Clean Water Act". *Laws & Regulations* <https://www.epa.gov/laws-regulations/summary-clean-water-act> (February 02, 2018).

Fuerst, John and Sagulenko, Evgeny (2011). "Beyond the Bacterium: Planctomycetes Challenge our Concepts of Microbial Structure and Function." Nature Reviews *Microbiology*, 9, 403-413.

Geider, R. J. and La Roche, J. (2002). "Redfield Revisited: Variability of C [ratio] N [ratio] P in Marine Microalgae and its Biochemical Basis." *European Journal of Phycology*, 37(1), 1-17.

Gregor, J. and Marsalek, B. (2004). "Freshwater Phytoplankton Quantification by Chlorophyll a: a Comparative Study of In Vitro, In Vivo and In Situ Methods." *Water Research*, 38(3), 517-522.

Hamilton, Stephen, Bruesewitz, Denise, Horst, Geoffrey, Weed, David and Sarnelle, Orlando (2009). "Biogenic Calcite–Phosphorus Precipitation as a Negative Feedback to Lake Eutrophication." *Canadian Journal of Fisheries and Aquatic Sciences*, 66, 343-350.

Herrero, A., Muro-Pastor, A. M., and Flores, E. (2001). "Nitrogen Control in Cyanobacteria." *Journal of Bacteriology*, 183(2), 411-425.

Huang Q., Wang Z., Wang C., Wang S., and Jin X (2005). "Phosphorus Release in Response to pH Variation in the Lake Sediments with Different Ratios of Iron-Bound P to Calcium-Bound P." *Chemical Speciation & Bioavailability,* 17, 55-61.

Hudnell, H. K. (2008). "Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs." *Springer Science & Business Media*, 619.

Jakubowska, Natalia and Szelag-Wasielewska (2015). "Toxic Picoplanktonic Cyanobacteria—Review." *Marine Drugs,* 13, 1497-1518.

Jetten, M. S. (2008). "The Microbial Nitrogen Cycle." *Environmental Microbiology*, 10(11), 2903-2909.

Johnson, Z and Martiny, A. (2015). "Techniques for Quantifying Phytoplankton Biodiversity." *Annual Review of Marine Sciences*, 7, 299–324.

Kasinak,J. Holt, B. Chislock, M. Wilson, A. (2014). "Benchtop Fluorometry of Phycocyanin as a Rapid Approach for Estimating Cyanobacterial Biovolume." *Journal of Plankton Research,* 37 (1), 248-257.

Kenny, J. F., Barber, N. L., Hutson, S. S., Linsey, K. S., Lovelace, J. K., and Maupin, M. A. (2009). "Estimated Use of Water in the United States in 2005." *Circular 1405*. US Geological Survey, 1344.

Kraemer, R. A., Choudhury, K., and Kampa, E. (2001). "Protecting Water Resources: Pollution Prevention." *In Thematic Background Paper, Secretariat of the International Conference on Freshwater–Bonn.*

Lenton, T. M. (2013). "Environmental Tipping Points." *Annual Review of Environment and Resources*, 38, 1-29.

Li, Haiyan, Liu, Liang, Li, Mingyi, and Zhang, Xiaoran (2013). "Effects of pH, Temperature, Dissolved Oxygen, and Flow Rate on Phosphorus Release Processes at the Sediment and Water Interface in Storm Sewer." *Journal of Analytical Methods in Chemistry*, 2013.

Louati, I., Pascault, N., Debroas, D., Bernard, C., Humbert, J. F., and Leloup, J. (2015). "Structural Diversity of Bacterial Communities Associated with Bloom-Forming Freshwater Cyanobacteria Differs According to the Cyanobacterial Genus." *PLoS One*, 10(11).

Lukkari, K., Hartikainen, H., and Leivuori, M. (2007). "Fractionation of Sediment Phosphorus Revisited. I: Fractionation Steps and their Biogeochemical Basis." Limnology and Oceanography: *Methods*, 5(12), 433-444.

Marcarelli, A.M., Baker, M.A., and Wurtsbaugh, W.A. (2008). "Is In-Stream N2 Fixation an Important N Source for Benthic Communities a9nd Stream Ecosystems?" *Journal of North American Benthological Society*, 27, 186–211.

de Montigny, C., and Prairie, Y. T. (1993). "The Relative Importance of Biological and Chemical Processes in the Release of Phosphorus from a Highly Organic Sediment." *Hydrobiologia*, 253(1-3), 141-150.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baurès, E., and Thomas, O. (2013). "State of Knowledge and Concerns on Cyanobacterial Blooms and Cyanotoxins." *Environment International*, 59, 303-327.

Moro, I., Rascio, N., La Rocca, N., Di Bella, M., and Andreoli, C. (2007). "Cyanobacterium Aponinum, a New Cyanoprokaryote from the Microbial Mat of Euganean Thermal Springs (Padua, Italy)." *Algological Studies*, 123(1), 1-15.

Murphy J. and Riley J. P. (1962). "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters." *Analytica Chemica Acta,* 27, 31 – 36.

National Science and Technology Council (NSTC) (2016). "Harmful Algal Blooms and Hypoxia Comprehensive Research Plan and Action Strategy: an Interagency Report."<https://cdn.coastalscience.noaa.gov/page-attachments/research/FINAL_HABs%20Hypoxia%20Research%20Plan%20and%20Action.pdf> (March 7, 2018).

Newton, Ryan, Jones, Stuart, Eiler, Alexander, McMahon, Katherine, and Bertilsson, Stefan (2011). "A Guide to the Natural History of Freshwater Lake Bacteria." *Microbiology and Molecular Biology Reviews*, March 2011, 14-49.

Nübel, U., Garcia-Pichel, F., and Muyzer, G. (1997). "PCR Primers to Amplify 16S Rrna Genes from Cyanobacteria." *Applied Environmental Microbiology*, 63, 3327–3332.

Oki, T. and Kanae, S. (2006). "Global Hydrological Cycles and World Water Resources." *Science*, 313(5790), 1068-1072.

Paerl, H. W. (2017). "Controlling Cyanobacterial Harmful Blooms in Freshwater Ecosystems." *Microbial Biotechnology*, 10(5), 1106-1110.

Paerl, H. (2008). "Nutrient and Other Environmental Controls of Harmful Cyanobacterial Blooms Along the Freshwater–Marine Continuum." *In Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer, New York, NY. 217-237.

Paerl, H. W., Fulton, R. S., Moisander, P. H., and Dyble, J. (2001). "Harmful Freshwater Algal Blooms, with an Emphasis on Cyanobacteria." *The Scientific World Journal*, 1, 76-113.

Paerl, H. W. and Otten, T. G. (2016). "Duelling 'CyanoHABs': Unravelling the Environmental Drivers Controlling Dominance and Succession among Diazotrophic and Non-N2-Fixing Harmful Cyanobacteria." *Environmental Microbiology*, 18(2), 316-324.

Paludan, Claus and Jensen, Henning (1995). "Sequential Extraction of Phosphorus in Freshwater Wetland and Lake Sediment: Significance of Humic Acids." *Wetland,* 15(4), 365-373.

Pfaff, John (1993). "Method 300.0 Determination of Inorganic Anions by Ion Chromatography." *USEPA: Inorganic Chemistry Branch, Chemistry Research Division* < https://www.epa.gov/sites/production/files/2015-08/documents/method_300-0_rev_2-1_1993.pdf> (January 28, 2018).

Pimentel, D., Berger, B., Filiberto, D., Newton, M., Wolfe, B., Karabinakis, E., and Nandagopal, S. (2004). "Water Resources: Agricultural and Environmental Issues." *BioScience*, 54(10), 909-918.

Psenner, R., Bostrom, B., Dinka, M., Pettersson, K,. Puscko, R., and Sager, M. (1988). "Fractionation of Phosphorus of Suspended Matter and Sediment." *Archiv für Hydrobiologie–BeiheftErgebnisse der Limnologie*, 30, 98-103.

PSOMAS and SWCA (2007). "Utah Lake TMDL: Pollutant Loading Assessment & Designated Beneficial. Use Impairment Assessment." State of Utah Division of Water Quality.

Qin, B., Gao, G., Zhu, G., Zhang, Y., Song, Y., Tang, X., and Deng, J. (2013). "Lake Eutrophication and its Ecosystem Response." *Chinese Science Bulletin*, 58(9), 961-970.

Reynolds, C. S., Huszar, V., Kruk, C., Naselli-Flores, L., and Melo, S. (2002). "Towards a Functional Classification of the Freshwater Phytoplankton." *Journal of Plankton Research*, 24(5), 417-428.

Rigosi, Anna, Carey, Cayelan, Ibelings, Bas, and Brookes, Justin (2014). "The Interaction Between Climate Warming and Eutrophication to Promote Cyanobacteria is Dependent on Trophic State and Varies Among Taxa." *Limnological Oceanographhy* 59, 99-114.

Scherer, Pia, Raeder, Uta, Geist, Juergen, and Zwirglmaier, Katrin (2016). "Influence of Temperature, Mixing, and Addition on of Microcystin-LR on Microcystin Gene Expression in Microcystis Aeruginosa." *Microbiology Open,* 6(1).

Smith, V.H., Tilamn G.D., and Nekola, J.C. (1999). "Eutrophication: Impacts of Excess Nutrient Inputs on Freshwater, Marine, and Terrestrial Ecosystems." *Environmental Pollution*, 100, 179-196.

Smith, V. H. and Schindler, D. W. (2009). "Eutrophication Science: where do we go from here?" *Trends in Ecology & Evolution*, 24(4), 201-207.

Sondergaard, Martin and Jeppesen, Erik (2003). "Role of Sediment and Internal Loading Phosphorus in Shallow Lakes." *Hydrobiologia,* 506-509, 135-145

Stackelberg, Nicholas (2016). "Utah Lake Nutrient Model Selection Report." Utah Department of Water Quality. < https://deq.utah.gov/legacy/destinations/u/utah-lake/docs/2016/Utah-Lake-Model-Selection.pdf>. (February 5, 2018).

Teubner, K., Feyerabend, R., Henning, M., Nicklisch, A., Woitke, P., and Kohl, J. G. (1999). "Alternative Blooming of Aphanizomenon Flos-Aquae or Planktothrix Agardhii Induced by the Timing of the Critical Nitrogen: Phosphorus Ratio in Hypertrophic Riverine Lakes (With 8 figures and 2 tables)." *Ergebnisse der Limnologie*, *54*, 325-344.

Utah Department of Agriculture and Food. (2016) "Lab Tests Confirm Possible Health Risks." *Farmers and ranchers advised not to use water taken from Utah Lake or the Jordan river* < https://www.ag.utah.gov/home/news/614-farmers-and-ranchers-urged-to-use-caution-with-water-taken-from-utah-lake.html> (February 5, 2018).

Utah Department of Water Quality. (2016). "Utah Lake, Jordan River, Canals Algal Bloom 2016." < https://deq.utah.gov/Divisions/dwq/health-advisory/harmful-algal-blooms/bloom-events/bloom-2016/utah-lake-jordan-river/index.htm> (January 18, 2018).

Utah Division of Water Quality (UDWQ) (2014). "Standard Operating Procedure for Collection of Lake Water Samples. State of Utah." Department of Environmental Quality. Revision 0. Effective May 1, 2014.

Utermöhl, H. (1958). "Zur Vervollkommnung der Quantitativen Phytoplankton-Methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel." *Internationale Vereinigung für Theoretische und Angewandte Limnologie: Mitteilungen*, 9(1), 1-38.

Winder, M. and Sommer, U. (2012). "Phytoplankton Response to a Changing Climate." *Hydrobiologia*, 698(1), 5-16.

Wu, Y., Li, L., Zheng, L., Dai, G., Ma, H., Shan, K., and Song, L. (2016). "Patterns of Succession between Bloom-Forming Cyanobacteria Aphanizomenon Flos-Aquae and Microcystis and Related Environmental Factors in Large, Shallow Dianchi Lake, China." *Hydrobiologia*, 765(1), 1-13.

Ye, Wenjin, Tan, Jing, Liu, Xianglong, Lin, Shengqin, Pan, Jianliang, Li, Daotang, and Yang, Hong (2011). "Temporal Variability of Cyanobacterial Populations in the Water and Sediment Samples of Lake Taihu as Determined by DGGE and Real-Time PCR." *Harmful Algae*, 10, 472-479.

Zhang, R., Wang, L., and Wu, F. (2015). "Phosphorus Speciation in Surface Sediments of a Hypertrophic Lake, Southwestern China: Insights from Fractionation and 31P NMR". *Chinese Journal of Geochemistry*, 34(2), 167-176.