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Factors Affecting Phytoplankton Biodiversity and Toxin Production

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Factors Affecting Phytoplankton Biodiversity and Toxin Production

by

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A Dissertation submitted in partial satisfaction of
the requirements for the degree of
Doctor of Philosophy in Biology

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June 2011

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ABBREVIATIONS

Abs	Absorbance
ALK	Alkalinity
NH4	Ammonium
Chl-a	Chlorophyll-a
DIN	Dissolved inorganic nitrogen
DON	Dissolved organic nitrogen
DOP	Dissolved organic phosphorus
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DWL	Drinking water limits
EC	Electrical conductivity
EPA	Environmental Protection Agency
H′	Shannon Index for Richness
HCl	Hydrochloric acid
IPCC	Intergovernmental Panel on Climate Change
IRWD	Irvine Ranch Water District
J′	Pielou's Index for Evenness
LC/MS	Liquid-chromatography/mass spectrometry
MERHAB	Monitoring and Event Response for Harmful Algal Blooms
NO_3	Nitrate
NO_2	Nitrite
OD	Optical density
PN	Particulate nitrogen
PP	Particulate phosphorus
PPIA	Protein phosphatase inhibition assay
PO_4	Phosphate
SRP	Soluble Reactive Phosphorus
TDN	Total dissolved nitrogen
TDP	Total dissolved phosphorus
TDS	Total dissolved solids
TN	Total nitrogen
TP	Total phosphorus
UCD	University of California, Davis
WHO	World Health Organization

ABSTRACT OF THE DISSERTATION

Factors Affecting Phytoplankton Biodiversity and Toxin Production

by

Tracey Magrann

Doctor of Philosophy, Graduate Program in Biology
Loma Linda University, June 2011
Dr. Stephen G. Dunbar, Chairperson

This dissertation reports a survey of water quality, algal biodiversity, and toxin production in Southern California lakes, ponds, bays, and lagoons along the California coastline, in five counties from Santa Barbara to San Diego. Samples were analyzed for pH, electrical conductivity, total dissolved solids, dissolved oxygen, alkalinity, chlorophyll–a, total phosphorous, phosphate, total dissolved phosphorous, dissolved organic and inorganic phosphorous, particulate phosphorous, total nitrogen, total dissolved nitrogen, dissolved organic and inorganic nitrogen, nitrate, nitrite, and ammonium. Algal assays were conducted for phytoplankton identification and numbers, and indices of biodiversity (richness and evenness) were calculated to ascertain relationships between nutrients and algal growth, especially beneficial Chlorophyta and toxic Cyanophyta.

After identifying several sites which contained algal toxins in excess of drinking water limits and high numbers of the cyanobacterium, *Microcystis*, research was continued to ascertain the impact of *Microcystis* on algal biodiversity. A series of beakers was inoculated with *Microcystis*, in addition to the genera of algae that were most commonly seen throughout our study sites, and a control set of beakers was prepared without *Microcystis*. Weekly algal assays were conducted for five weeks, and richness

and evenness were calculated. Having demonstrated that *Microcystis* negatively impacts algal biodiversity, we tested the effectiveness of a Blue Pro™ water treatment facility in removing this colonial organism from a small, freshwater lake, in addition to removal of dissolved nutrients required for its growth. While using this technology at Mason Lake (Irvine, CA, USA), PO_4 levels and algal compositions were evaluated in one sample of lake water before and after treatment. A PO_4 restriction assay on the algae in Mason Lake at that time revealed the PO_4 threshold needed for algal growth was 0.02 mg $\cdot$ L^{-1}. Our study demonstrated that water chemistry factors are correlated with the presence of the cyanobacterium, *Microcystis*, and that removal of that species may allow green algae to increase in numbers, improving biodiversity in ponds and small lakes. The application of this knowledge will benefit zooplankton, fishes, endangered birds, and other organisms in those habitats.

CHAPTER ONE

INTRODUCTION

The purpose of the study was to evaluate the safety of a number of sites along the Southern California coastline which are of ecological importance to fishes, birds, other animals, and humans. The objectives were to ascertain relationships between nutrient loads and eutrophication, evaluate the impact of the cyanobacterium, *Microcystis*, on algal biodiversity, determine the effectiveness of new technology to remove dissolved nutrients and colonial toxic algae, and find the phosphate threshold needed for algal growth.

Hypotheses

- Algal compositions and toxin production in Southern California lakes, ponds, bays, and lagoons covary with ambient water column nutrient concentrations.
- Biodiversity of phytoplanktonic taxa in Southern California lentic habitats declines and shifts towards more pollution tolerant species (Cyanophyta) as nutrient loads increase.
- *Microcystis* negatively impacts algal biodiversity.
- Blue Pro™ technology decreases phosphate and removes toxic algae from ponds.
- There is a phosphate threshold needed for algal growth at Mason Lake.

These hypotheses are important to investigate, especially along the Pacific Flyway, extending from Alaska to Patagonia, which is a major migratory route for many endangered species of birds. This pathway provides critical food sources, breeding grounds, rest stops, and habitats for birds, fishes, and amphibians. Southern California coastal lakes, ponds, bays, and lagoons are an important segment of this migratory route, and evaluation of water quality is essential. Nuisance algal blooms and their toxins are frequent threats to this important ecological system. This chapter will discuss nutrient loading as a major systemic stressor significantly impacting lentic ecosystems, toxins produced by the cyanobacterium, *Microcystis*, and the impact of nutrients and *Microcystis* on algal biodiversity.

Nutrient Loads

Water quality of ponds and lakes reflects the health of aquatic and terrestrial ecosystems, as well as the stability of the larger watershed. Lakes with dilute water are especially sensitive to environmental conditions because small changes can result in pronounced ecological effects, such as rapid fluctuations in pH and changes in algal community compositions (Heard *et al.* 2008). Long–term eutrophication is often caused by nitrogen and phosphorous inputs, which alter nutrient cycles, and cause shifts in phytoplankton communities (Goldman *et al.* 1993, Sickman *et al.* 2003). Eutrophication can be increased in some lakes due to atmospheric deposition of nutrients (Sickman *et al.* 2003).

Nitrogenous Nutrients

Nitrogen is generally the growth limiting nutrient in estuaries and coastal waters (Wetzel 2001), and is present as nitrate (NO_3), nitrite (NO_2), ammonium (NH_4), and nitrogen gas (N_2), which are biochemically inter–convertible components of the nitrogen cycle (Kaiser 1969). Total nitrogen (TN) represents all nitrogen found in an unfiltered water sample, and can be further divided into different forms, as illustrated in Figure 1.1.

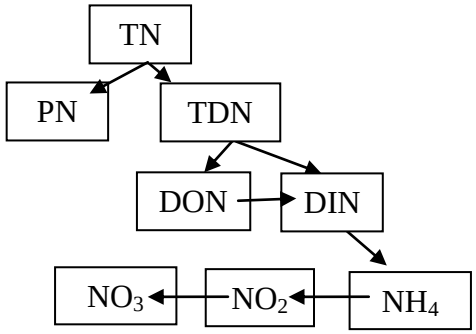

Fig. 1.1. Forms of nitrogen: total nitrogen (TN), particulate nitrogen (PN), total dissolved nitrogen (TDN), dissolved organic nitrogen (DON), dissolved inorganic nitrogen (DIN), nitrate (NO_3), nitrite (NO_2), and ammonium (NH_4). Arrows indicate direction of conversion from one form to another.

Particulate nitrogen (PN) is that part of the total nitrogen that remains in solid form. Therefore, total dissolved nitrogen (TDN) is measured after water is filtered. Dissolved nitrogen can be organic or inorganic. Dissolved organic nitrogen (DON) is found in the cells of all living things and is a component of proteins, peptides, and amino acids. When plants and animals die, proteins (which contain DON) are broken down by bacteria, causing DON to first become dissolved inorganic nitrogen (DIN), then ammonium (NH_4). Other bacteria convert NH_4 into nitrite (NO_2), which is rapidly

converted to nitrate (NO_3). Nitrates can then be used for growth by plants and algae. Therefore, ponds with dead leaves, insects, or other decaying organic material will have high values of these forms of nitrogen. Excess nitrates have several deleterious effects on birds, including inhibition of thyroid hormone (Fidanci *et al.* 2010), impaired growth (Grizzle *et al.* 1997), liver and kidney damage, and immune dysfunction (Atefa *et al.* 1991). High concentrations of nitrite can produce "brown blood disease" in freshwater fishes (MSU 1998). In this case, nitrite enters the bloodstream through the gills and turns the blood a chocolate–brown color. The blood cannot transport oxygen effectively, and the fishes suffocate, despite adequate oxygen concentration in the water (MSU 1998).

Major sources of nitrogen in aquatic ecosystems include human and animal waste, fertilizers, fossil fuels, and cleaning products. Ammonia and organic forms of nitrogen are largely removed by wastewater treatment plants. However, these processes result in an increase in nitrate discharge, thus, no changes in total nitrogen takes place. Therefore, while general concerns about toxicity in fishes have decreased, the potential for eutrophication has not (Mueller and Helsel 1999).

Nitrate (NO_3) is the stable form of nitrogen that develops when oxygen is present (Stumm and Morgan 1981). Nitrate generally occurs in trace quantities in surface water, but may attain high levels in some groundwater (Bartsch and Gakstatter 1978). In excessive amounts, it contributes to methemoglobinemia in infants and must therefore be limited to 10 mg/L in drinking water (Lundquist 1975). It may be found in concentrations up to 30 mg/L in fresh wastewater (Moore and Thornton 1988). It is an essential nutrient for many photosynthetic autotrophs and may be a growth–limiting nutrient. Nitrate often contaminates groundwater when water originates from manure pits, fertilized fields,

lawns, or septic systems (Shaw *et al.* 2004). A concentration of nitrate–nitrogen (NO_3–N) plus ammonium–nitrogen (NH_4–N) of 0.3 mg/ L will support algal blooms if enough phosphorus is present (Shaw *et al.* 2004).

Nitrite (NO_2) rapidly converts to nitrate and is therefore an intermediate oxidation state of nitrogen (Stumm and Morgan 1981). Nitrite can enter a water supply system through its use as a corrosion inhibitor in industrially processed water (Shaw *et al.* 2004). When nitrite occurs in acidic solutions, it can react with secondary amines to form nitrosamines, many of which are carcinogenic (Shaw and Nimphius 1985).

Ammonium (NH_4) is a form of nitrogen found in organic materials and many fertilizers. It is the first form of nitrogen released when organic matter decays (DeZuane 1997). It can be used by most aquatic plants and is therefore an important nutrient. It converts rapidly to nitrate (NO_3) if oxygen is present (DeZuane 1997). The conversion rate is related to water temperature (Feldman 1956). Ammonia is toxic to fishes at relatively low concentrations in pH–neutral or alkaline water (Feldman 1956). Under acid conditions, non–toxic ammonium ions (NH_4^+) form, but at high pH values toxic ammonium hydroxide (NH_4OH) occurs (Shaw and Nimphius 1985). The water quality standard for fishes and aquatic life is 0.02 mg/ L of NH_4OH. At a pH of 7 and a temperature of 20° C, the ratio of ammonium ions to ammonium hydroxide is 250:1; at pH 8, the ratio is 26:1 (Shaw and Nimphius 1985). Ammonia is present naturally in surface waters. Its concentration is generally low in groundwater because it adsorbs to soil particles and is not leached readily from soils (Clesceri *et al.* 1989). It is mainly produced by hydrolysis of urea. Ammonia concentrations in freshwater vary from 10 to 30 µg/L (WDNR 1980).

Phosphorous Nutrients

While nitrogen is the growth limiting nutrient in coastal waters, phosphorus is often the growth limiting nutrient in freshwater lakes and rivers because it occurs in the least amount relative to the needs of plants (Wetzel 2001). Total phosphorus (TP) represents all phosphorous found in an unfiltered freshwater or seawater sample, and can be further divided into different forms, as illustrated in Figure 1.2.

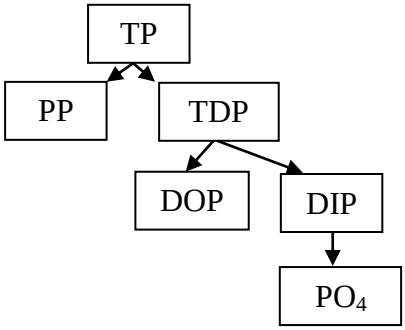

Fig.1 2. Forms of phosphorus: total phosphorous (TP), particulate phosphorous (PP), total dissolved phosphorous (TDP), dissolved organic phosphorous (DOP), dissolved inorganic phosphorous (DIP), and phosphate (PO_4). Arrows indicate direction of conversion from one form to another.

Total phosphorus (TP) includes particulate forms plus total dissolved phosphorous (TDP), which is the amount of phosphorus in solution (Sawyer 1947). Phosphorus (P) is the key nutrient that influences plant growth in lakes (Shaw *et al.* 2004). Every organism contains a large amount of phosphorous. Therefore, ponds with dead leaves, insects, or other decaying material will have a high Particulate Phosphorous (PP) value. Water from sites with high particulate phosphorous (PP) can be filtered to

remove the solid organic material. This will prevent the phosphorous from being released into its dissolved form, which is then available for algae to use. Dissolved phosphorous is either organic or inorganic. Dissolved organic phosphorus (DOP) is bound to plant or animal tissue, usually in the form of body waste (feces) and food residues (Sawyer 1947). Dissolved inorganic phosphate (DIP) is not associated with living tissues, and may occur from detergents, pesticides, or fertilizers that wash into lakes. PO_4 is the form of dissolved phosphorous that results from erosion of particulate material (Wetzel 2001). It also results from fertilizers and commercial cleaning products which may ultimately be carried as runoff into natural waterbodies (Fitzgerald and Faust 1967). Phosphorus is necessary for the growth of most organisms and can be the nutrient that limits primary productivity in a body of water (Fitzgerald and Faust 1967). Excessive phosphate stimulates growth of nuisance algae in natural waterbodies (Sawyer 1947). Once phosphorous is dissolved in the water as DOP, DIP, or PO_4, it can no longer be mechanically filtered out. However, chemicals, such as ferric (iron) chloride, can be added to the water to bind the phosphorous, causing it to precipitate.

Eutrophication in nutrient–rich lakes increases as weather conditions become warmer. These conditions foster algal blooms, which block sunlight from reaching algae below the surface. Algae without sunlight soon die and sink to the bottom of the lake. Bacteria then decompose the cells in a process that consumes the available oxygen dissolved in the water, potentially suffocating zooplankton, fishes, and aquatic plants. Mitigation efforts are more effective after information is obtained regarding water chemistry and biodiversity status of sites.

Features of Ponds and Lakes Affect Algal Biodiversity

After water has been evaluated for nutrient content, algal assays should be conducted to ascertain biodiversity status of a lentic habitat. Richness and evenness are statistical measures of biodiversity in an ecosystem. Richness refers to the number of species in the site, and evenness refers to the relative abundance or proportion of individuals in the site. A site with healthy biodiversity is one that contains a large variety of organisms. A site with normal richness will have a Shannon Index of 1 – 4 (Shannon 1948). A site with ideal evenness will have a Pielou's Index of 100% (Pielou 1974), indicating an even distribution of each genus within the site.

Ponds and lakes are differentiated by their size and depth, and these features also affect the phytoplankton community structure they support. Biggs *et al.* (1994) lists the following features of natural ponds: all ponds support plant and animal communities; natural ponds occur in all sizes and depths; most ponds created by natural processes are small and shallow, usually less than half a meter deep; many naturally formed ponds are short–lived, being created and filled in over a period of tens or hundreds of years; some naturally formed ponds, such as bog pools and temporary ponds, can be very stable, changing little over thousands of years; ponds are common in areas where water is abundant or near to the surface; ponds of all shapes, sizes, depths and degrees of permanence have the potential to provide valuable wildlife habitats; man–made and natural ponds support plant and animal communities that are essentially the same in both pond types.

Many of the small, freshwater habitats along the California segment of the Pacific Flyway are ponds. Because some of these sites are ephemeral, they are not

always recognized for their ecological importance. Since their hydroperiod and depth differ from lakes, management strategies for ponds are different. Researchers provide valuable information on management, specifically for ponds. Biggs *et al.* (1994) lists the following myths about ponds: drying–out is disastrous for pond communities; ponds should be at least 2m deep; all pond zones, from deep open water to shallow margins, should be created and maintained; larger ponds have better biodiversity; ponds should not be shaded by trees; ponds should be dredged to prevent them from being 'choked' with vegetation; pond water–level fluctuations should be minimized; livestock should be prevented from having access to ponds; and ponds are entirely self–contained systems, isolated 'islands' in a sea of dry land.

As a pond fills with sediment and becomes progressively shallower, the community it supports will also gradually change. However, there is no evidence that the pond's conservation value will inevitably decline; rather it will support a different species assemblage which is likely to be just as valuable (PondAction 1994). Silty ponds support many invertebrates, including the alderfly, *Sialis lutaria*, water beetles, such as *Haliplus laminatus*, and the Screech Beetle, *Hygrobia hermanni* (Biggs *et al.* 1994). Even when aquatic species are no longer present, the wet soil supports plants and semi–terrestrial wetland animals, including snails, such as *Oxyloma pfeifferi*, and many flies and ground beetles. Additionally, wet wooded hollows filled with leaves can have specific value (Biggs *et al.* 1994). Temporary ponds have fewer species than comparable perennial ponds, yet they support uncommon plant and invertebrate species (Nicolet 2001). Since drying is a natural fluctuation in ponds, many species inhabiting shallow ponds are adapted to this circumstance, although some may be

unable to survive droughts, opening a niche for new organisms (Biggs *et al.* 1994). Anthropogenic drainage causes unnatural disturbances by reducing water levels permanently, increasing the likelihood of sustained damage to wildlife communities (Biggs *et al.* 1994).

Lakes are larger and deeper than ponds, and are divided into three zones based on amount of sunlight. The littoral zone is where sunlight can reach the bottom. The limnetic zone refers to the open waters. The profundal zone is the deep water where sunlight does not penetrate. The amount of light influences community composition of plants and algae, which provide important habitats for organisms in the ecosystem (EPA.gov 2010). Phytoplankton (algae that are not attached to substrate) are primary producers that rely on photosynthesis, and therefore they mainly occur in the littoral and limnetic zones. They are important constituents in aquatic ecosystems since they oxygenate the water and are ingested by zooplankton, which are ingested by small insects and fishes, which are, in turn, ingested by larger fishes, insects, amphibians, and other animals. The number and type of phytoplankton is influenced by nutrient concentrations (EPA.gov 2010).

The water column of lakes is further divided into three zones called the epilimnion (surface water), hypolimnion (bottom water), and the metalimnion (or thermocline), which is the transitional area between the bottom and surface water (EPA.gov 2010). Although phytoplankton require sunlight for photosynthesis, some species are motile and can travel throughout the water column. They may be affected by temperature changes. Therefore, the depth of a lake affects the phytoplankton composition (UCD 1996). Deep water in lakes may become oxygen depleted in the summer because the thermocline does not allow mixing of layers. Mixing in the spring

and fall occurs when the temperature of the lake becomes more uniform from top to bottom. This allows oxygen to reach the bottom and releases nutrients to the surface (UCD 1996).

Phytoplankton biodiversity differs between lakes and ponds, since water depth and permanence are major influences on community types. Varying these factors at a site to create a mixture of permanent, semi–permanent and seasonal pools, provides habitats for a far greater variety of plants, invertebrates, amphibians, birds and mammals than non–varied conditions (Williams *et al.* 1997). Between winter and summer, pond waters rise and fall, creating a draw–down zone of variable wetness and high biological diversity. Williams *et al*. (1997) also states that the wildlife potential of a pond can be considerably improved by extending the area of the draw–down zone, which needs to slope evenly down to deeper water. Increasing the depth of a pond to accommodate fishes and attract birds can be useful for increasing biodiversity (Williams *et al.* 1997).

Nuisance Algae

Green algae are a good food source for zooplankton and small fishes. Diatoms can be beneficial to ponds and lakes, although their silica frustules make them unsuitable as food for most fishes. When diatoms decompose, empty silica frustules aerate the benthic strata, fostering growth of aquatic plants. Certain genera of cyanobacteria or blue–green algae, such as those that form stromatolites, are beneficial to aquatic communities due to their diversity, complexity and environmental associations (Allwood *et al.* 2006). However, other cyanobacteria are a nuisance because they secrete toxins, are not a

suitable food source, and out–compete other algae, causing an imbalance in the community structure. The toxic cyanobacterium, *Microcystis*, has been documented in a variety of aquatic habitats, including lakes, rivers, estuaries, oceans, and water supply reservoirs. Based on World Health Organization (WHO) guidelines, low risk for microcystin toxins is < 10 μg $\cdot L^{-1}$, moderate risk is >10 – 20 μg $\cdot L^{-1}$, and high risk is > 20 μg $\cdot L^{-1}$ (WHO 2003). The first known reported incidence of cyanobacterial toxin poisoning was in China 1000 years ago when General Zhu Ge–Ling reported mortality in troops that drank water from a river in southern China that was green (Wang *et al.* 2009). Another report of intoxication was from an Australian lake in 1878 (Falconer 2001). With increasing eutrophication of lakes due to increased agricultural use and population pressures, the number of cyanobacterial blooms may be increasing. A high incidence of primary liver cancer in China has been attributed to drinking water contaminated with cyanobacteria (Feng *et al.* 2006). In a survey of 66 lake–river systems in NW Russia from Lake Ladoga to the Barents Sea, 19% of the total algal composition was Cyanophyta, the phylum of toxin–producing cyanobacteria (Komulaynen 2008). Severe hepatotoxicity caused by the microcystins from the tropical cyanobacterium, C*ylindrospermopsis raciborskii*, was traced to a domestic water supply reservoir on Palm Island, Australia (Hawkins *et al.* 1985). Toxic *Microcystis* strains have also been found in the Swan River, Australia (Orr *et al.* 2004), Iguassu River Estuary, Pernambuco State, Brazil (Leao *et al.* 2008), and in marine environments (Hecky and Kilham 1988). As part of the National Lake Assessment Project, a survey of 50 lakes in Minnesota was conducted after reports of severe nuisance algal blooms (Lindon and Heiskary 2009). That research documented high levels of microcystin toxins in 11% of the sites. The

California Regional Water Quality Board issued a press release that 11 dog deaths occurred shortly after contact with fresh waterbodies in three Northern California rivers in Humboldt and Mendocino counties since 2001, and cyanobacterial toxins were the suspected cause of death (Creagher 2009). Microcystin toxins have also been implicated in the death of Central California southern sea otters (Miller *et al.* 2010).

In September 2005, the EPA organized a review of cyanobacterial harmful algal blooms (HABs) in the United States for the purpose of developing a national standard for these toxins in recreational and drinking water (EPA 2005). This project led to the first regional Monitoring and Event Response for Harmful Algal Blooms (MERHAB), a tier–based monitoring system for cyanobacterial toxins. This is a significant advancement since the five US/Canadian Great Lakes represents 21% of the world's freshwater (Tilzer and Serruya 1990).

In many lakes, cyanobacterial cell counts are linearly related to phosphate concentration. Cyanophyta have a higher affinity for phosphorus than green algae, and therefore outcompete green algae under conditions of phosphate limitation (Falconer 2004). According to Falconer (2004), unlike most green algae, cyanobacteria can descend vertically in the water column where phosphate availability is higher. That study found that *Microcystis* is especially adept at phosphate storage and variable buoyancy. Under favorable nutrient conditions and sunlight, Falconer found that Chlorophyta can grow at double the rate of Cyanophyta. However, as cell density impedes light penetration, the Cyanophyta that fix nitrogen tend to proliferate and outcompete Chlorophyta, even in nitrogen–depleted waters (Falconer 2004).

Since many endangered birds use the coastal lakes of Southern California during their migratory route along the Pacific Flyway, it is important to ascertain the safety of these waters. It is necessary to evaluate water samples from these sites for nutrient loads and toxin content. Once baseline information is obtained, proper management strategies can be implemented to reduce nutrient loading by anthropogenic disturbances, remove algal toxins, and precipitate dissolved nutrients to inhibit excess algal growth.

Utilization of phosphate and nitrate removal systems in small lakes may be of great benefit in limiting algal growth. Since the toxins are contained within the cell walls of the cyanobacteria, removal of Microcystis colonies from small lakes may also decrease toxin levels. In 2003, an Engineer at the University of Idaho designed Blue Pro™, a sand filtration system infused with ferric chloride. This system was patented and incorporated into a company called Bluewater Technologies. It was designed for use in water treatment facilities, but its use for inhibition of nuisance algal growth by phosphate restriction on lakes is an untapped resource for lake management. Further studies using this technology may reveal a solution for removal of *Microcystis* and its toxins.

Summary of Dissertation

This research began with a survey of 40 lakes, bays, ponds, and lagoons in five counties along the Southern California coastline (Chapter 2). Samples were evaluated for nutrient content, phytoplankton composition, and algal toxin levels. Analyses ascertained relationships between water chemistry factors and algal biodiversity and toxin production. Our second project evaluated the impact of *Microcystis* on algal biodiversity under controlled, laboratory conditions. Thereafter, a pilot study was conducted at Mason

Lake using new technology to filter colonial algae and precipitate dissolved phosphate (Chapter 3). During the pilot study, a phosphate restriction assay was performed to determine the phosphate threshold needed for algal growth at Mason Lake. A review of the ecological impact of Southern California ponds and small lakes, and strategies for their conservation is provided in Chapter 4. Results from our study may assist in land management decisions, especially regarding California coastal ponds and lakes which provide critical habitats for endangered species.

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CHAPTER TWO

WATER CHEMISTRY FACTORS AFFECTING PHYTOPLANKTON BIODIVERSITY AND TOXIN PRODUCTION IN SOUTHERN CALIFORNIA LENTIC HABITATS

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Abstract

California coastal waters constitute an important segment of the Pacific Flyway and provide habitats to many threatened and endangered birds. Excess nutrient loads and warm temperatures cause eutrophication, which fosters growth of toxic cyanobacteria. In this study, water samples were collected in 40 lakes, ponds, bays, and lagoons along the California coastline from Santa Barbara to San Diego counties. Samples were analyzed for nutrients, pH, alkalinity, electrical conductivity, total dissolved solids, dissolved oxygen, and chlorophyll–a. Algal assays were conducted to ascertain phytoplankton genera identification and numbers, and indices of biodiversity (richness and evenness) were calculated. The most predominant genera at the study sites were *Microcystis*, *Euglena*, and *Navicula*. Five of the 40 sites contained excess microcystin toxins, and two sites contained anatoxin–a in excess of drinking water limits. We found that TN and DO positively correlated with toxin levels, and sites with toxins in excess of drinking water limits had significantly higher dissolved organic carbon (DOC) levels than other sites. Cyanophyta positively correlated with TP and negatively correlated with EC and DOC, while Euglenophyta positively correlated with EC and DOC. We demonstrate that algal compositions and toxin production in Southern California lakes, ponds, bays, and lagoons covary with ambient water column nutrient concentrations and that diversity of phytoplanktonic taxa in Southern California lentic habitats decline and shift towards more pollution tolerant species (Cyanophyta) as nutrient load increases. Information obtained in this study may be helpful in evaluating and managing land use around sites of ecological importance. Additionally, sites of particular concern can be targeted for ongoing monitoring of toxin and nutrient loads.

Introduction

The Pacific Flyway is a major avian migratory route, extending from Alaska to Mexico. This pathway provides food sources, breeding grounds, rest stops, and habitats for many birds, including endangered species. Southern California coastal lakes, ponds, bays, and lagoons constitute an important segment of this pathway. Endemic fishes, some of which are endangered, also rely on these waters. Many of these costal sites are popular for human recreational activities.

In ponds and lakes, the relative abundances of Cyanophyta (cyanobacteria, or blue–green algae), Chlorophyta (green algae), Bacillariophyta (diatoms), Euglenophyta (Euglenoids), Pyrrhophyta (dinoflagellates), and Cryptophyta (flagellated, like dinoflagellates) depends on multiple water quality parameters. Factors affecting water quality include nutrient concentration, salinity, temperature, pH, dissolved organic carbon, and particular elements required by certain algae. However, analysis of phytoplanktonic ecosystem dynamics can be complicated by biological competition as nutrient load changes.

Urban lakes are prone to eutrophication from long water residence time and high nutrient loads from birds, fishes, humans, dead organic vegetation, and nutrient run–off from adjacent areas. Sites of similar size and location may develop different phytoplankton communities due to variations in water chemistry factors. As pollution gradients of phosphorous, nitrogen, and ammonium increase, there is often a shift in phytoplankton composition towards lower species diversity and more pollution tolerant organisms (Carrick and Lowe 1988). One such phylum is Cyanobacteria, a blue–green alga, some species of which are known for secreting toxins. Even when pollution

gradients are level, certain nutrient ratios may foster growth of Cyanobacteria, which may vary in number and type of genera present, due to differences in nutrient consignment (Xavier *et al.* 2007). Therefore, water chemistry analyses may be helpful in predicting algal disturbances.

Eutrophication manifests as an increase in nutrients and cell counts, and a decrease in richness and evenness of phytoplankton biodiversity. One reason for the decrease in biodiversity is that Cyanophyta (blue–green algae) may out–compete Chlorophyta (green algae) at higher nutrient levels. Falconer (2004) found that Cyanophyta bind PO_4 with higher affinity than Chlorophyta, and PO_4 depletion inhibits growth of green algae. Sites with excess nutrients are often dominated by Cyanophyta, and are subsequently at risk for toxicity. Other studies have contributed information about the effect of nutrients on predominance of Cyanophyta (Barnese and Schlelske 1994, Downing *et al.* 2001, Gikuma-Njuru and Hecky 2005).

Three common cyanobacterial toxins are microcystin, cylindrospermopsin, and anatoxin–a. Microcystin and cylindrospermopsin are protein phosphatase inhibitors (Carmichael and An 1999) which cause liver toxicity and promote tumor formation in fishes, birds, and humans. Anatoxin–a is a neurotoxin which causes convulsions and death in fishes, birds, and humans (Gácsia *et al.* 2009). Microcystin toxin is often produced by the colonial cyanobacterium, *Microcystis*, which contains gas vacuoles and accumulates on surface water. This represents a particular hazard for humans, as it forms concentrated levels of toxins within easy reach of children and adults engaging in shoreline recreation. Disease due to cyanobacterial toxins ranges from mild to severe, depending on whether exposure was by physical contact or ingestion. Symptoms include

skin irritation, malaise, anorexia, vomiting, headache, sore throat, muscle and joint pain, bloody diarrhea, and damage to kidneys and liver (WHO 2001). Many birds have died from ingestion of cyanobacterial toxins. Doñana National Park in south-west Spain is designated as a World Heritage Site, and is home to representatives of more than 70% of all European bird species (Magrann *et al.* Submitted). Lopez-Rodas *et al*. (2008) reported that in 2004, thousands of fish were found floating in the lagoon at Doñana National Park, with hundreds of dead herbivorous waterfowl nearby. Several days later, piscivorous birds in the area died, and within two weeks, at least 6,000 birds, including endangered species, such as the white-headed duck and marbled teal, had succumbed. Post-mortem findings were consistent with microcystin toxicity. Other documented instances of wild bird mortality caused by algal toxins include ducks, geese, coots, mallards, songbirds, Franklin's gulls, American wigeons, brown pelicans, Brandt's cormorants, and common terns (Schindler 1977), and massive deaths of Lesser Flamingos (*Phoeniconaias minor*) are periodically reported at hot springs in East Africa (Gupta 2007).

Although cyanotoxins cause severe ecological impacts, and their predominance is affected by nutrient loads, little is known about phytoplankton community composition and status of eutrophication in Southern California lentic habitats. Since most of these sites are near urban areas, land use may drive an increase in nutrient loading in these habitats, causing a change in dominance of phytoplankton in freshwater ponds and lakes (Hecky and Kilham 1988). The ecological importance of these locations is being undermined by potential threats from imbalanced algal community structures and algal toxins. Data on algal community compositions are useful to assess water quality (Lowe

1974). Southern California government agencies have measurements on nutrient concentrations of local lentic ecosystems (Busse *et al.* 2003, Schnetzer *et al.* 2007, Sutula *et al.* 2007), yet little information exists regarding algal community compositions and toxin quantification. Without such data, correlations cannot be drawn about how land use and other anthropogenic infringements contribute to toxic algal blooms.

This study evaluated the diversity of phytoplanktonic taxa in Southern California lentic habitats, and assessed covariance of community structure with ambient nutrient concentrations. The purpose of this paper is to report the prevalence of microcystin toxins in Southern California lakes, ponds, bays, and lagoons. Data obtained in this study may provide valuable information needed to improve biodiversity in lentic ecosystems.

Materials and Methods

Sixty–six grab samples were collected within three meters of the shoreline in 40 lakes, ponds, bays, and lagoons from Santa Barbara to San Diego counties, along the Pacific Flyway (Fig. 2.1). The first series was collected from 30 sites during early summer (June – July) 2009. This series was repeated in late summer (August – September) 2009. Six sites were no longer accessible in late summer, due to the ephemeral nature of these ponds. Ten additional sites were added to the sampling regime in late summer. Toxin analyses were conducted on 40 of the late summer series. Sites were selected based on their variance in nutrient levels and for their ecological importance to wildlife and human recreational activities. Several sites were also selected for their proximity to urban land use.

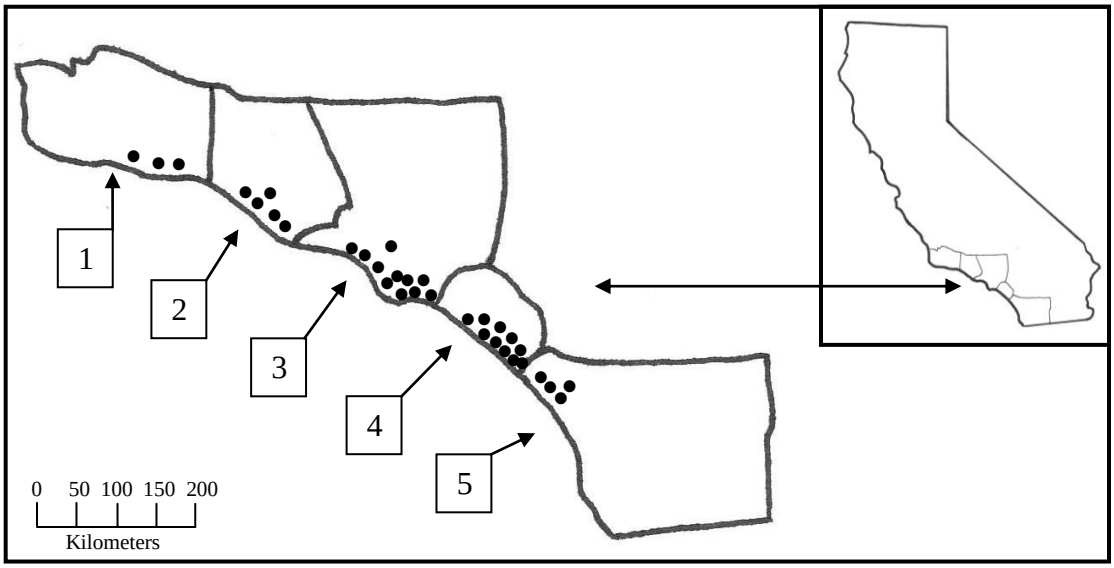

Fig. 2.1. Study sites in Southern California Counties of Santa Barbara (1), Ventura (2), Los Angeles (3), Orange (4), and San Diego (5).

At the point and approximate time of sample collection, measurements of temperature, pH, electrical conductivity (EC), and total dissolved solids (TDS) were acquired with a Hanna HI98129 Digital Tester Meter (Hach Company, CO, USA, supplier), and dissolved oxygen (DO) was recorded with a Milwaukee SM600 DO Meter (Hach Company, CO, USA, supplier). A Secchi disc was also lowered into the water until the black and white lines were no longer visible, and depth (i.e., visibility) was recorded in centimeters. Between sites, equipment was bathed in 2M HCl for 5 minutes, then rinsed with Milli–Q water and allowed to air dry. Samples were obtained at approximately three sites per day, three days a week. Samples were transported in cool, dark bins and processed within six hours. Additional details of sample acquisition are found in Appendix A. Alkalinity was measured with a Hach Digital Titrator Model 16900

test kit (Hach Company, CO, USA, supplier), titrated with sulfuric acid to a colorimetric end point. Total alkalinity reflected all carbonate, bicarbonate and hydroxide present. Additional details on the alkalinity method are provided in Appendix B.

Algal assays were conducted and total cells · mL^{-1} calculated with the following equation:

$$Cells \cdot mL^{-1} = (cells\ in\ 10\ fields)\ (coverslip\ area / 10 \cdot field\ area)\ (1 / mL\ sample\ used) \quad (1)$$

The Shannon Index (Shannon 1948) was used to calculate diversity (H′) of genera and phyla at each site. Pielou's Index (Pielou 1974) was used to calculate evenness (J′) of genera and phyla of each site, by dividing H′ by the natural log of the total number of genera in the sample (H'_{max}). Additional details of the cell count method are provided in Appendix C, and a sample bench sheet is provided in appendix D. Algal counts were conducted separately for Bacillariophyta, Euglenophyta, Chlorophyta, Cyanophyta, Pyrrhophyta, and *Microcystis*. Relative abundance was calculated by dividing cells · mL^{-1} of each genera by the total cells · mL^{-1} in the site.

Samples were analyzed for chlorophyll–a using a model 10AU fluorometer (Turner Designs, CA, USA, supplier). Chlorophyll–a was calculated by EPA Method 445.0 (Rigler 1956), using the equation,

$$Chlorophyll\ a = (F_m / F_m - 1)\ (F_b - F_a)\ (v / V) \quad (2)$$

where F_m was average acid ratio (F_b/F_a) of pure chlorophyll–a standard, F_b was fluorescence before acidification, F_a was fluorescence after acidification, v was extract volume (in liters), and V was volume filtered (in liters).

Samples were analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), total dissolved phosphate (TDP), total phosphorus (TP), and total nitrogen (TN). Total dissolved nitrogen and TDP were analyzed by using persulfate to digest unfiltered and filtered water samples to convert nitrogen from all N components into nitrate, and phosphorus from all P components into orthophosphate for simultaneous determination of TN and TP. The resulting digests were analyzed by automated colorimetry for nitrate–N and orthophosphate using a Colorimeter (Alpkem, TX, USA, supplier) by APHA standard methods (APHA 1999). Soluble Reactive Phosphorus (SRP) was analyzed via the automated ascorbic acid reduction method, using a QuikChem 8000 Flow Injection Analyzer (Lachat, CO, USA, supplier). Samples were analyzed for nitrate + nitrite (NO_3 + NO_2) using cadmium reduction, nitrite (NO_2) using colorimetry, ammonium (NH_4) using gas diffusion, and phosphate (PO_4) using the molybdate method (Piper and Lovell 1981). From those results, values were calculated for dissolved organic phosphorus (DOP), particulate phosphorous (PP), nitrate (NO_3), dissolved inorganic nitrogen (DIN), and dissolved organic nitrogen (DON). Protein Phosphatase Inhibition Assays (PPIA) were conducted to detect concentrations of microcystin toxins using the methodology of Carmichael and An (1999). Positive samples were confirmed and variants determined using liquid chromatography–mass spectrometry (LC–MS).

Statistical Analyses

We used multiple regression analyses (Mertler and Vannatta 2004) to identify potential predictors of the following ten dependent variables: visibility (Secchi depth); biomass (Chlorophyll-a); relative abundance of each algal group or species (Bacillariophyta, Euglenophyta, Chlorophyta, Cyanophyta, and *Microcystis*); and measures of algal biodiversity (phyla and genera richness [H'] and evenness [J']). The predictors for each of the 10 models included TP, TN, DOC, EC, DO, and Alk. For these analyses, we used all 66 samples, assuming independence for the 14 bodies of water measured twice (in spring and fall), since they yielded highly dissimilar measures. All of the dependent variables except the biodiversity measures, and all of the predictors except alkalinity, required normalization with rank transformation prior to analysis. We examined bivariate scatterplots, residuals, and multicollinearity (tolerance > 0.20) to assure parametric assumptions were met. Relative abundance of Pyrrhophyta was dichotomous (present, absent), so we subjected it to logistic regression (Mertler and Vannatta 2004) using the same predictors. We also used the same predictors in regression models of the two toxins, microcystin and anatoxin-a (both rank-transformed); however, these measures were obtained from only 40 samples. In addition to overall model significance, we also interpreted the effect sizes of individual predictors, which corresponded to higher absolute values for the beta coefficients of multiple regression, and greater departure from 1.0 (<1.0 for negative relationship, >1.0 for positive relationship) for the log-odds ratios of logistic regression. We also conducted a bivariate Pearson's correlation (r), but expressed the result as a coefficient of determination (r^2). All effect sizes (beta coefficients, difference of log-odds ratios form 1.0, and coefficient

determination) can be loosely interpreted as the proportion of variance in one variable explained by the other.

We used *t*-tests (Zar 1996) to compare the water chemistry, *Microcystin* density, and biodiversity measures of toxic versus non-toxic sites. We defined a toxic site as having microcystin levels > 1.0 $\mu g \cdot L^{-1}$ or anatoxin-a levels >3 $\mu g \cdot L^{-1}$; these values exceed the established human drinking water limits (WHO 2003). We obtained toxicity measures from 40 sites sampled in late summer, so our analysis was restricted to these. We computed effect sizes as Cohen's *d* using pooled standard deviation, with values of ~0.2 generally deemed small, ~0.5 moderate, and ~0.8 large (Cohen 1988).

All tests other than Cohen's *d* computations were conducted using SPSS 13.0 for Windows (Statistical Package for the Social Sciences, Inc., Chicago, 2004), with alpha = 0.05. We computed Cohen's *d* using a spreadsheet. Following Perneger (Perneger 1998) and Nakagawa (Nakagawa 2004), we did not apply Bonferroni adjustments of alpha to the multiple tests.

Results

Water Chemistry Factors Affecting Algal Biodiversity

The 66 samples showed considerable variation in water chemistry, biodiversity measures, and toxin levels. Five of the 11 regression models for water visibility and algal abundance and diversity proved significant (Table 2.1), as indicated by high adjusted R^2 values. Water visibility ($P < 0.001$) was negatively associated with TN and DO. Relative abundance of Bacillariophyta ($P = 0.024$) was negatively associated with TP, whereas relative abundance of Cyanophyta ($P = 0.022$) was positively associated with TP and

negatively associated with DOC. *Microcystis* density (P = 0.029) was negatively associated with EC. Genera evenness (P = 0.034) was positively associated with DOC. Effect sizes (beta coefficients and log-odds ratios for Pyrrhrophyta) for relative abundance of the different algal groups in Table 2.1 were always higher for TP than TN, which suggests that phosphate had a greater influence on algal abundance than nitrogen. However, the most striking result was the contrast between the positive association of Cyanophyta (including *Microcystis* density) with TP and the negative association of all other algal groups (including Pyrrhophyta, indicated by log-odds ratio < 1) with TP. Bivariate relationships between the four algal groups (Bacillariophyta, Euglenophyta, Chlorophyta, Cyanophyta; Pyrrhophyta excluded) and TP are shown in Fig. 2.2 (for non-transformed data).

Table 2.1. Results of multiple regression models for dependent variables, including model fit (adjusted R^2) and beta coefficients; $N = 66$.

Model (Dependent Measures)	**Model Adjusted R^2**	**TP**	**TN**	**DOC**	**EC**	**DO**	**Alk**
Visibility (Secchi depth)	0.36***	-0.01	-0.43*	-0.19	0.11	-0.21*	-0.02
Chl–*a*	0.09	-0.27	0.37	0.20	0.15	0.03	0.13
Bacillariophyta	0.14*	-0.35*	-0.14	0.27	0.25	-0.09	0.14
Euglenophyta	0.09	-0.31	-0.07	0.38*	0.27*	-0.10	-0.02
Chlorophyta	0.04	-0.24	-0.18	0.20	-0.08	-0.06	0.16
Cyanophyta	0.14*	0.37*	0.19	-0.35*	-0.20	0.06	-0.10
Pyrrhophyta[(a)]	0.30	0.94	0.99	1.10	1.04	0.99	0.92
Microcystis	0.13*	0.20	0.19	-0.11	-0.31*	0.14	0.03
# genera	0.06	-0.16	-0.11	0.24	-0.23	0.08	0.20
Genera richness (H′)	0.10	-0.27	-0.23	-0.23*	0.13	-0.04	0.17
Genera H′ w/o *Microcystis*	0.04	-0.18	-0.21	0.35*	-0.06	-0.07	0.12
Genera evenness (J′)	0.12*	-0.27	-0.26	0.32*	0.21	-0.03	0.12
Genera J′ w/o *Microcystis*	0.05	0.14	-0.41*	0.36*	0.12	-0.04	0.01
Microcystin	0.31**	-0.10	0.38	0.31	0.11	0.20	0.08
Anatoxin–a	0.50***	-0.13	0.52**	0.28	0.05	0.25	0.04

(a) Presence analyzed by logistic regression with log-odds ratios of 1.0 indicating no effect; all other models multiple linear regression.
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P < 0.001$
TP = total phosphorous; TN = total nitrogen; DOC = dissolved organic carbon; EC = electrical conductivity; DO = dissolved oxygen; Alk = alkalinity.

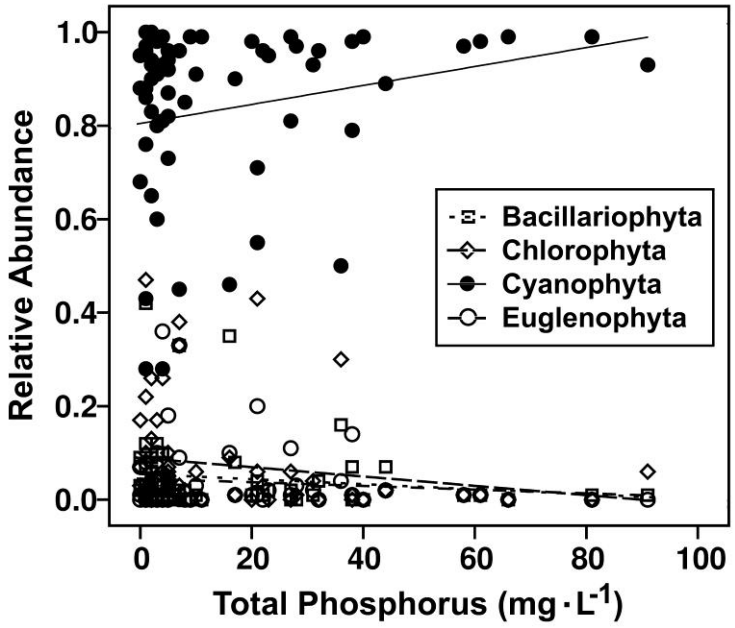

Fig. 2.2. Relative abundance of four algal phyla in relation to total phosphorus (*N* = 66 sites).

Pearson's correlations were conducted to ascertain relationships among nutrients. Phosphorus forms had significant positive correlations with each other, nitrogen forms had significant correlations with each other, and many phosphorus and nitrogen factors significantly correlated with each other, as shown in Table 2.2.

Biological factors demonstrated several correlations. Cyanophyta exhibited a significant positive correlation ($r^2 = 0.24$; $p = 0.05$) with PO_4, yet Chlorophyta exhibited a negative correlation ($r^2 = -0.15$ $p < 0.05$) with PO_4 (Fig. 2.2). Linear regression analysis demonstrated a trend that Cyanophyta numbers increased ($r^2 = 0.06$; $p = 0.05$) while Chlorophyta numbers decreased ($r^2 = -0.15$ $p < 0.05$), especially at $PO_4 < 2$ $\mu g \cdot L^{-1}$.

Table 2.2. Significant Pearson's correlations among water chemistry factors.

	TDP	**TP**	**PO_4**	**DOP**	**PP**	**TN**	**TDN**	**DIN**	**DON**	**NO_3**	**NH_4**
TDP	X	0.90	0.85	0.83	0.43	0.51	0.41	NS	0.47	-0.26*	0.32
TP	0.90	X	0.75	0.72	0.70	0.67	0.42	NS	0.46	-0.37	0.92*
PO_4	0.85	0.75	X	0.55	0.30	0.33	NS	0.24	NS	NS	0.38
DOP	0.83	0.72	0.55	X	0.31*	0.45	0.38	NS	0.55	-0.37	NS
PP	0.43	0.70	0.30	0.31*	X	0.66	0.34	NS	0.33	-0.23	NS
TN	0.51	0.67	0.33	0.45	0.66	X	0.84	NS	0.66	NS	0.23*
TDN	0.41	0.42	NS	0.38	0.34	0.84	X	NS	NS	NS	NS
DIN	NS	NS	0.24	NS	NS	NS	NS	X	NS	0.59	NS
DON	0.47	0.46	NS	0.55	0.33	0.66	NS	NS	X	-0.37	NS
NO_2	NS	NS	0.35	NS	NS	NS	0.44	0.57	NS	NS	0.33
NO_3	-0.26*	-0.37	NS	-0.37	-0.23	NS	NS	0.59	-0.37	X	NS
NH_4	0.32	0.92*	0.38	NS	NS	NS	NS	NS	NS	NS	X
Secchi	-0.33	-0.36	NS	-0.26*	-0.36	-0.59	NS	NS	NS	0.28*	NS
DOC	0.41	0.47	NS	0.42	0.51	NS	NS	NS	NS	-0.49	NS
pH	NS	NS	NS	NS	0.46	0.42	NS	NS	NS	-0.31	NS
EC	NS	NS	NS	0.35	NS	NS	NS	NS	NS	-0.27	NS
Alk	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TDS	NS	NS	NS	0.31*	NS	NS	NS	NS	NS	NS	NS

$P \leq 0.01$; * $P \leq 0.05$

Water Chemistry Factors Affecting Toxin Production

Five of the 40 sites (12.5%) contained microcystin in excess of drinking water limits (> 1 $\mu g \cdot L^{-1}$; WHO, 2003). Two of these locations, Andree Clark Bird Refuge (Fig. 2.3) and Mason Lake (Fig. 2.4), contained particularly elevated levels of the toxin. Two sites, Andree Clark Bird Refuge and IRWD Pond C (Fig. 2.5), contained anatoxin–a in excess of drinking water limits (3 $\mu g \cdot L^{-1}$; WHO, 2003). No sites contained any other algal toxins in excess of drinking water limits. Toxin results are shown in Table 2.3.

Table 2.3. Toxin levels (microcystin and anatoxin–a) at the six sites where they were found in excess (bold) of drinking water limits (DWL) as defined by the World Health Organization (WHO 2003). The degree of excess is reflected as **Δ** DWL.

Lake Name	Micro-cystin ($\mu g \cdot L^{-1}$)	SD	Δ DWL ($\mu g \cdot L^{-1}$)	ATX–a ($\mu g \cdot L^{-1}$)	SD	Δ DWL ($\mu g \cdot L^{-1}$)
Andree Clark Refuge	**18.64**	8.5	+ 17.64	**3.52**	0.1	+ 0.52
IRWD Pond C	0.12	–	- 0.88	**3.52**	0.1	+ 0.52
Mason Lake	**19.60**	1.2	+ 18.60	1.76	–	- 1.24
Newport Valley	**1.20**	0.1	+ 0.20	0.29	–	- 2.71
Peters Canyon Lake	**3.11**	0.5	+ 2.11	1.76	–	- 1.24
San Joaquin Marsh, Pond 9	**4.71**	0.8	+ 3.71	2.51	–	- 0.49

Fig. 2.3. Andree Clark Bird Refuge, Santa Barbara, CA

Fig. 2.4. Mason Lake, Irvine, CA

Fig. 2.5. Irvine Ranch Water District, Pond C

Regression analyses provided significant models for both toxins (Table 2.1). Both toxins were negatively, but not significantly, associated with phosphorus. The strongest predictors for microcystin ($P = 0.005$) and anatoxin-a ($P < 0.001$) were TN and DOC. However, these only approached significance for microcystin, and only TN was significant for anatoxin-a. The two toxins were strongly and positively correlated with each other ($r^2 = 0.870$, $P < 0.40$, $N = 40$).

When the 40 sites were dichotomized according to being above or below established drinking water limits for microcystin or anatoxin-a ("non-toxic;" $N = 34$; "toxic;" $N = 6$), *t*-tests revealed no differences in the water chemistry, abundance of *Microcystis*, or biodiversity of the two groups (Table 2.2). However, effect sizes (Cohen's *d* values) were moderate to large for nearly all of the dependent variables, suggesting that real differences exist in most water chemistry measures, that *Microcystis* density is greater at toxic sites, and that algal diversity and evenness are reduced at toxic sites.

Table 2.4. Results of t–tests for water chemistry and biodiversity measures for toxic vs. non–toxic sites.

Variable[a]	Non-toxic Sites (N = 34)		Toxic Sites (N = 6)		Significance[b]		
	mean	SE	mean	SE	$t_{(38)}$	*P*	Cohen's *d*
TP	19.7	4.2	27.3	7.7	1.65	0.107	0.73
TN	251.9	48.3	470.5	126.2	1.83	0.075	0.81
DOC	25.4	4.5	54.0	20.7	1.48	0.146	0.62
EC	10198	2683	12328	5139	1.10	0.278	0.49
DO	9.0	1.1	8.3	1.9	0.11	0.911	0.05
Alkalinity	177	97	217	70.7	1.24	0.223	0.55
Microcystis	1231	234	2071	657	1.36	0.183	0.74
# genera	9.1	0.83	5.8	1.54	1.55	0.130	0.69
Genera H'	0.56	0.09	0.21	0.11	1.49	0.144	0.64
Genera J'	0.26	0.04	0.12	0.06	1.59	0.119	0.71

[a] TP = total phosphorus; TN = total nitrogen; DOC = dissolved organic carbon; EC = electrical conductivity; DO = dissolved oxygen; *Microcystis* density (cells · mL^{-1}); H' = Shannon's diversity index; J' = Pielou's evenness index.

[b] *t*-tests (with water chemicals and *Microcystis* rank-transformed prior to analysis); note the relatively large effect sizes (Cohen's *d*: ~0.2 = small, ~0.5 = moderate and ~0.8 = large; Cohen, 1988).

Phytoplankton Biodiversity (Richness and Evenness)

We found that the overall distributions of phyla throughout the study sites were Cyanophyta (92 %), Chlorophyta (3 %), Bacillariophyta (3 %), Euglenophyta (2 %), Pyrrhophyta (0.03 %), and Cryptophyta (0.02 %). In 96% of the study sites, the cyanobacterium, *Microcystis* (Fig 2.6a), was the predominant genus. In 3% of the study sites, *Euglena* (Fig 2.6b) was predominant, and the diatom, *Attheya* (Fig. 2.6c) was predominant in one site. Pearson's correlations for relationships between phyla demonstrated that Cyanophyta exhibited strong negative correlations with Chlorophyta (r^2 = -0.84; p = 0.01), Bacillariophyta (r^2 = -0.80; p = 0.01), and Euglenophyta (r^2 = -0.76; p = 0.01).

The five genera with the highest cell counts throughout all the study sites combined were the Cyanophyta, *Microcystis* (Fig 2.6a; $\bar{x}$ = 1.9 x 10^5 cells·mL^{-1} ± 1.2 x 10^6 SE, range = 0 – 7.9 x 10^5 cells·mL^{-1}), *Euglena* (Fig 2.6b; $\bar{x}$ = 3.3 x 10^3 cells·mL^{-1} ±

2.2 SE x 10^5, range = 0 – 4.0 x 10^4), the Cyanophyta, *Merismopedia* (Fig 2.6d; $\bar{x}$ = 2.6 x 10^3 cells·mL^{-1} ± 1.8 x 10^4 SE, range = 7.5 x 10^3 – 1.3 x 10^5), the Chlorophyta, *Chlamydomonas* (Fig 2.6e; $\bar{x}$ = 2.5 x 10^3 cells·mL^{-1} ± 1.6 x 10^5 SE, range = 0 – 8.6 x 10^4), and the Bacillariophyta, *Nitzschia* (Fig 2.6f; $\bar{x}$ = 1.8 x 10^3 cells·mL^{-1} ± 1.2 x 10^5 SE, range = 0 – 3.5 x 10^4). Genera with the lowest cell counts were the Chlorophyta, *Spirogyra* (Fig 2.6g), and the Bacillariophyta, *Melosira*, (Fig 2.6h) each with $\bar{x}$ = 125 cells·mL^{-1} ± 1.2 x 10^2 SE. Additional photos of algae seen throughout the study sites are illustrated in Appendix E, total cells · mL^{-1} of all genera in the study sites are listed in Appendix F, and study sites sorted by genera predominance are listed in Appendix G.

The number of genera throughout the sites ranged from 1 – 20 ($\bar{x}$ = 9 ± 2 SE). The Shannon Index was used to calculate richness (H′) of phyla and genera for each site. Ideal values are an index of 1 – 4. Throughout the study sites, H′ values for richness of phyla ranged from 0 – 1.31 ($\bar{x}$ = 0.40 ± 0.91 SE). H′ values for richness of genera throughout the study sites ranged from 0 – 2.03 ($\bar{x}$ = 0.61 ± 1.42 SE). To understand the impact of *Microcystis* upon algal richness, H′ was then computed without the numbers for *Microcystis*. H′ values for richness of phyla without *Microcystis* ranged from 0 – 1.29 at each site, with $\bar{x}$ = 0.87 ± 0.38 SE for all the sites combined. H′ values for richness of genera without *Microcystis* ranged from 0 – 2.54 at each site, with $\bar{x}$ = 1.48 ± 1.06 SE for all sites combined.

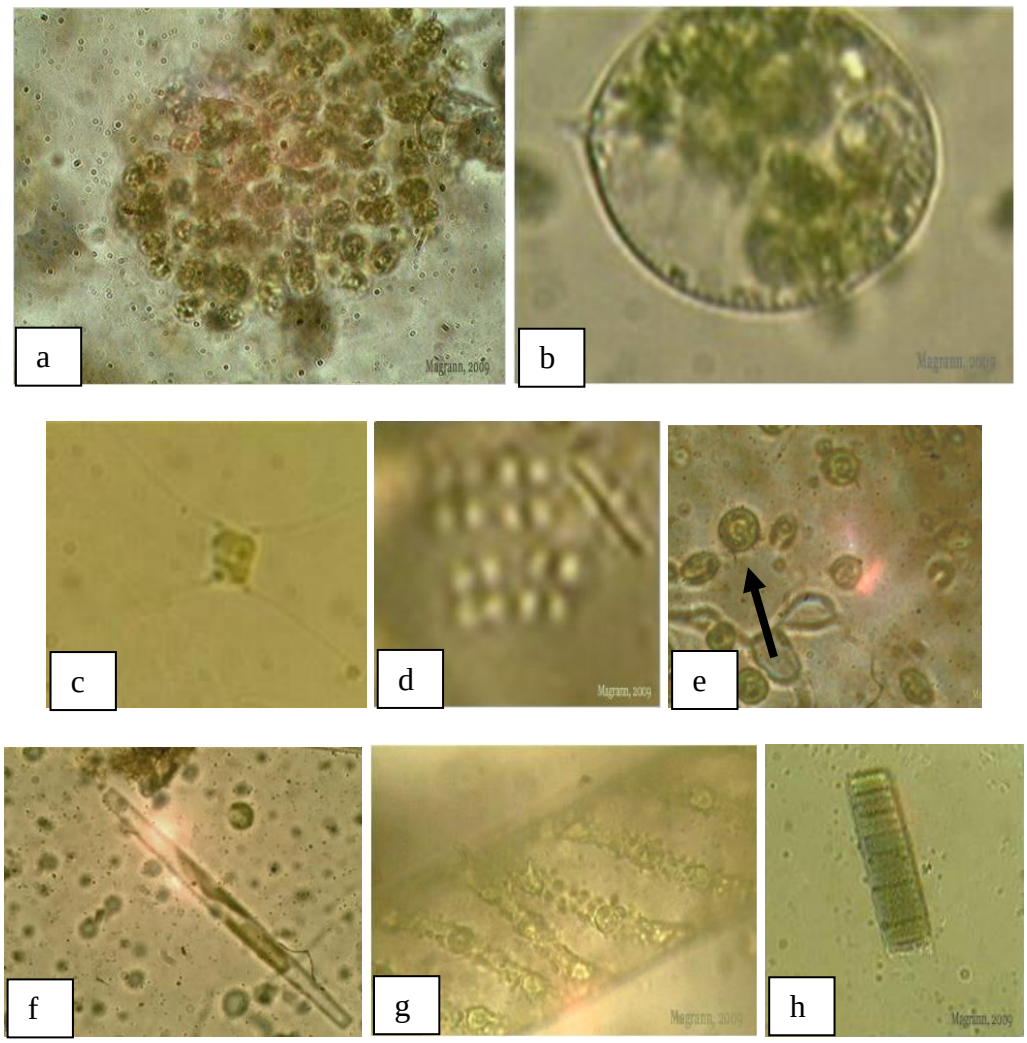

Fig 2.6. Algal photos. *Microcystis* (a), *Euglena* (b), *Attheya* (c), *Merismopedia* (d), *Chlamydomonas* (e), *Nitzschia* (f), *Spirogyra* (g), and *Melosira* (h).

Pielou's Index was used to calculate evenness (J′) of phyla and genera for each site. Ideal evenness is 100%. J′ values for evenness of phyla throughout the study sites ranged from 0 – 100% ($\bar{x}$ = 29% ± 71% SE) for all sites combined. J′ values for evenness of genera ranged from 0 – 88% ($\bar{x}$ = 28% ± 60% SE) for each site. To understand the impact of *Microcystis* upon algal evenness, J′ was calculated without the numbers for *Microcystis*. J′ values for evenness of phyla without *Microcystis* ranged from

0 – 100% at each site, with $\bar{x}$ = 66% ± 34% SE for all sites combined. J′ values for evenness of genera without *Microcystis* ranged from 0 – 91% at each site, with $\bar{x}$ = 69% ± 22% SE) for all sites combined. Richness (H') and evenness (J') of genera and phyla, with and without *Microcystis*, sorted by H' of genera, is listed in Appendix H.

Twenty–six samples from 17 sites were nitrogen–dependent, with N:P ratios ranging from 2 – 14 ($\bar{x}$ = 8 ± 6 SE). Forty samples from 25 sites were phosphorous dependent, with N:P ratios ranging from 16 – 150 ($\bar{x}$ = 46 ± 104 SE). Ratios of N:P for all study sites are listed in Appendix I.

Discussion

Eutrophication and nutrient load usually increase in lakes as temperatures rise. This manifests as increased nutrients and cell counts. We found that high levels of TN and TP significantly correlated with the presence of Cyantophyta. The primary nutrients that limit algal growth are nitrogen and phosphorous, while TN:TP ratios may affect algal community composition. Studies have shown that Bacillariophyta and Chlorophyta tend to dominate systems with higher nitrogen–phosphorous ratios, and Cyanophyta tend to dominate at lower ratios (Falconer 2004, Gikuma-Njuru and Hecky 2005), although our study found no significant correlations between those ratios and algal phyla.

The current study found that, as PO_4 levels increased, Cyanophyta numbers increased while Chlorophyta numbers decreased, especially at lower PO_4 levels (< 2 $\mu g \cdot L^{-1}$). This may indicate the former out–compete the latter at PO_4 levels < 2 $\mu g \cdot L^{-1}$. This finding is supported by work from Falconer (2004), who stated that Cyanophyta have a higher affinity for phosphorus than Chlorophyta, and outcompete green algae

under conditions of PO_4 limitation. We also found that Cyanophyta negatively correlated with EC and DOC, while TN, DO, and DOC were positively correlated with toxin levels.

In many lakes, Cyanophyta cell counts are linearly related to PO_4 concentration, which may be impacted by oxygen levels. Well oxygenated conditions have been shown to keep PO_4 strongly bound to mineral particles, whereas anoxic depths release PO_4 into solution, favoring growth of Cyanophyta (Gikuma-Njuru and Hecky 2005). According to Falconer (2004), cyanobacteria, unlike most green algae, can descend vertically in the water column where PO_4 availability is higher. Falconer's 2004 study also demonstrated that Chlorophyta can grow at double the rate of Cyanophyta under favorable nutrient conditions and sunlight. However, he found that as cell density impedes light penetration, Cyanophyta that fix nitrogen proliferate and outcompete Chlorophyta. Of the 66 samples in our study, 26 had high PO_4 levels ($> 1\ \mu g \cdot L^{-1}$). It was expected that overall cell counts would increase as PO_4 levels increased, yet a decrease in Chlorophyta numbers was seen. Fu, *et al*. (2005) found that PO_4 was an important source of phosphorus for Cyanophyta in non–tidal areas, but those in the open ocean relied on dissolved organic phosphorus (DOP).

In our study, Cyanophyta positively correlated with TP and negatively correlated with DOC. Other studies have found positive correlations with TP and Cyanophyta. Smith *et. al* (1987) found that TP and TN were principal predictors of Cyanophyta in four Swedish lakes. Kuffner and Paul (2001) also found that TP positively correlated with cyanobacteria in a lagoon in Guam. Schladow and Hamilton (1997) also demonstrated trends that high TP levels corresponded with Cyanophyta production in lakes and reservoirs in Australia.

Nitrate and phosphate levels alone may not be sufficient indicators of algal growth. Other researchers have found that multiple water chemistry parameters may influence algal growth. Marino *et. al* (1990) found that molybdenum availability impacts abundance of cyanobacteria in saline ecosystems, but only in the presence of sulfate. Phytoplankton communities in clear, oligotrophic lakes are often dominated by algae that may only be competitive under specific light conditions (Wehr 1993). Temperature requirements vary among algal species (Hoham 1975) and therefore act to restrict geographic distribution of freshwater blue–green algae (Hoffmann 1996). Marks and Lowe (1993) found that phosphorus enrichment increased periphyton accumulation only when nitrogen levels were high. Shifts in vegetation along shorelines change DOC levels, and may significantly affect proportions and variation in algal compositions (Pienitz and Smol 1993). Although algae produce oxygen in the presence of light, they consume it during dark hours, and there may also be a minimum DO requirement for proliferation of algal cells. In the current study, we found that sites with DO < 3.0 $\mu g \cdot L^{-1}$ generally had fewer cells (17 – 46,000 per mL^{-1}) than the rest of the sites.

EC is an indicator of salinity. Several researchers have demonstrated a salinity tolerance of up to 14 $g \cdot L^{-1}$ in *Microcystis* (Orr *et al.* 2004, Liu 2006, Verspagen *et al.* 2006, Tonk *et al.* 2007), and Prinsloo and Pieterse (1994) found a strain that was tolerant of salinity up to 25 $g \cdot L^{-1}$. Since we found that *Microcystis* negatively correlated with EC, the strains in our study are unlikely to be salt tolerant, although we did not directly test salinity tolerance.

The pH of a lentic ecosystem may also influence type and quantity of algae present. Alkaline ponds and lakes foster proliferation of some taxa of phytoplankton,

while areas which receive acid precipitation tend to contain algae that prefer a low pH. One study found that some strains of Chlorophyta grow optimally at pH 4.0 – 5.0, while other strains only tolerate ranges of 4.5 – 5.0 (Hoham and Mohn 1985). In our study, alkalinity negatively correlated with NO_3, and pH positively correlated with PP, TN, and NO_3. Although pH was similar at each site in our study, alkalinity was quite variable. This indicates that buffering capacity differs among sites that are otherwise similar. Sites with lower alkalinity have more free ions in solution. This can lead to rapid fluctuations in pH, which may be unsuitable for sensitive organisms. Thus, higher alkalinity sites may be more ecologically stable. One site (Irvine Regional Park Pond, Irvine) had an alkalinity measure of zero. This site also had the lowest PO_4 level (0.004 $\mu g \cdot L^{-1}$) and an almost equal balance of Cyanophyta and Chlorophyta. The combination of low alkalinity, PO_4, and DO (3.0 $\mu g \cdot L^{-1}$), may prevent Cyanophyta from outcompeting and overwhelming Chlorophyta growth.

Our study showed no correlation between Chlorophyta and nutrients, except a non–significant trend at low levels (< 2 $\mu g \cdot L^{-1}$) of PO_4. However, other studies found either positive or negative correlations between Chlorophyta and nitrogen levels. Abou-Walya *et al*. (2000) found that TN and DON are important forms of nitrogen that affect Chlorophyta growth in the Nile River. Conversely, Barnese and Schelske (1994) found that when nitrigen and carbon were added in combination, chlorophyta growth declined in a Florida lake. Perhaps these differences are due to factors that vary between lentic and non–lentic habitats, or factors present in lakes that are not present in rivers.

We found that Bacillariophyta numbers were positively correlated with EC, but were negatively correlated with TP. This indicates that diatoms in our study were more

numerous in higher saline gradients, which were nitrogen dependent systems. The predominant diatoms throughout our study sites were *Nitzschi*a and *Navicula*. The presence of these genera typically indicates eutrophic, alkaline conditions, high ionic concentration, and intense organic pollution (Guerrero and Rodriguez 1991).

We found a positive correlation between Euglenophyta numbers and DOC, indicating that Euglena in our study may be utilizing DOC, while this nutrient had a negative correlation with Cyanophyta numbers. Euglenophyta in our study also demonstrated a positive correlation with EC, possibly indicating a higher salinity tolerance in Euglenophyta than Chlorophyta and Cyanophyta.

We also found significant evidence that high levels of certain nutrients may impact algal biodiversity. Our study revealed that, as levels of DOC increased, richness (H′) declined while evenness (J′) increased. We also found that TN negatively correlated with evenness. We found no significant corelations between biodiversity and nitrogen–phosphorous ratios. This differs from the study of a shallow lake in the Philippines, where Cuvin-Aralar *et al*. (2004) found a decrease in algal genera when nitrogen–phosphorous ratios were low. This may indicate that tropical conditions affect nutrient ratios. In our study, visibility by Secchi depth negatively correlated with most nutrients, but positively correlated with NO_3. This is similar to the findings of Lau, *et al.* (1995) in primary settled waste water, which may present comparable conditions to the lentic systems in our study**.**

Lagoons and bays in our study had higher genera biodiversity than most lakes and ponds. Lagoon and bay biodiversity increased in the warmer season, while pond and lake biodiversity decreased. The increase in genera biodiversity in lagoons and bays may be

indirectly attributed to salinity. Our study found that Euglenophyta numbers increased significantly as electrical conductivity increased, and there were fewer *Microcystis* colonies at those sites. It is possible that the presence of many Euglenoids may inhibit establishment of *Microcystis* colonies. It is unlikely the salinity of lagoons and other brackish waters directly inhibits *Microcystis*, since it was present in large numbers at Sims Pond (Long Beach, CA), a site with high salinity (EC > 3999 $\mu S \cdot cm^{-1}$). Changes in salinity across freshwater, estuarine, and marine environments affects the nutrient storage capacity in many species of algae (Herbst and Bradley 1989). Throughout the study sites, mean Shannon Index values for richness of phyla ($H' = 0.40$) and genera ($H' = 0.61$) were quite low. Magrann *et al*. (Submitted) demonstrated a significant negative impact of *Microcystis* colonies on algal biodiversity, predicting the benefits of an algal removal system. Therefore, we computed biodiversity values without the numbers for *Microcystis* to ascertain the effect on algal biodiversity by using new technology (Blue Pro™) to remove nuisance colonial algae. Performing additional calculations using the Shannon Index and Pielou's Index without including numbers for *Microcystis* demonstrated that, throughout the study sites, overall mean values were much higher for richness of phyla ($H' = 0.87$) and genera ($H' = 1.48$), and evenness of phyla ($J' = 0.66$) and genera ($J' = 0.69$). This may predict an improvement in algal biodiversity if *Microcystis* could be removed.

Sites with *Microcystis* and high PO_4 levels are at an increased risk of cyanobacterial algal blooms, which pose a health threat to humans and endangered birds. Removal of PO_4 from lakes and waters that drain into oceans may inhibit algal growth. Blue Pro™ (Bluewater Technologies, Idaho, USA) is an algal filtration system which can

be installed in lakes and at mouths of small rivers. It uses ionized sand infused with alum to filter colonial algae, and ferric chloride to precipitate PO_4, producing a non–toxic, iron–phosphate cake which can be disposed in landfill. It also transforms nitrate/nitrite into nitrogen gas. Magrann *et al*. (2009) used this technology on Mason Lake (Irvine) and demonstrated a 94% decrease in *Microcystis* colonies, and 96% reduction in PO_4 after one pass through the unit. Although we suggest this technology could also be useful in improving algal biodiversity, these measures result from only a single sample during a preliminary pilot study and must be interpreted with caution.

Microcystis was present in high numbers in many of our sites. However, not all cyanobacteria produce toxins, and when toxins are present, they are not always in excess of drinking water limits. Multi–regression analysis showed no significant correlation between nutrients and microcystin toxin levels, yet t–test results of sites dichotomized into toxic and non–toxic showed a positive correlation between DOC and sites containing toxins in excess of drinking water limits. Effect sizes (Cohen's *d*) were large for TN and moderate for TP and alkalinity in relation to toxic sites, indicating a positive correlation, although not enough to be statistically significant. We also found that DO and TN positively correlated with anatoxin–a and cylindrospermopsin levels. Other researchers have found that nitrogen and phosphorous levels limit microcystin production (Oh *et al.* 2000, Kameyama *et al.* 2002), and Crossetti and Bicudo (2005) found that low alkalinity and nutrient depletion decreased photosynthetic productivity in cyanobacteria in Garcas Pond, Brazil.

Our study found 52 genera of algae in a variety of concentrations, and several nutrients significantly correlated with algal composition, cyanobacterial numbers, and toxic sites. Our data support the suggestion that algal compositions and toxin production in Southern

California lakes, ponds, bays, and lagoons covary with ambient water column nutrient concentrations. We also suggest that diversity of phytoplanktonic taxa in Southern California waterbodies declines and shifts towards more pollution tolerant species (Cyanophyta) as nutrient load increases.

Five of our 40 sites contained microcystin toxins in excess of drinking water limits. Two of these locations (Andree Clark Bird Refuge and Mason Lake) contained particularly elevated levels, and are sites of ecological importance. Andree Clark Bird Refuge (Santa Barbara, CA) is a 0.12 km^2 wildlife refuge for migrating birds. Our study found that these avian species are being exposed to dangerously high levels of microcystin toxins. The site is encircled by a public walking path, which may also expose humans and pets to toxins. Mason Lake, in Mason Regional Park (Irvine), is a 37,000 m^2 water body located just 1.6 km away from Newport Back Bay Estuary, a habitat for a number of rare and endangered birds. It is likely that some birds stop at Mason Lake, possibly causing cross contamination of the estuary with cyanobacterial toxins. Since *Microcystis* contains gas vacuoles, the toxic cells accumulate at the surface of the lake. People and animals can easily come into direct contact with high toxin levels. Mitigation efforts are needed to remove nutrients, reduce toxins, and increase biodiversity. This may be done by filtering out toxic *Microcystis* and precipitating dissolved PO_4 from water, inhibiting future algal growth. Removal of excess nutrients and toxic algae from lakes and ponds is an essential step in protecting valuable habitats for endangered species.

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CHAPTER THREE

IMPACTS OF MICROCYSTIS ON ALGAL BIODIVERSITY AND USE OF NEW TECHNOLOGY TO REMOVE MICROCYSTIS AND DISSOLVED NUTRIENTS

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Abstract

This study was undertaken to ascertain the impact of the cyanobacterium, *Microcystis*, on algal biodiversity. Under laboratory conditions, we found that the presence of *Microcystis* decreased phyla richness by 58%, phyla evenness by 47%, genera richness by 66%, and genera evenness by 51%. Analysis by mixed ANOVAs demonstrated a significant interaction between treatment and time, and confirmed a significant reduction in richness and evenness of phyla and genera. We also conducted a PO_4 restriction assay on the algae in Mason Lake (Irvine, CA, USA), and found that the PO_4 threshold needed for algal growth there was 0.02 mg $\cdot$ L^{-1}. A pilot study was then conducted to test the effectiveness of the Blue Pro™ water treatment facility in removal of this colonial organism from Mason Lake, in addition to removal of dissolved nutrients required for its growth. We measured a 97% reduction in *Microcystis* cells, a 72% reduction in Chlorophyll–a, and a 96% reduction in PO_4 after just one 10–minute cycle through the unit. Our study demonstrated that removal of *Microcystis* colonies may allow green algae to increase in numbers. This may improve algal biodiversity, which will benefit zooplankton and fishes.

Introduction

Microcystis is a cyanobacterium known for producing carcinogenic toxins (Boyer 2007). Phosphate (PO_4) and nitrate (NO_3) are the major nutrients needed for its growth (Kameyama *et al.* 2002). Colonial *Microcystis* and high nutrient loads are often the major factors causing eutrophication in freshwater lakes. Phosphate pollution enhances cyanobacterial algal blooms, causing fish kills worldwide, especially impacting the shellfish industry (Tatrai *et al.* 2005). Estuaries and public lakes with high levels of cyanobacteria pose a health threat to humans (WHO 2003) and endangered birds (Magrann *et al.* Submitted). Decreasing nutrient levels from lakes and waters that drain into the ocean may inhibit establishment of cyanobacterial colonies.

Growth of cyanobacteria is stimulated by nitrogen and phosphorus containing nutrients, warm temperatures, stagnant water, and intense sunlight (Budde 2004). The toxicity and increased occurrence of cyanobacterial blooms in the Great Lakes led the U.S. Environmental Protection Agency (USEPA) to include cyanobacterial toxins on the Drinking Water Contaminant Candidate List for further study and future regulatory determination (Budde 2004). Cyanobacterial toxins have been associated with taste and odor problems in drinking water, and with the poisoning of birds and livestock, as well as human fatalities (Boyer 2007). These toxins can also impact the food web of an entire lake by affecting zooplankton feeding and reproduction. The diversity of toxic species and the number of these toxins is great. According to Boyer (2007), there are over 80 different types of microcystins alone. Furthermore, a given species may produce multiple types of toxins.

According to the World Health Organization (WHO 2001), people may be exposed to cyanobacterial toxins by bathing in contaminated water, although the most frequent and serious health effects are caused by drinking water containing the toxins, including ingestion during recreational water contact. Their report also states that, although the gas vacuoles of many species form a surface scum, high concentrations may also be present throughout affected water. They concluded that surface accumulations of cyanobacteria are particularly hazardous to human health because of their high toxin content. Cyanobacterial toxins cause mild to severe forms of disease, depending on whether exposure was by physical contact or ingestion. Humpage (2002) states that death is due to hypovolemic shock, and chronic exposure has been shown to enhance the growth of hepatic and colonic pre–cancerous lesions, suggesting that microcystins act as tumor promoters. Honkanen *et al.* (1990) stated that the onset of symptoms is rapid, with death occurring in a few hours if a lethal dose is taken.

Since sites with excess nutrients are often dominated by Cyanophyta, they are subsequently at risk of toxicity. Growth of cyanobacteria is usually determined by PO_4 levels (Schindler 1977). For growth to occur, the ambient PO_4 concentration must fluctuate around a threshold value (Rigler 1956, Falkner *et al.* 1984). In the study by Kameyama *et al*. (2002), production of microcystins in culture decreased with decreasing PO_4 concentrations. Thus, lowering PO_4 levels will likely inhibit microcystin production, even in viable cells. Phosphorous levels can sometimes predict whether or not an algae bloom will occur. However, studies vary in their findings regarding the minimum amount of PO_4 needed for algal growth. Shen and Song (2007) indicated that the growth of four unicellular and one small colonial *Microcystis* strain was significantly inhibited at a PO_4

concentration of 0.2 mg · L^{-1}. However, growth of the large colonial *Microcystis* strains was not inhibited. Tátrai *et al*. (2005) stated that total phosphorus concentrations in excess of 0.1 mg · L^{-1} provides sufficient nutrient enrichment in lakes for there to be a probability of eutrophication. Oh *et al*. (2000) also found a phosphorus threshold of 0.1 mg · L^{-1} was needed for *Microcystis* growth. Xavier *et al*. (2007) indicated that the typical PO_4 value for lakes with nuisance algae is 0.02 mg · L^{-1}. Water chemistry factors influence phytoplankton community compositions, and Magrann *et al.* (Submitted) demonstrated that high levels of PO_4 foster the growth of toxic cyanobacteria. Removal of dissolved nutrients may inhibit cyanobacterial growth, allowing an increase in numbers of beneficial green algae. Blue Pro™ technology is a sand filtration system infused with ferric chloride to precipitate and remove dissolved PO_4. It was designed for cleaning waste water, although it may be useful for removing nutrients and filtering out colonial cyanobacterial colonies in small lakes and ponds.

The goals of this research were to evaluate the impact of *Microcystis* upon algal diversity under laboratory conditions, estimate the PO_4 threshold needed for growth of algae at Mason Lake, and to evaluate the effectiveness of Blue Pro™ technology in decreasing PO_4 and removing toxic algae. If cyanobacterial cell numbers can be decreased in small lakes and ponds, overall algal biodiversity would be improved. This will benefit zooplankton, fishes, endangered birds, and other species in aquatic habitats.

Materials and Methods

Microcystis and Algal Biodiversity

Algal Cultures

One hundred mL of modified BG–11 medium containing f/2 vitamin solution and silica (Canadian Phycological Culture Centre, Waterloo, Ontario) was added to ten, 150–mL beakers. Five beakers were inoculated with 5 mL of each of the following strains of algae (Canadian Phycological Culture Centre, Ontario): one diatom, *Navicula pelliculosa* (CPCC 552), two green algae, *Scenedesmus obliquus* (CPCC 5) and *Chlamydomonas reinhardtii* (CPCC 243), one Euglena, *Euglena gracilis* (CPCC 95). The other five beakers were identically inoculated, with the addition of 5 mL of the cyanobacterium, *Microcystis aeruginosa* (CPCC 300). These strains were chosen because they were the most predominant species in our study sites. Both sets of five beakers received aeration from a small aquarium air pump (Petco, San Diego, CA) attached to a five–gang valve (Penn Plax, Hauppauge, NY). Each flask was covered with non–absorbent cotton to maintain sterility. Continuous lighting was provided by one daytime fluorescent and one cool–white bulb. Room temperature was regulated between 20 – 25 °C. Algal assays were conducted on each of the ten samples on the first day and weekly thereafter. The Shannon Index (Shannon 1948) was used to calculate richness (H′) of genera and phyla of each beaker. Pielou's Index (Pielou 1974) was used to calculate evenness (J′) of genera and phyla of each beaker, by dividing H′ by the natural log of the total number of genera in the sample (H'_{max}). Algal assays were conducted on the first day, and every week for five weeks. Total cells · mL $^{-1}$ was calculated using the equation

$$Cells \cdot mL^{-1} = (cells\ in\ 10\ fields)\ (coverslip\ area / 10 \cdot field\ area)\ (1 / mL\ sample\ used) \quad (1)$$

Statistical Analyses

We subjected each of the four biodiversity measures (Richness and evenness of phyla and genera) to a 2 x 6 (condition x time) mixed analysis of variance model (Mertler and Vannatta 2004), treating condition (2 levels; with and without *Microcystis*) as a between-subjects factor and time (6 levels; first day and weekly for 5 weeks) as a within-subjects factor. Data were normally distributed but heteroscedastic, and homoscedasticity was not achieved through transformations. Two of the four ANOVA models failed the assumption of sphericity (multivariate homoscedasticity), but the results were so robust that, for sake of consistency, we applied no adjustment (e.g., Greenhouse–Geisser) to the degrees of freedom. We computed effect sizes as partial eta-squared (η^2), with values of ~0.06 generally regarded moderate and ≥ 0.14 large (Cohen 1988). Because η^2 values for the main effects summed to >1.0, we adjusted these by dividing each η^2 value by the sum of all values. Statistical tests were conducted using SPSS 13.0 for Windows (Statistical Package for the Social Sciences, Inc., Chicago, 2004), with alpha = 0.05.

Phosphate Restriction Assay at Mason Lake

Ten mL of water obtained from one location at Mason Lake was placed into each of 11 plastic, 15 mL, graduated conical tubes with screw–top lids. Samples were centrifuged at 3K for 4 minutes and the supernate decanted and discarded with a micropipette. Pure phosphate standard in a dilution series of 0.1 – 0.01 was added to these tubes. No growth was observed, therefore, the experiment was repeated using a

phosphate standard derived from filtered water from Mason Lake. This standard was prepared using 0.1 µm filters to obtain 1000 mL of lake water supernate. The PO_4 of this water measured 10.01 mg · L^{-1} of PO_4. Then 10 mL of filtered lake water was added to 90 mL of reverse osmosis water. The PO_4 level was tested again and found to be 1.0 mg · L^{-1} of PO_4. To dilute the standard further, 10 mL of the 1.0 mg · L^{-1} standard was added to 90 mL of reverse osmosis water. The PO_4 level was tested again and found to be 0.1 mg · L^{-1} of PO_4. This standard phosphate solution was then used as the dilution series for this experiment. Algae pellets were obtained by centrifuging 10 mL of lake water. One pellet was placed in each of 11 tubes, and a PO_4 dilution series from 0.01 – 0.10 mg · L^{-1} was created, as was a control tube. The tubes were tightly capped and gently inverted until the algae pellets had evenly distributed within the solution. The cap of each tube was loosened so air exchange could occur, and placed in direct sunlight to incubate. The observation of growth (nearest 0.1 ml accumulation in tube bottom) and color in each tube was recorded once a week for six weeks. We used curve-fitting regression analysis in SPSS to evaluate the influence of PO_4 dilution on algal growth.

Blue Pro™ Pilot Study at Mason Lake

A temporary Blue Pro™ treatment facility was rented for three weeks for a pilot study at Mason Lake (Fig. 3.1). One cycle through the unit was divided into two phases, where influent water in the first phase received an injection of alum, a flocculent that precipitates algae. The treated water was pumped to the top of the sand bed tank and then pumped down a tube in the center of the tank until it reached the bottom of the sand bed. The water was dispersed by horizontal distribution arms, forcing water out of the pipe

and into the ionized sand bed which filtered the solid particles as water was forced back toward the top of the tank (Fig. 3.2). The filtered water was then gathered in a hose and carried back to the treatment facility for the second phase, which consisted of an injection of ferric chloride to precipitate out most of the dissolved PO_4. The treated water was again pumped through the sand bed filtration system, which removed the iron–phosphate solids, allowing only the cleaned water to be evacuated out of the effluent hose to be returned to the lake. Waste products of the system were dewatered to form a non–toxic (CH2MHILL 2006) sludge that was disposed of by Irvine Ranch Water District.

Fig. 3.1. Temporary Blue Pro™ water treatment facility at Mason Lake, April 2008. In a permanent facility, the sand towers can be embedded in the ground, and the trailer can either be camouflaged or decorated as an attraction for public education.

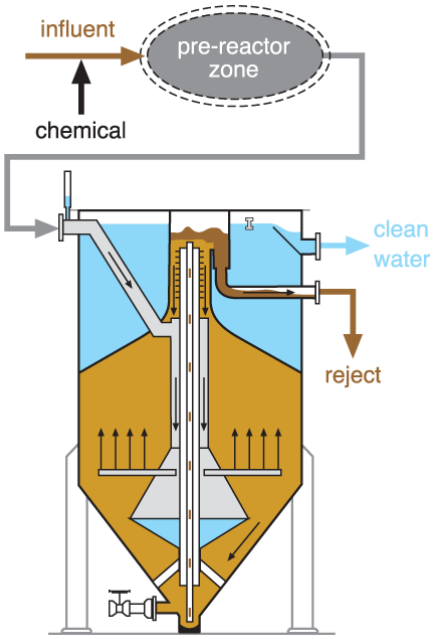

Fig. 3.2. Internal diagram of Blue Pro™ sand tower (BlueH2O.com)

Sample Acquisition

Lake water was evaluated before and after treatment. A grab sample (labeled "influent") was obtained directly from Mason Lake in April, 2008. The Blue Pro™ technician provided two more samples. One sample (labeled "Phase 1") was obtained after the first pass through the Blue Pro™ system (after treatment with alum), and one sample (labeled "Phase 2") was obtained after the second pass through the system (after treatment with ferric chloride). Together, these two latter samples constituted one cycle through the Blue Pro™ unit. Phosphate analysis was conducted on all three samples using the molybdate method (Piper and Lovell 1981). We also conducted algal assays and chlorophyll measurements on each of the three samples.

Algal Assay Technique

Three Utermöhl settling chambers were fixed to their base plates with high vacuum seal grease. Five mL of lake water from each sample was added to a chamber and allowed to settle for eight hours. The chamber was removed from the base plate and replaced simultaneously with a thick glass lid. The coverslip of the base plate was placed over the oil immersion lens of an inverted microscope and observed at 1000x. Ten fields were viewed, algal cells were counted and identified to genus, and cells·mL^{-1} calculated.

Chlorophyll Assay Technique

A filtration flask was assembled with a 50 mm perforated glass funnel in the top. A glass–fiber Whatman GF/F 25 mm filter paper was placed into the funnel. The lake water was dripped onto the filter paper with a disposable plastic pipette. Only 15 mL of the Influent water could be used before the filter became clogged. Therefore, this amount was used for the other two samples as well. The filtered water was noted to be clear, and was then discarded. The filter was removed with clean forceps and placed into a Pyrex Kontes tube. A clean glass rod was used to push the filter to the bottom and 2 mL of 90% acetone were added. A motorized Teflon pestle attached to a hand drill was inserted, and was used to grind the filter paper while keeping the pestle immersed in acetone. The pulverized solution was poured into a 15 mL conical centrifuge tube, the Kontes tube was rinsed with 1 mL of acetone which was added to the centrifuge tube. The final yield was 3 mL of acetone in the centrifuge tube with the macerated filter. The tube was centrifuged at 3k for 5 minutes. Quartz ultraviolet cuvettes were used in a spectrophotometer at 630, 647, 664 nm absorbance for each sample, and 750 nm absorbance for the 90% acetone

blank. The following trichromatic calculations (Jeffrey 1975) were used for determining chlorophylls in a mixed phytoplankton assemblage in which both chlorophyll b and c containing organisms are present,

$$\text{Chlorophyll } a = 11.85OD_{664}\text{-}1.54OD_{647}\text{-}0.08OD_{630} \quad (2)$$

$$\text{Chlorophyll } b = 21.03OD_{647}\text{-}5.43OD_{664}\text{-}2.66OD_{630} \quad (3)$$

$$\text{Chlorophyll } c = 24.52OD_{630}\text{-}1.67OD_{664}\text{-}7.60OD_{647} \quad (4)$$

where OD_x indicates optical density at each wavelength. Each optical density (OD) value was corrected for absorbance at 750 nm using the equation

$$OD_{664} = Abs_{664} - Abs_{750} \quad (5)$$

The values were multiplied by volume of acetone extract and divided by the volume filtered (all in milliliters). The results are reported in milligrams per liter.

Results

Algal Biodiversity Results

Richness (H′) and evenness (J′) of phyla and of genera were each subjected to 2 x 6 (condition x time) mixed ANOVAs. There was a significant interaction between condition and time in the four measures of richness and evenness of phyla and genera, as shown in Table 3.1 and Fig. 3.3. In the control batch (without *Microcystis*), mean phyla and genera richness (H′) and evenness (J′) changed negligibly (1.1 – 4.4%) during the

five-week period. However, in experimental tubes containing *Microcystis*, mean phyla and genera richness and evenness decreased markedly (47.2 – 65.8%). For the latter condition (with *Microcystis*), the two green algae, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*, died during the final week.

Table 3.1. Percentage decreases (mean ± SE) in richness (H′) and evenness (J′) associated with absence (control) or presence (experimental) of *Microcystis* in laboratory pure cultures during a 35–day period. Results from 2 x 6 (condition x time) mixed ANOVAs, with *P*–values and adjusted partial eta–squared (η^2) effect sizes provided.

Dependent measure	**Decrease (%; SD)**		**Condition**		**Time**		**Interaction**	
	Control	**Experimental**	***P***	η^2	***P***	η^2	***P***	η^2
Phyla H′	4.4 ± 0.0	58.1 ± 0.4	<0.001	0.24	<0.001	0.38	<0.001	0.38
Phyla J′	3.7 ± 1.5	47.2 ± 21.0	<0.001	0.34	<0.001	0.33	<0.001	0.33
Genera H′	1.6 ± 0.0	65.8 ± 0.5	<0.001	0.34	<0.001	0.33	<0.001	0.33
Genera J′	1.1 ± 0.5	50.5 ± 24.0	<0.001	0.34	<0.001	0.33	<0.001	0.33

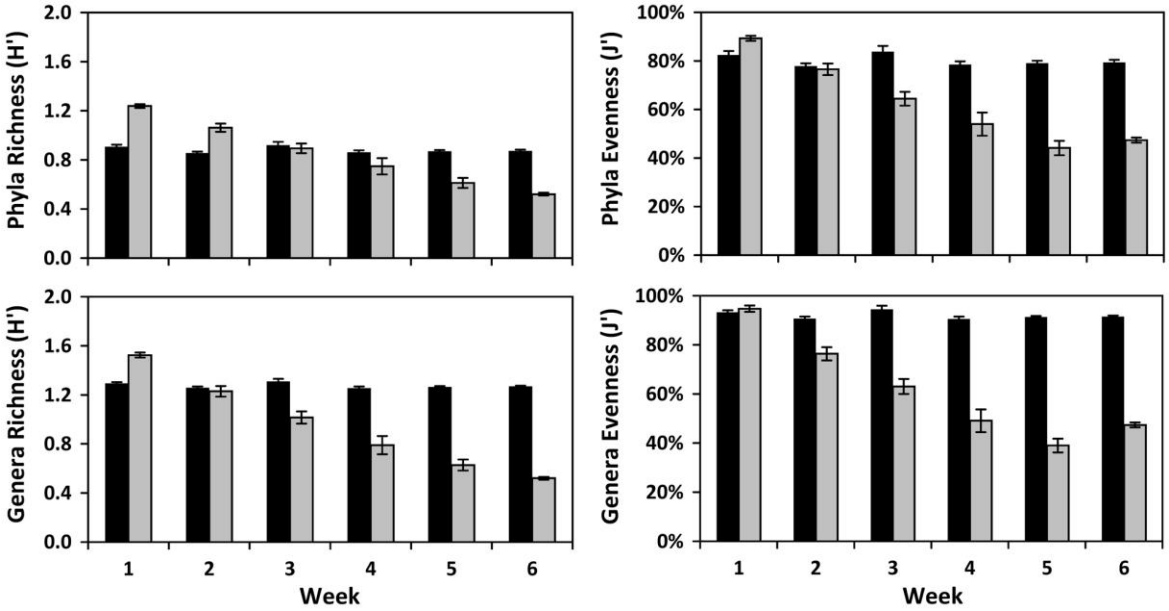

Fig. 3.3. Richness and evenness of phyla and genera. Dark columns are control batches, light bars are experimental batches. Vertical black lines are standard deviation. Significance is described in Table 3.1.

Phosphate Restriction Assay at Mason Lake

Algal growth was seen throughout the six week period in PO_4 dilutions down to 0.4 mg · L^{-1}. At PO_4 dilutions of 0.03 mg · L^{-1}, some algae survived, but growth was limited, with algae turning yellow. At PO_4 dilutions of 0.02 mg · L^{-1} there was no growth, algae turned yellow by the second week, and became clear cells (death) by the fourth week. We saw no evidence of growth at PO_4 concentrations below 0.02 mg · L^{-1}. A quadratic regression equation provided the best fit ($-0.22 + 3.70x - 1.03x^2$; $P < 0.001$; Fig. 3.4), confirming the PO_4 limitation of algal growth for Mason Lake water.

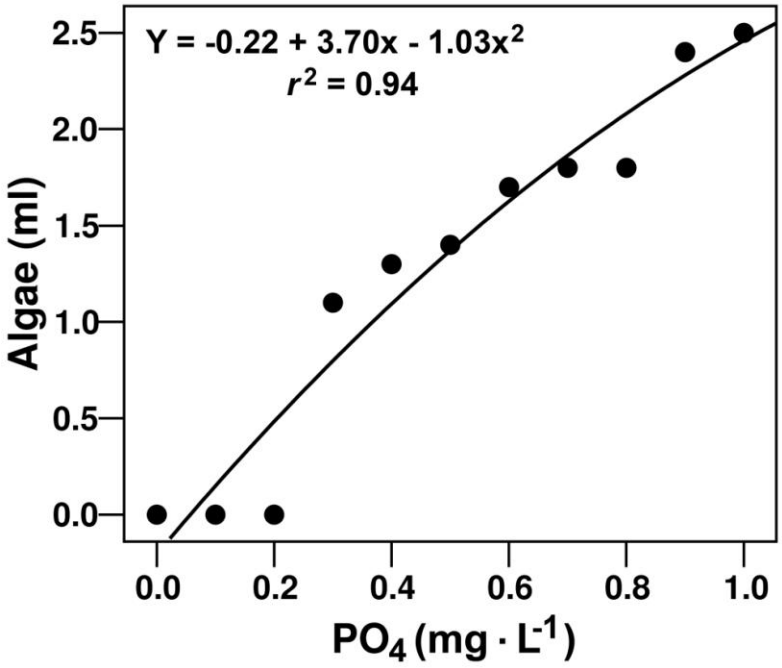

Fig. 3.4 Growth of algae diminished as PO_4 levels decreased.

Algal Assay Results

The number of algal cells before treatment (Influent) was 6,189,948 cells · mL $^{-1}$. There were few Bacillariophyta (diatoms), and no Chlorophyta (green algae), Chrysophyta (golden brown), or Pyrrhophyta (red algae including dinoflagellates) were seen. The predominant phylum was Cyanophyta (cyanobacteria), with 5,254,200 cells · mL $^{-1}$ of *Microcystis*, plus a smaller number of other cyanobacteria. In order of abundance from most to least, the genera were *Microcystis, Merismopedia, Anabaena,* and *Aphanizomenon*.

After treatment with alum (Phase 1) total cells were 587,970 cells · mL $^{-1}$ (91% reduction in cells). There were few Bacillariophyta and Chlorophyta, and no Chrysophyta or Pyrrhophyta. Most of the *Microcystis* was removed, and the *Microcystis* cell count was reduced to 490,392 cells · mL $^{-1}$. In order of abundance, from most to least, the genera were *Microcystis, Aphanizomenon, Anabaena,* and *Merismopedia*. With the vast reduction in *Microcystis*, some Chlorophyta (green algae) were now seen. The Chlorophyta genera, in order of abundance, were *Chlamydomonas* and *Scenedesmus*.

The number of algal cells after treatment with ferric chloride (Phase 2) was 366,543 cells · mL $^{-1}$ (38% reduction in cells from Phase 1, and an overall 94% reduction in cells compared with the Influent). No Bacillariophyta, Chrysophyta, or Pyrrhophyta were seen. There were a few Chlorophyta, all *Chlamydomonas*. The *Microcystis* cell count was reduced to 150,120 cells · mL $^{-1}$ (97% overall reduction in *Microcystis*). Decrease in total cells and in *Microcystis* after treatment with alum and ferric chloride is

shown in Table 3.2. The experiment was not repeated to obtain multiple samples. Therefore, statistical analysis could not be conducted.

Table 3.2. Changes in cells and nutrients after Blue Pro™ treatment.

	Influent	Alum	Ferric Chloride	Decrease
Total cells · mL $^{-1}$	6,189,948	587,970	366,543	94%
***Microcystis* cells · mL $^{-1}$**	5,254,200	490,392	150,120	97%
Chl–a µg·mL^{-1}	0.15	0.05	0.04	72%
PO_4 mg · L^{-1}	10.09	7.30	0.42	96%

Chlorophyll Assay Results

Chlorophyll–b (typical of green algae) and chlorophyll–c (typical of diatoms and dinoflagellates) were negligible in all samples. Chlorophyll–a (typical of green algae and cyanobacteria) before treatment (Influent) was 0.15 µg·mL^{-1}. After treatment with alum (Phase 1), Chlorophyll–a was 0.05 µg·mL^{-1} (69% reduction). After treatment with ferric chloride (Phase 2) Chlorophyll–a was 0.04 µg·mL^{-1} (9% reduction from Phase 1 and overall reduction of 72% from the Influent).

Phosphate Results

The PO_4 level before treatment (Influent) was 10.09 ± 0.04 mg · L^{-1}. After treatment with alum (Phase 1) it was 7.30 ± 0.03 mg · L^{-1}. This represents a 28% reduction in PO_4. After treatment with ferric chloride (Phase 2), the PO_4 level dropped to 0.42 ± 0.01 mg · L^{-1}, representing a 94% decrease from Phase 1, and an overall reduction of 96% from the Influent. These results are shown in Table 3.2.

Discussion

To ascertain the impact of *Microcystis* upon algal biodiversity, and to predict the effectiveness of an algal removal system in lakes, pure cultures of a mixed assemblage of phytoplankton were exposed to this cyanobacterium, compared to a control batch without *Microcystis*. While cell counts in the control batch remained stable throughout the study, richness and evenness of phyla and genera decreased significantly in the experimental batch containing *Microcystis*. Two factors contributed to this decline in biodiversity. The first issue was an increase in *Microcystis*, causing a decline in evenness, and the second was death of both green algal species toward the end of the five week period, causing a decrease in richness.

Chlorophyta and Cyanophyta are impacted by nutrient levels (Nwankwo *et al.* 2008). Nutrient depletion may be a factor that relates to *Microcystis* causing a decrease in richness and evenness of phytoplankton biodiversity. Cyanophyta (blue–green algae) have been shown to out–compete Chlorophyta (green algae) at higher nutrient levels, causing a decrease in biodiversity (Falconer 2004). In that study, Falconer also found that Cyanophyta bind PO_4 with higher affinity than Chlorophyta, and PO_4 depletion inhibits the growth of green algae. Riemann *et al*. (1989) found that chlorophyll content of green algae were lower during nitrogen and phosphorus deficiency. Hecky and Kilham (1988) stated that the relative proportions of nutrients supplied to phytoplankton can be a strong selective force shaping phytoplankton communities. Although nutrient levels were not measured in our study, PO_4 is assumed to have diminished during the five week experiment. Since Cyanophyta out–compete green algae under conditions of nutrient

deficiency, this may be why green algae died during the last few weeks of our algal biodiversity study, while *Microcystis* flourished.

Magrann *et al.* (Submitted) demonstrated that nutrient loading fosters the growth of *Microcystis*. Having demonstrated in our current study, that *Microcystis* negatively impacts algal biodiversity, we then directed our research towards testing the effectiveness of a Blue Pro™ water treatment facility in removing this colonial organism from a small, freshwater lake, in addition to removal of dissolved nutrients required for its growth. The study site was Mason Lake, in Mason Regional Park (Irvine, CA), which is a 37,000 m^2 freshwater lake, approximately 20 years old, and very eutrophic. Mason Lake has had extensive algal problems for years, resulting in poor visibility, malodorous water, and a slimy surface film. The park is owned by the County government and is intended for public fishing and toy sailboat regattas. However, the water has become so overgrown with algae that most of the fishes have died, the surface of the lake is covered with a thick film with the odor of sulphur, and the overgrowth impedes the motors of toy sailboats. At any given time, approximately 300 ducks and other waterfowl can be seen on the lake. Although some of the excess PO_4 is due to the use of reclaimed water, the excrement from waterfowl is probably the major source of PO_4 loading. The major nuisance alga is *Microcystis* (Magrann *et al.* 2010). Mason Lake is located just 1.6 km away from the Newport Back Bay Estuary, a habitat for a number of rare and endangered birds. These include the California Least Tern, Light–Footed Clapper Rail, Brown Pelican, Peregrine Falcon, Belding's Savannah Sparrow, and the Coastal California Gnatcatcher (NewportBay.org 2008). According to their website, up to 30,000 migrating birds can be seen there on any day during the winter months. It is likely that some of them stop at

Mason Lake, possibly cross–contaminating the estuary with cyanobacterial toxins. The results of this study were to aid in the decision of whether or not to proceed with the installation of a permanent Blue Pro™ water treatment system in Mason Lake.

Previous algal treatment at Mason Lake included copper sulfate, which induces cyanobacterial cell lysis, followed by release of toxins into the surrounding waters (Gumbo *et al.* 2008). This necessitates the use of expensive toxin removal processes, such as activated carbon and/or oxidative ozone and chlorine (Haider *et al.* 2003). Additionally, copper can dissociate in anaerobic conditions, be consumed by fishes, and accumulate as toxins in the food chain (Mason 1996). Since use of copper sulfate is restricted due to its toxicity, an alternative is the use of alum (aluminum), which binds with PO_4, yet leaves a gelatinous precipitate that builds up in the lake. Blue Pro™ is a water treatment system used to remove PO_4, metals, and other impurities in water from human sewage treatment facilities (BlueH2O.net 2009). It includes an ionized sand filtration system to precipitate PO_4 using ferric chloride, producing a non–toxic, iron–phosphate cake, external to the lake, which can be disposed of in a landfill.

The Blue Pro™ system reduced PO_4 by 96%. The PO_4 restriction assay on the algal communities of Mason Lake showed a PO_4 threshold of 0.02 mg $\cdot$ L^{-1} needed for growth This is a much lower threshold than 0.2 mg $\cdot$ L^{-1} found by Shen and Song (2007), and lower than 0.1 mg $\cdot$ L^{-1} found by Tátrai *et al*. (2005) and Oh *et al*. (2000). However, Xavier *et al*. (2007) also found a PO_4 threshold of 0.02 mg $\cdot$ L^{-1} was needed for growth of nuisance algae in two man-made urban lakes in Portugal. Since Mason Lake is 37,000 m^2, we estimated it would take approximately three months to filter the entire volume of lake water through the Blue Pro™ filter once, which should remove most of the nuisance

algae. Therefore, ongoing treatment with ferric chloride would be required. Since the system removed 97% of the *Microcystis* colonies, toxin levels will also decrease, since the toxins are embedded in the cell walls (Carmichael and An 1999). There were 27 $\mu g \cdot L^{-1}$ of microcystin toxins before treatment, exceeding acceptable limits for recreational water by 35%. Toxin levels were not evaluated after treatment. Theoretically, if the toxins were also reduced by 97%, the toxin level would be approximately 0.8 $\mu g \cdot L^{-1}$, which is below the limit of drinking water. After completion of the Mason Lake pilot study, Blue Water Technologies developed a system that also removes nitrogen, called Blue Nite™. This unit can be used in series with the Blue Pro™ unit to simultaneously remove algae, PO_4, and nitrogen. The limitation of our Blue Pro™ and PO_4 restriction studies was that the experiments were carried out on single samples only. Therefore, results related to this preliminary Blue Pro™ study should be evaluated with caution, since no variances or trends could be determined. Further research on this technology is needed at ponds, small lakes, and river mouths to evaluate the effectiveness in eliminating PO_4 loads from urban runoff.

Conclusions

Our study demonstrated that the presence of *Microcystis* negatively impacts algal biodiversity by decreasing richness and evenness of algal phyla and genera under laboratory conditions. Studies vary in the PO_4 threshold required for algal growth. Our experiment on a single sample from one lake found a PO_4 threshold of 0.02 $mg \cdot L^{-1}$ was required by algae at that site. Our pilot study, using a filtration system which also removes dissolved nutrients, demonstrated a 97% reduction of the *Microcystis* colonies.

However, since only one sample was examined, statistics could not be generated for that study. Further research *in situ* is needed to ascertain the long–term effects of removal of nutrients and colonial cyanobacterial colonies. We suggest that removal of *Microcystis* from small lakes and ponds will improve algal biodiversity, benefiting zooplankton, fishes, endangered birds, and other species in lentic habitats.

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CHAPTER FOUR

ECOLOGICAL IMPACTS OF SOUTHERN CALIFORNIA PONDS AND SMALL LAKES, AND STRATEGIES FOR THEIR CONSERVATION

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Abstract

Southern California ponds and small lakes are of ecological importance to native flora and fauna. They are threatened by air pollution, climate change, nutrient loads, and anthropogenic disturbances. The distinction between ponds and lakes is based on size, which has not been universalized. Phytoplankton biodiversity is different in ponds and shallow lakes, compared to larger lakes. Since light penetrates to the bottom of shallow waterbodies, nutrient enrichment rapidly fosters the growth of cyanobacteria, and algal toxins are of particular concern. Due to their small size and simple community structure, ponds function as early warning systems for long term effects on larger aquatic systems. There are causal associations between communities of organism groups (macrophytes, zooplankton, macroinvertebrates and water birds) and a variety of ecologically relevant gradients, such as nutrient load, hydroperiod, surface area, salinity, and connections between ponds. Therefore, protection of environmental gradients is essential for the conservation of biodiversity in ponds as well as the total landscape. Risk factors from surrounding land use must be identified, such as nutrient loads, erosion, and pesticide contamination. Impacts from climate change are also of concern. Adaptation measures in land use can increase the capacity of small waterbodies to adapt to variations in weather. Installation of buffer zones and islands also contribute to conservation of shallow water ecosystems. New filtration technology is available to remove dissolved nutrients and colonial cyanobacteria from ponds and small lakes. Such mitigation efforts may improve aquatic health in most sites, yet negative consequences may occur in sites containing organisms which actually benefit from the presence of cyanobacteria.

Introduction

Southern California lakes and ponds are of significant ecological and economic importance due to their impact on the local biodiversity and provision of recreational opportunities. Human pursuits are often focused on immediate intent rather than cumulative impact. Thus, little is done to monitor and improve water conditions in smaller lakes and ponds. Since water quality determines the distribution and abundance of many plants and animals, examination of factors impacting the quality of Southern California lakes and ponds is vitally important to maintain healthy ecosystems on a large scale.

Southern California ponds and small lakes provide habitats for many endangered species of invertebrates, reptiles, plants, and birds. Ponds and small lakes are threatened by climate change and anthropogenic modifications which occur during residential development. Ponds are distinguished from lakes according to size, but the demarcation line is controversial. Much of the water that supplies Southern California waterbodies comes from two sources, and the quality of that water affects biodiversity and distribution of aquatic and terrestrial species downstream. Water depth affects temperature and sunlight penetration, which influences algal compositions. Shallow waterbodies, such as ponds and small lakes may be particularly sensitive to eutrophication. Decreases in phytoplankton biodiversity foster the growth of cyanobacteria, such as *Microcystis*. Although some cyanobacteria are not a nuisance to aquatic ecosystems, *Microcystis* is a is of particular concern, since it produces a deadly toxin that impacts the entire food web of the ecosystem (Oh *et al.* 2000). Conservation and management of ponds and small lakes must address several factors, including protection of aquatic species from climate

change, excess nutrient loads, erosion, and pesticides. Sites contaminated with *Microcystis* may benefit from filtration technology. However, the ecosystem should be evaluated for organisms that may be negatively impacted by the removal of cyanobacteria, such as fish larvae which use cyanobacterial mats for protection against predation (Engström-Öst *et al.* 2006). Initiating conservation efforts for eutrophic ponds and small lakes must be done gradually to prevent shock to desirable species. Buffer zones should be created and maintained wherever possible, and installation of islands in existing habitats may be an excellent conservation strategy for ponds and small lakes.

Ecological Importance of Southern California Ponds and Small Lakes

Southern California is home to thousands of plant and animal species, many of which are found nowhere else on Earth. Approximately 200 species of plants and 200 species of animals are currently considered threatened or sensitive by government agencies and conservation groups in Southern California (Bond and Bradley 2005). Any disturbance that causes a change in landscape may place species and communities at risk. The US Department of Fish and Game actively seeks methods and strategies for sustaining species viability and ecological integrity (Stephenson and Calcarone 1999).

Water diverted from streams, rivers, and lakes of the Sierra Nevada mountains supplies a large portion of Southern California lakes and ponds (Heard *et al.* 2008). The quality of this water determines distribution and abundance of many plants and animals. Lakes and streams support rich communities of native organisms in the water and in adjoining riparian areas. Links within these habitats are multifaceted, and are fundamentally dependent on natural flows of water. Constituents of this water (mineral

particles, organic matter, solutes, biota) are highly variable over time, changing markedly between seasons (UCD 1996). Native biota is well adapted to these seasonal extremes, but anthropogenic modifications of these habitats occur during residential development (Heard *et al.* 2008). Such alterations include volume of water, flood peaks, duration of low flows, seasonal timing, sediment supply, amounts of nutrients and organic matter, and water temperature (UCD 1996). Introduction of exotic fishes and reptiles, conversion of streams to lakes, and conversion of riparian zones to roads and structures also have deleterious effects (Sickman *et al.* 2003). Scientists have documented a decline in distribution and abundance of native aquatic and riparian organisms, which has led to deterioration of biotic integrity and sustainability of aquatic systems in Southern California (UCD 1996). Deterioration of aquatic habitats contributes to loss of native species, which negatively impacts ecosystem functions.

Although much attention is given to larger aquatic sites, ponds are also of vital importance in protecting freshwater biodiversity. Ponds may support populations of two–thirds of all wetland plants and animals (Williams *et al.* 1997). Many species, particularly invertebrates and amphibians, are largely restricted to ponds, which are also home to some of our rarest freshwater plants and animals (Gee *et al.* 1994). Unpolluted ponds have more uncommon species than polluted ponds, richer aquatic plant communities, and fewer problems with nuisance levels of plants, such as duckweeds and algae, or alien species, such as Water Fern (Williams *et al.* 1997). It is therefore important to protect and adequately maintain ponds as important freshwater resources. The ecology of ponds is evaluated according to their wetland plant and

macroinvertebrate communities, physiochemical characteristics, and their value as a biodiversity resource (Nicolet 2001).

Ponds and Small Lakes Are Being Threatened

Ephemeral ponds have fewer species of plants and invertebrates than comparably sized perennial ponds. However, unpolluted ephemeral ponds may have more species per site than ponds which have been degraded by human activities, suggesting that degradation may have a worse effect than drying out on species richness of ponds (Bennett 1997). The most striking difference between ephemeral and perennial ponds is that ephemeral ponds have a significantly greater number of mobile species, such as water beetles, and perennial ponds have a significantly higher proportion of less mobile species, such as water snails (Nicolet 2001). Although ephemeral ponds generally support fewer wetland plant and invertebrate species per site than are found in perennial ponds, they are especially important for amphibians and certain rare species (Bratton 1990, Collinson *et al.* 1995, Williams *et al.* 2001). Lakes are valued for their importance for ecological considerations, wilderness character, recreational uses, and regional water supplies. Water resources are subjected to natural and anthropogenic disturbances that have potential to modify systems and degrade water quality (Sickman *et al.* 2003). Some of these stressors are localized, such as visitor use, which threatens specific waterbodies, whereas other stressors are systemic, such as drought or flood conditions, which threaten regional ecosystems.

Differences between Lakes and Ponds

A lake is a body of relatively still fresh or salt water, localized in a basin that is surrounded by land (Moss *et al.* 1996). Ponds are natural or man–made waterbodies between 25 m^2 and 2 ha in area, and may be perennial or seasonal (Collinson *et al.* 1995). The technical distinction between a pond and a lake has not been universally standardized. Definitions for a pond include "bodies of water where light penetrates to the bottom" (EPA.gov 2010), or "bodies of water which lack wave action on the shoreline" (LakeManagement.org 2010), and some definitions describe only size differences (Moss *et al.* 1996, Williams *et al.* 2004). Even among those who distinguish lakes from ponds by size alone, there is no universally recognized standard for the maximum size of a pond. The International Ramsar Wetland Convention sets the upper limit for pond size as 8 ha (Ramsar.org 1999); the British Pond Conservation defines pond size between 1 m^2 and 2 ha (Collinson *et al.* 1995); the European Pond Conservation Network sets the upper size limit at 5 ha (De Meester *et al.* 2005, DeMeester *et al.* 2005, EPCN 2008), and some much larger bodies of water, such as Walden Pond in Concord, MA, measure 25 ha (Mass.gov 2010).

Natural vs. Man–Made Lakes and Ponds

Natural lakes are generally found in mountainous areas, rift zones, and areas with ongoing glaciation, yet others are found in endorheic basins or along courses of mature rivers (EPA.gov 2010). All lakes eventually evaporate, erode, fill in with sediments or plant growth, or spill out of their basins (Baxter 1972). Many lakes are artificial and are constructed for industrial or agricultural use, for power generation, domestic water

supply, or for aesthetic or recreational purposes (EPA.gov 2010). Natural ponds are fed by underground springs, although most Southern California ponds are man–made.

Ephemeral vs. Perennial Ponds

Due to their size, lakes in Southern California are not ephemeral. Ephemeral ponds are lentic waterbodies with a recurrent dry phase (Williams *et al.* 2001). Although ephemeral ponds dry out annually, they can recur for many decades. Despite this ephemeral nature, these habitats may be the most stable of freshwater environments, filling in much more slowly than larger and deeper ponds and lakes (Gray 1988). Many ephemeral ponds do not accumulate the sediment loads of perennial waterbodies because organic matter is rapidly oxidized during the dry period (Biggs *et al.* 1994). Ephemeral ponds are of particular ecological importance for their specialized faunal assemblages and the considerable numbers of rare and endemic species they support (Bratton 1990). Despite their persistence under natural conditions, ephemeral ponds are highly vulnerable to anthropogenic damage. Their small volumes make them particularly susceptible to water pollution (Williams *et al.* 2001) and climate change (Bailey-Watts *et al.* 2000). Further, land drainage rapidly damages the ecosystems of ephemeral ponds due to their shallow depths. Because of declining numbers of ephemeral ponds, many specialized species associated with them have become threatened or rare (Biggs *et al.* 1994).

The EPA lists several ways in which waterbodies may be formed (EPA.gov 2010). Glacial lakes occur where basins have been excavated or altered by moving ice. Oxbows form when river meanders are cut off from the main channel. Levees result

when the water levels of rivers become too high and deposit enough sediment to form a completely separate body of water. Levees may also be man–made to restrain flow of water from another source. Sinkholes are ponds formed in the depression left by dissolving organic materials such as salt and limestone; they are often alkaline and hypersaline. Barrier lakes occur behind sand bars in coastal regions; they are brackish due to tidal flow or sea spray. Beaver ponds are constructed and maintained by beavers and are often abandoned when food supplies run low.

Sources of Water That Sustain Southern California Lakes and Ponds

Although most lakes in Southern California are manmade, some natural lakes still exist. For example, the only natural lakes in Orange County are the three in James Dilley Preserve, of which Barbara Lake is the largest (CAopenspace.org 2010). Natural lakes are mainly formed from rivers. There are three main water sources that supply the lakes of Southern California: the Los Angeles aqueduct, the California aqueduct, and the Colorado aqueduct (MWD 2011). According to the Metropolitan Water District (MWD), the Los Angeles aqueduct was constructed in 1913 and is fed by Owens River, which originates from snowmelt in the Easter Sierra mountains, 375 km from Los Angeles (MWD 2011). They also state that water diverted from Owens Lake caused water levels to drop, and since it had no natural outlet, its sulfates, carbonates, and chlorides, became concentrated. Under certain weather conditions, these salts evaporated into clouds which caused some of the worst pollution problems in the entire United States (MWD 2011). The MWD documents that water was then diverted from Mono Lake, which also became hypersaline. Since Mono Lake is an important temporary habitat for migratory birds,

water diversion was limited in the 1980's, when Mono Lake's hypersalinity was recognized as a dangerous environmental impact; since then, the lake level has stabilized (MWD 2011).

Most lakes in Southern California are supplied by the Metropolitan Water District. Water for those sites originates from the Colorado River aqueduct, at Lake Havasu in Arizona, and terminates after 390 km in Lake Matthews in Riverside County (MWD 2011). The district treats that water at its five water filtration plants in Southern California (MWD 2011). Ponds of Southern California are supplied by three types of sources: surface runoff, groundwater and inflows (Sophocleous 2002). Many ponds are fed by several of these sources, the importance of which may vary during the year. Surface run–off in agricultural and urban areas often contains high levels of soluble pollutants (e.g., nitrate, phosphate, biocides), metals, and toxins. Ponds in natural landscapes that are fed by surface run–off are the least impacted by this disturbance. Ponds in areas of intensive land use can be protected from pollution by installation of buffer zones, such as marsh plants or filtration devices (Williams *et al.* 1997). Groundwater ponds contain moving water, and are often less polluted than surface run–off ponds because water is partly filtered by sediments, and soluble pollutants are continually diluted and carried away. Inflow ponds may contain clean or polluted water, depending on quality of the inflow's catchment. Anthropogenic changes in hydrology threaten the existence of ephemeral (temporary) ponds by causing excessive or depleted water levels. Ephemeral ponds are inherently shallow and are destroyed, even by limited drainage for agriculture or urban development (Baskin 1994).

A pond with no water can be good for aquatic wildlife. Adding water to ephemeral ponds in an effort to keep a pond from drying out will result in an inflow of pollutants, build up of organic sediments, and changes in vegetation composition and structure (Biggs *et al.* 2001). Making ephemeral ponds perennial also allows colonization by animals that cannot tolerate periods of drought, such as waterfowl, which will be able to make use of the pond throughout the year, depositing additional nutrients through their feces. Stocking a pond with fishes also negatively impacts biota and physical structure of the pond by increasing turbidity and reducing plant and algal growth (Collinson *et al.* 1995). Deepening of ephemeral ponds is equally damaging. When aquatic vegetation is removed, subsequent changes to phytoplankton composition occur, which may foster overgrowth of cyanobacteria. One option is to leave the site alone and create another deeper, more perennial pond nearby to add to the range of habitats available. However, this option may result in an increase in amphibian communities, which may disrupt ecological balance (Bratton 1990). As length of the hydroperiod increases, ephemeral ponds may convert into semi–perennial ponds that dry only in drought years, and then into perennial ponds. As their sizes increase, ephemeral ponds may become ephemeral lakes and marshes (Williams *et al.* 2001).

Phytoplankton Biodiversity in Ponds and Shallow Lakes

Algal species vary with trophic level. Diatoms, for example, require fewer nutrients than other algae, and therefore are common in oligotrophic (nutrient–poor) waterbodies (UCD 1996). Zooplankton are more numerous in lakes and ponds that are rich in phytoplankton. These invertebrates are a good indicator of the health of an aquatic

system. Shifts in composition of invertebrate communities suggest changes in aquatic habitat or water quality. Common zooplankton found in lakes and ponds include rotifers, water fleas, and copepods. Insects such as water beetles and larval mosquitoes are also common. Various freshwater fishes and amphibians feed on the zooplankton and algae. A variety of birds such as ducks, coots, osprey, and raptors feed on the abundant food resources of a lake or pond (UCD 1996). The health of an aquatic ecosystem depends on the quality of the upstream watershed. Road construction is one disturbance that results in excessive sedimentation, which washes excess nutrients into the water (UCD 1996). High levels of nutrients have been shown to foster the growth of toxic cyanobacteria (Magrann *et al.* Submitted-b).

Ponds and lakes differ with respect to phytoplankton composition and biodiversity. Ponds significantly contribute to an ecosystem by creating links between existing aquatic habitats, providing nutrient interception, and regulating hydrological processes (Ce´re´ghino *et al.* 2008). Ponds are numerous, typically outnumbering larger lakes by a ratio of about 100 to 1 (Oertli *et al.* 2005). Ponds function differently from lakes (Sondergaard *et al.* 2005), are richer in biodiversity (Williams *et al.* 2004), and show greater biotic and environmental amplitudes than rivers and lakes (Davies 2005). Compared to lakes, ponds support more macroinvertebrate and zooplankton species (DeBie *et al.* 2007) and more uncommon species (Oertli *et al.* 2007). Ponds outside nature reserves are significantly degraded and support fewer plant species than those which are in protected areas. Environmental factors that correlate with species number and rarity include area, isolation, pH (and the related chemical measures alkalinity, calcium, conductivity), and abundance of vegetation (Biggs *et al.* 2005). Degraded ponds

are those which have few algal species and few rare species. This condition is often caused by intensive land use and excessive nutrient levels (Biggs *et al.* 2005).

Ponds and lakes differ in depth, causing variations in light and temperature which influences algal compositions. Red algae have chloroplasts containing accessory pigments called cyanoxanthins. Red algae absorb every color wavelength except red. Since red wavelengths are absorbed in the first few meters of water, red algae thrive in waters deeper than several meters (Konopka and Brock 1978). Blue–green algae (cyanobacteria) possess chloroplasts containing the blue pigment, phycocyanin. Blue wavelengths are more readily absorbed if the water contains average or abundant amounts of organic material, so these algae thrive in such conditions (Konopka and Brock 1978). Green algae chloroplasts contain mostly chlorophyll. Blue and green wavelengths are predominant in deep water. Since green algae absorb every color except green, they thrive in shallower waters and cannot survive at depths (Konopka and Brock 1978). Yellow and brown algae contain chloroplasts with carotenoids and fucoxanthin pigments, strongly absorbing green wavelengths and preferring deeper water (Konopka and Brock 1978). The temperature range for optimal algal growth varies by species. Some blue–green algae grow best between 15°C and 30°C (Konopka and Brock 1978), whereas some red algae prefer temperatures of 5°C (Brody and Emerson 1959). Since water depth influences what algal phyla are present, it may influence production of cyanobacterial toxins.

Algal Toxins from Cyanobacteria

Cyanobacteria share some characteristics with plants as well as bacteria. They are photosynthetic like plants, yet are capable of producing endotoxins like many pathogenic bacteria. Some cyanobacteria are beneficial to the environment by converting nitrogen gas to usable ammonium or nitrate (nitrogen fixation), serving a vital role in the nitrogen cycle (Tomitani *et al.* 2006). Stromatolites are another type of cyanobacteria which are beneficial to aquatic communities due to their diversity, complexity and environmental associations (Allwood *et al.* 2006). However, other cyanobacteria produce neurotoxins, such as saxitoxin or anatoxin–a, both of which cause paralytic shellfish poisoning (PSP), and still others produce hepatotoxins, such as microcystin (Dell-Aversano et al. 2004). A list of cyanobacteria and their toxins is provided in Table 4.1.

Cyanobacteria have been linked to illness in various regions of the world, including North and South America, Africa, Australia, Europe, Scandinavia and China (CDC 2010). Diehnelt *et al*. (2005) stated that cyanobacteria have likely been transported globally by natural and anthropogenic processes, and their growth is stimulated by nitrogen and phosphorus, warm temperatures, stagnant water, and intense sunlight. The toxicity and increased occurrence of cyanobacterial blooms led the U.S. Environmental Protection Agency (USEPA) to include cyanobacterial toxins on the drinking water Contaminant Candidate List for further study and regulation (Antoniou *et al.* 2005).

Table 4.1. Cyanobacteria and their toxins, adapted from Gupta (2007).

Category	Toxin	Cyanobacteria
Neurotoxins	Anatoxin–a	*Anabaena* *Pseudanabaena* *Aphanizomenon* *Oscillatoria* *Planktothrix* *Cylindrospermopsis* *Phormidium* *Trichidesmium* *Woronichinia*
	Anatoxin–a(s)	*Anabaena* *Oscillatoria*
	Saxitoxin	*Anabaena* *Aphanizomenon* *Cylindrospermopsis* *Lyngbya* *Planktothrix*
	Neosaxitoxin	*Aphanizomenon*
Hepatotoxins	Microcystin	*Microcystis* *Anabaena* *Gloeotrichia* *Nostoc* *Oscillatoria* *Planktothrix* *Anabaenopsis* *Hapalosiphon*
	Nodularin	*Nodularia*
	Cylindrospermopsin	*Cylindrospermopsis* *Aphanizomenon* *Raphidiopsis* *Umezakia*
Tumor promoters	Microcystin	*Microcystis* *Cylindrospermopsis* *Gloeotrichia* *Nostoc* *Oscillatoria* *Planktothrix* *Anabaenopsis* *Hapalosiphon*
	Cylindrospermopsin	*Cylindrospermopsis*
	Aplysiatoxin	*Lyngbya* *Oscillatoria* *Schizothrix* *Symploca*
	Lyngbyatoxin	*Lyngbya*
	Oscillapeptin–J	*Planktothrix*
Cytotoxins	Lipopolysaccharides	*Gloeotrichia* *Lyngbya* *Nostoc* *Hapalosiphon*
	Scytophycin	*Scytonema*

There are many types of microcystin toxins, and it is not uncommon for one pond to contain many different varieties. Microcystin and other cyanobacterial toxins have been associated with taste and odor problems in drinking water, and with poisoning of birds and livestock (WHO 2001). These toxins can also impact the food web of an entire lake by affecting zooplankton feeding and reproduction. According to Dietrich and Hoeger (2005), diversity of toxic species and their toxins is great, with over 80 different types of microcystins alone. They also found that a given species may not produce toxins, and those that do, may produce multiple types of toxins.

The WHO (2003) stated that people may be exposed to cyanobacterial toxins by bathing in contaminated water, although the most frequent and serious health effects are caused by drinking water containing the toxins, including ingestion during recreational water contact. They also warned that "surface scums" are particularly hazardous due to their high toxin content, and that children should be especially protected. The WHO (2001) stated that disease due to cyanobacterial toxins varies according to the type of toxin and type of exposure (drinking vs. skin contact). They listed symptoms of microcystin toxicity in humans as skin irritation, fever, sore throat, headache, muscle and joint pain, oral blisters, diarrhea, vomiting, weakness, and pallor. Chronic exposure has been shown to enhance growth of hepatic and colonic pre–cancerous lesions, suggesting that microcystins may act as tumor promoters (Burch 2008). The WHO (2001) stated that onset of symptoms occurs rapidly, with death resulting in a few hours after a lethal dose. They also stated that damage to the liver is rapid and irreversible, and dialysis or liver transplants may be the only effective treatments. The antibiotic isoniazid (Rifampin) has been shown to be somewhat effective as a prophylactic in animal studies (Lakshmana

Rao *et al.* 2004). Microcystins may be inactivated by high levels of chlorine (Xagoraraki *et al.* 2006). Temperature also affects microcystin concentrations. Oh *et al*. (2000) found that microcystin levels of *Anabaena* and *M. aeruginosa* were highest at 25°C and between 20 and 24°C, while temperatures beyond optimal levels decreased the number of those algal cells.

The gene cluster for microcystin toxin (McyS) has been identified (Pearson *et al*., (2010), and is now possible to detect within cyanobacteria. Toxic and non–toxic cyanobacterial strains have different genes that can be determined from PCR amplification assays. This procedure can discern differences in geographical origins for species of morphological similarity (Pearson *et al*., 2010). Wilson *et al*. (2005) demonstrated that *Microcystis aeruginosa* strains were genetically diverse within and among 14 lakes in 9 counties in Michigan, but the microcystin toxin gene was found in strains from all of the lakes studied. Further genetic research would be useful to determine factors that affect microcystin production.

Several factors control the dominance of *Microcystis* relative to other phytoplankton species in ponds and lakes. Diatoms and green algae tend to dominate systems with higher N:P ratios, and cyanobacteria tend to dominate at lower ratios (Falconer 2004). Salinity is another factor that may influence growth of cyanobacteria (Liu 2006). Tonk *et al*.(2007) successfully cultured *Microcystis aeruginosa* in salinities up to 18 $g \cdot L^{-1}$, yet Orr *et al*. (2004) successfully cultured the same species in salinities up to 21.2 $g \cdot L^{-1}$. Magrann *et al*.(Submitted-b) identified *Microcystis* colonies in several Southern California bays with salinities of 35 $g \cdot L^{-1}$. These differences in salinity tolerance might be attributed to variations in salt tolerance among strains or subspecies of

Microcystis aeruginosa. Lindon and Heiskary (2009) reported a significant positive correlation between microcystin toxins and pH, yet a significant negative correlation between microcystin and alkalinity.

According to Sawyer *et al.* (2003), algae consume carbon dioxide in their photosynthetic activity, causing high pH conditions in lakes. Those authors also found that algae can continue to extract carbon dioxide from water until an inhibitory pH is reached, usually in the range of pH 10 to 11. In serial reuse systems, Colt *et al*. (2009) found that excretion of metabolic carbon dioxide has a significant impact on ambient pH, carbon dioxide, and unionized ammonia concentrations. They also found that this impact depends strongly on alkalinity and loss of carbon dioxide. While testing nutrient removal techniques, Crossetti and Bicudo (2005) found that space liberated by blue–green species that did not adapt to nutrient–impoverished conditions, was gradually occupied by other algal species. They found that nutrient impoverishment induced increased levels of CO_2, while alkalinity and pH decreased. They concluded that these conditions contributed to decreased photosynthetic activity of all algal species present.

Ecological Effects of Harmful Algal Blooms

The presence of *Microcystis* can cause ecological discord. Magrann *et al*. (Submitted-a) demonstrated that *Microcystis* causes a significant negative impact on algal biodiversity. Terrestrial organisms are also at risk. Rodas and Costas (1999) found that mice and livestock are unable to detect microcystin toxins in water, and have a preference for consuming dense cultures of *Microcystis*, rather than perfectly clear water, even though the toxins lead to their death. Zurawell *et al*. (1999) found that *Microcystis*

accumulates in pulmonate snails, which may introduce toxicity into other aquatic ecosystems. *Microcystis* has also been shown to cause fatal molting in *Daphnia* species which feed on them (Rohrlack *et al.* 2004). *Microcystis* in the presence of the zebra mussel, *Dreissena polymorpha*, can cause synergistic problems. Fields (2005) stated that *D. polymorpha* consume *Microcystis* amidst organic debris, and discharge the undesirable cyanobacteria along with its pseudofeces. The pseudofeces, in turn, releases nutrients that feed the *Microcystis*. Jang *et al.* (2004) discovered that several *M. aeruginosa* strains increased toxin production when exposed to fish, especially phytoplanktivorous species, even when fish appeared not to feed vigorously on toxic *Microcystis*.

Conservation and Management of Ponds and Small Lakes

The goal of ecosystem management is to use ecological knowledge to produce desired resources, products, and services while sustaining diversity and productivity of ecosystems (Stephenson and Calcarone 1999). In California, several groups cooperate to design management strategies for sites having ponds and lakes of ecological importance, including the U.S. Department of Agriculture, California Department of Fish and Game, the U.S. Department of Interior, Fish and Wildlife Service, and the Bureau of Land Management (Stephenson and Calcarone 1999). Status and trend information is invaluable in making management decisions, monitoring compliance with State water quality standards, influencing regional, state, and national environmental policies, and providing information to the public (IPCC 2007). Data required for lake management includes trends in composition, structure, and extent of ecological communities, as well as natural and human processes that are driving landscape change (Stephenson and

Calcarone 1999). Baldwin and Boulding (2008) recommend that phytoplankton data be obtained monthly, or more frequently during growing seasons. They also recommend that multiple sites at each waterbody be sampled, and at various depths, if possible. Bodini (2000) used a qualitative algorithm model to explain observed patterns of nutrient abundance from numerous inputs, and to predict subsequent effects on local food webs. The International Lake Environment Committee (ILEC) has outlined seven management principles (Table 4.2) specifically designed for lentic waters of lakes and reservoirs (ILEC 2005). Rast (2008) stated that one of the most difficult lake management strategies to universalize is governance, such as institutions, policy, stakeholder participation, public awareness, sustainable financing, and cultural values. These issues should be addressed in addition to physical landscape modifications.

Table 4.2. The seven principles of the World Lake vision. Source: ILEC (2005).

Principle 1	A harmonious relationship between humans and nature is essential for the sustainability of lakes.
Principle 2	A lake drainage basin is the logical starting point for planning and management actions for sustainable lake use.
Principle 3	A long–term, proactive approach directed to preventing the causes of lake degradation is essential.
Principle 4	Policy development and decision making for lake management should be based on sound science and the best available information.
Principle 6	Citizens and other stakeholders must participate meaningfully in identifying and resolving critical lake problems.
Principle 7	Good governance, based on fairness, transparency and empowerment of all stakeholders, is essential for sustainable lake use.

Possible Negative Consequences of Conservation Efforts to Mitigate Eutrophication

Mitigation of lake pollutants by nutrient restriction may decrease numbers of cyanobacteria. Reduction in cyanobacterial cell counts is beneficial in removing toxins, yet it poses a question regarding the subsequent impact on algal biodiversity. Cyanobacteria are not the primary food source for most organisms, since the function of cyanobacterial toxins is to prevent predation (Kirk and Gilbert 1992). Toxic cyanobacteria have a low sterol content, making them a poor food source (Wiegand and Pflugmacher 2005). However, problems may arise after removal of *Microcystis* from lakes and ponds. Those organisms that are able to cope with toxins may lose their dominance in that ecosystem, causing an imbalance. Herbivorous fishes, and therefore predatory fishes, avoid areas with high *Microcystis* concentrations. Although larvae of these fishes do not feed on cyanobacteria, the presence of that toxic alga may function as important refuge during predation pressure. Some organisms are able to tolerate ingestion of cyanobacteria to varying degrees (Table 4.3). Considering the variety of species that consume *Microcystis* as a food source, experimental studies under laboratory conditions, such as that done by Magrann *et al*. (Submitted-a), are appropriate to ascertain the effect of removal of *Microcystis* on algal biodiversity at individual sites before using filtration technology.

Initiating Conservation Efforts for Eutrophic Ponds and Small Lakes

Ce´re´ghino *et al* (2008) stated that direct threats to ponds include habitat destruction (in–filling ponds; deepening of ephemeral pools so that they become

perennial) or other forms of strong human impact (e.g. urban runoff, acidification, diffuse agricultural pollution, introduction of exotic species, excessive trampling by livestock). Biodiversity in ponds can be protected when threats from surrounding land use are identified. Risk factors include nutrient loading, increased erosion, and pesticide contamination (Declerck *et al.* 2006). Conservation efforts for ponds and lakes must also consider climate change impacts. Adaptation measures in land–use planning and infrastructure design can increase the capacity of a small waterbody to adapt to variations in weather (IPCC 2007).

There are numerous strategies to initiate conservation efforts for small lakes which are regularly affected by toxic algal blooms. Biggs *et al*. (1994) stated that the first principle of pond management is to make use of existing habitats, even if they are not aesthetically pleasing. They asserted that muddy surrounds, shaded banks, bare sand, floating grasses and dense areas of emergent plants provide opportunities for biodiversity. They also recommended avoidance of making all ponds look the same, to maintain a variety of stages of succession and depths. They warned against making a sudden change in the management regime of a pond or its surrounds (e.g., by drastic deepening or tree clearance), due to risk of damage to community structure. They stated that buffer zones should be created and maintained wherever possible.

Table 4.3. Species tolerance of *Microcystis*.

Category	Organism	Tolerance	Reference
Bacteria	*Bacillus cereus*	Lyses *Microcystis* cells and consumes them	Gumbo et al.(2008)
	Pseudomonas	Detoxifies microcystin	Jones *et al.* (1994)
	Ochromonas	Feed on cyanobacteria	Burkert *et al.* (2001)
Protozoa	Amoeba *Nuclearia delicatula*	Feed on cyanobacteria	Wright *et al.* (1981) Sigee *et al.* (1999)
	Ciliate *Nassula tumida*	Feed on cyanobacteria	Sigee *et al.* (1999)
Invertebrates	Copepods	Feed on cyanobacteria	Yoshioka and Wada (1994)
		Consume only non–toxic strains of cyanobacteria	DeMott and Moxter (1991)
		Small ones avoid cyanobacteria, but large ones feed on them	Kirk and Gilbert (1992)
	Rotifers	Small ones avoid cyanobacteria, but large cladocerans feed on them	Kirk and Gilbert (1992)
	Cladocerans (water fleas) *Boeckella triarticulata*, *Moina micrura*, *Ceriodaphnia dubia*	Graze and survive on cyanobacteria, although may avoid toxic strains	Boon *et al.* (1994)
		Decomposed *Microcystis* seemed to be utilized as an important food source	Hanazato and Yasuno (1987)
		Small ones avoid cyanobacteria, but large cladocerans feed on them	Kirk and Gilbert (1992)
Crustaceans	Mysid shrimp *Mysis mixta*	Feed on cyanobacteria	Engström-Öst *et al.* (2001)
Fishes	Mullets *Liza dumerili*, *Mugil cephalus*, and *M. curema*	Have stomach pH of 7.0 – 8.0 to digest cyanobacteria	Gehrke and Harris (1995)
	Rainbow trout *Oncorhynchus mykiss*	Unaffected by the presence of cyanobacteria	Deblois *et al.* (2008)
	Tilapia *Oreochromis niloticus* and *Tilapia rendalli*	Can feed on *Microcystis* and deactivate its toxins	Deblois *et al.* (2008)
	Herbivorous fish *Oreochromis niloticus*	Feed on cyanobacteria	Getachew (1987)
	Three–spined stickleback fish *Gasterosteus aculeatus*	Preferred a toxic cyanobacterial habitat to a chemical predator signal	Engström–Öst *et al.* (2006)

Williams *et al*. (1997) found that semi–natural buffers were most effective around surface fed waterbodies where most flows to the pond can be intercepted, yet groundwater fed ponds may also benefit from buffer zones because, as these ponds age and accumulate sediments, their subsurface flows may reduce and the immediate surroundings become more important in determining water quality. However, they stated that around inflow–fed ponds, buffer zones have little impact, since the watercourses draining into the pond primarily determine its quality. They recommended sediment traps and reedbed filters to remove pollutants from inflow streams and pollution prevention measures throughout the upstream catchment.

Researchers have contributed guidelines for designing buffer zones as a conservation measure for ponds and small lakes (Table 4.4). Health of eutrophic ponds can also be enhanced by installation of islands. These provide good habitats for plants and macro–invertebrates, especially if the margin of the pond is shaded, grazed, or trampled (Williams *et al.* 1997). Islands are particularly valuable for large ponds, since they are a preferred habitat for wetland birds, providing safe areas for resting, feeding, and nesting (Williams *et al.* 1997). Islands should be designed with gentle slopes near water level, and low, flat areas that are submerged in spring to provide mud banks which are valuable for wetland plants and birds (Andrews and Kinsman 1990). According to Williams *et al*. (1997) deep, steep–sided irrigation ponds may support good aquatic plant communities if they can be kept pollution free. They also stated that, in heavily stocked fish ponds, dense marginal vegetation can provide invertebrates with protection from fishes. UCD (1996) reported that a watershed approach allows connections to be made between upstream actions and downstream consequences and benefits. They concluded

that it is therefore important to identify potential sources of pollution from influent water, especially land disturbance from erosion, streambank instability, loss of riparian habitat, and loss of large woody debris.

Table 4.4. Guidelines for designing buffer zones for conservation of ponds and small lakes.

	Design Features	
1	Buffer zone width of at least 30 – 50 m	(Stienstra 2000)
2	Buffer zone wider on the side of hill slopes from which most water and sediments will be derived	(Stienstra 2000)
3	Removal of tile drainage and surface runnels	(Stienstra 2000)
4	Add protection of muddy edges and annual plants from insecticide spray drift	(Williams *et al.* 1997)
5	Add wetland vegetation	(Williams *et al.* 1997)
6	Add good drawdown zones	(Williams *et al.* 1997)
7	Shallow ponds need marshy margins	(Williams *et al.* 1997)
8	Deep ponds need submerged aquatic plants	(Williams *et al.* 1997)

A new approach to conservation of small lakes was demonstrated in the study of Magrann *et al.* (2009), which used an algal filtration and phosphate removal system called Blue Pro™ (Bluewater Technologies, Idaho). This technology relies on ionized sand infused with alum to filter colonial algae, and ferric chloride to precipitate phosphate. The resulting non–toxic, iron phosphate cake can be disposed of in landfill. The study took place at a 37,000 m^2 lake in Irvine, CA, and demonstrated a 94% decrease in *Microcystis* colonies and 96% reduction in PO_4 after one pass through the unit. However, these results should be interpreted with caution since these measures resulted from a single sample during a preliminary study. Since PO_4 is one of the main nutrients

required for algal growth, this technology may improve algal biodiversity and reduce toxin production in small lakes.

Landscape conservation efforts are more effective if based on a solid knowledge of factors that affect pond community structure and diversity. Several studies have documented clear associations between communities of organism groups (macrophytes, zooplankton, macroinvertebrates and water birds) and a variety of ecologically relevant gradients, such as nutrient load (Soranno 1997), hydroperiod (Boix *et al.* 2007, Della Bella *et al.* 2007), surface area (Ce´re´ghino *et al.* 2008), salinity (Boix *et al.* 2007), and connections between ponds (Oertli *et al.* 2007, Gasco´n *et al.* 2010). If these associations are causal, conservation of environmental gradients is essential for conservation of biodiversity in ponds and total landscape (Ce´re´ghino *et al.* 2008). Due to their small size and simple community structure, ponds may also function as early warning systems for long term effects on larger aquatic systems (Ce´re´ghino *et al.* 2008).

Conclusions

Southern California ponds and small lakes are of great ecological importance, yet they are threatened by anthropogenic disturbances. Excess nutrient deposition is one of the main factors that contribute to eutrophication. Phytoplankton biodiversity is subsequently affected, fostering the growth of cyanobacteria. While not all species of cyanobacteria are nuisance strains, some, such as *Microcystis*, secrete deadly toxins. Overproduction of toxic species leads to disruption of natural aquatic and terrestrial

communities, in addition to toxin accumulation throughout the food web. Adaptation measures in land use, such as buffer zones and installation of islands, may contribute to conservation of shallow water ecosystems. Filtration of dissolved nutrients and colonial cyanobacteria may also be beneficial in those sites which do not contain organisms that depend on that algal phylum for sustenance or protection.

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CHAPTER FIVE

CONCLUSIONS

California coastal waters constitute an important segment of the Pacific Flyway, providing permanent and temporary habitats to endangered birds and other species. Excess nutrient loads and warm temperatures cause eutrophication, which fosters growth of toxic cyanobacteria, especially in lentic habitats. This research began with a survey of 66 water samples collected from 40 lakes, ponds, bays, and lagoons along the California coastline, in five counties from Santa Barbara to San Diego. Samples were analyzed for temperature, pH, EC, TDS, DO, alkalinity, chlorophyll–a, TP, PO_4, TDP, DOP, PP, TDN, TN, NO_3, NO_2, DIN, DON, and NH_4. Algal assays were conducted for phytoplankton identification and numbers, and indices of biodiversity (richness and evenness) were calculated to ascertain relationships between nutrients and algal growth, especially for beneficial Chlorophyta and toxic Cyanophyta.

We found that Cyanophyta exhibited a significant positive correlation with PO_4, while Chlorophyta exhibited a negative correlation with PO_4. Cyanophyta also exhibited strong negative correlations with Chlorophyta, Bacillariophyta, and Euglenophyta. The most predominant genera at study sites were *Microcystis* and *Euglena*. Five of the 40 sites contained excess microcystin, and two sites contained anatoxin–a in excess of drinking water limits. We found that TN and DO positively correlated with toxin levels, and sites with toxins in excess of drinking water limits had significantly higher levels of DOC. Cyanophyta positively correlated with TP and negatively correlated with EC and

DOC, while Euglenophyta positively correlated with EC and DOC. We found that DOC negatively correlated with richness (H′) of genera, yet positively correlated with evenness of genera, and TN negatively correlated with evenness (J′) of genera. During this phase of our study, we demonstrated that algal compositions and toxin production in Southern California lakes, ponds, bays, and lagoons covary with ambient water column nutrient concentrations and that diversity of phytoplanktonic taxa in Southern California lentic habitats decline and shift towards more pollution tolerant species (Cyanophyta) as nutrient load increases.

After we identified several sites along the Southern California coastline which contained algal toxins in excess of drinking water limits and high numbers of the cyanobacterium, *Microcystis*, we tested the impact of *Microcystis* on algal biodiversity. We inoculated one series of beakers with *Microcystis,* in addition to the type of algae that was most commonly seen throughout our previous study sites, and prepared an identical set of beakers without *Microcystis*. Weekly algal assays were conducted for five weeks, and richness and evenness were calculated. In the control batch (without *Microcystis*), average phyla richness (H′) declined from 0.91 to 0.87, a decrease of 4%. Average phyla evenness (J′) declined from 82% to 79%, a decrease of 4%. Genera richness declined from 1.29 to 1.27, a decrease of 2%. Genera evenness declined from 93% to 92%. In the experimental batch (with *Microcystis*), the two green algae, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii,* died during the final week, affecting richness and evenness results. Average phyla richness (H′) declined from 1.24 to 0.52, a decrease of 58%. Average phyla evenness (J′) declined from 89% to 47%, a decrease of 42%. Genera richness declined from 1.52 to 0.52, a decrease of 66%. Genera evenness declined from

95% to 47%, a decrease of 48%. This phase of our study demonstrated that the presence of *Microcystis* negatively impacts algal biodiversity by decreasing richness and evenness of algal phyla and genera under laboratory conditions.

Having demonstrated that *Microcystis* negatively impacts algal biodiversity, we assessed the effectiveness of a Blue Pro™ water treatment facility in removing this colonial organism from a small, freshwater lake, in addition to removal of dissolved nutrients required for its growth. Our phosphate restriction assay on a single sample from Mason Lake (Irvine, CA, USA), determined that a PO_4 threshold of 0.02 mg · L^{-1} was required by algae at that site. Using Blue Pro™ technology at Mason Lake, we evaluated PO_4 levels and algal composition of one sample of lake water before and after treatment. We measured a 97% reduction in *Microcystis* cells, a 72% reduction in Chlorophyll–a, and a 96% reduction in PO_4 after just one cycle through the unit. Although we recommend caution in the interpretation of these results, we do suggest that this phase of our study demonstrated that removal of *Microcystis* colonies may allow green algae to increase in numbers. This would improve algal biodiversity, benefiting zooplankton, fishes, endangered birds, and other species in lentic habitats. Information obtained in our study may be helpful in evaluating and managing land use around sites of ecological importance. Additionally, sites of particular concern can be targeted for ongoing monitoring of toxin and nutrient loads.

APPENDIX A

SAMPLE ACQUISITION AND FIELD PROCESSING

One sample from each site was acquired from June 5-July 23, 2009. Photos were taken of each site; date, time, and GPS coordinates were recorded on a bench sheet. A Secchi disc was lowered into the water until the black and white lines were no longer visible, and depth was recorded in centimeters. Visibility was determined by Secchi depth. Measurements of temperature, pH, EC, and TDS were obtained with a Hanna HI98129 Digital Tester Meter at the point and approximate time, of water sample collection. Dissolved oxygen readings were obtained with a Milwaukee SM600 DO Meter at the point and approximate time, of water sample collection. Grab samples were obtained from each site and transferred to separate tubes for laboratory analysis. Used equipment was bathed in 2M HCl for 5 minutes, then rinsed with Milli-Q water and allowed to air dry. The Milwaukee Meter (DO) probe was cleaned with the cleaning solution and stored in the storage solution included in the meter kit. Samples were obtained at approximately three sites per day, three days a week.

APPENDIX B

ALKALINITY DETERMINATION TECHNIQUE

Alkalinity was measured with a Hach Digital Titrator Model 16900 test kit. The sample was titrated with sulfuric acid to a colorimetric end point corresponding to a specific pH. Phenolphthalein alkalinity was determined by titration to a pH of 8.3, as evidenced by the color change of phenolphthalein indicator, which reflects the total hydroxide and one half the carbonate present. T (total) alkalinity was determined by titration to a pH between 3.7 and 5.1, as evidenced by the color change of bromcresol green indicator, which reflects all carbonate, bicarbonate and hydroxide present. The total alkalinity test results were recorded in $mg \cdot L^{-1}$ $CaCO_3$.

APPENDIX C

PHYTOPLANKTON IDENTIFICATION AND CELL COUNTS

Each algal assay was prepared on the same day as its acquisition. Utermöhl settling chambers were fixed to their base plates with Dow Corning high vacuum seal grease. Fifty ml of sample water was added to each chamber and allowed to settle overnight. The chamber was removed from the base plate and replaced simultaneously with a thick glass lid. The coverslip of the base plate was placed over the oil immersion lens of an inverted microscope and observed at 1000x. Ten fields were viewed; algal cells were counted and identified to genus (with assistance from Phycotech, Inc.) and cells · ml $^{-1}$ were calculated using the equation,

$$\text{Cells} \cdot \text{ml}^{-1} = (\text{cells in 10 fields}) \cdot (\text{coverslip area} \cdot \text{Field area}^{-1}) \cdot (\text{ml sample used})^{-1}$$

Calculation of Cells · ml $^{-1}$

Field Diameter Under 1000x plus camera: 100 µm = 0.1mm

Field Area: π (r^2) => (0.05 x 0.05) (3.14) = .00785 mm

Coverslip Radius: 25mm

Coverslip Area: π (r^2) => (12.5 x 12.5) (3.14) = 491mm

APPENDIX D

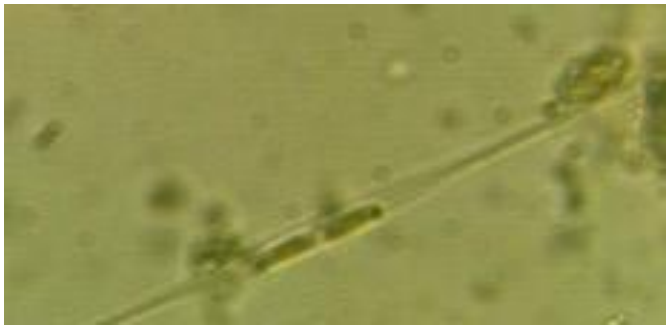
ALGAL ASSAY BENCH SHEET

Site:											
Date:											
TAXON	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**TOTALS**
BACILLARIOPHYTA											
Nitzchia 											**0**
Cymbella 											**0**
Navicula 											**0**
											0
CHLORPHYTA											
Ankistrodesmus 											**0**
Chlamydomonas 											**0**
Cosmarium 											**0**
Scenedesmus											**0**
											0
CYANOPHYTA											
Merismopedia 											**0**
Anabaena 											**0**
Aphanizomen  											**0**
Cylindrospermopsis											**0**
Microcystis 											**0**
											0
CHRYSOPHYCEAE											
Synura 											**0**
PYRROPHYTA											
Dinoflagellates 											**0**
TOTALS:	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
TOTALS (Cells/ml)											**0**

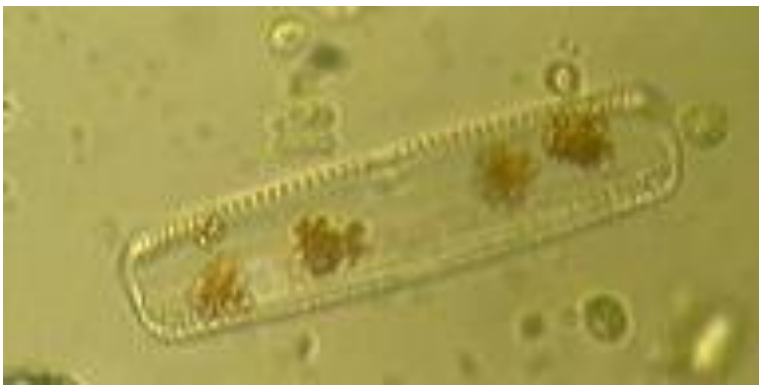
APPENDIX E

PHOTOS OF ALGAE IN OUR STUDY SITES

DIATOMS (Bacillariophyta)

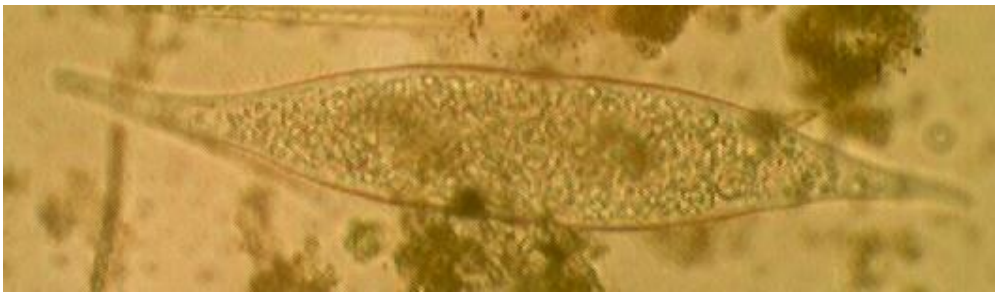


Nitzschia

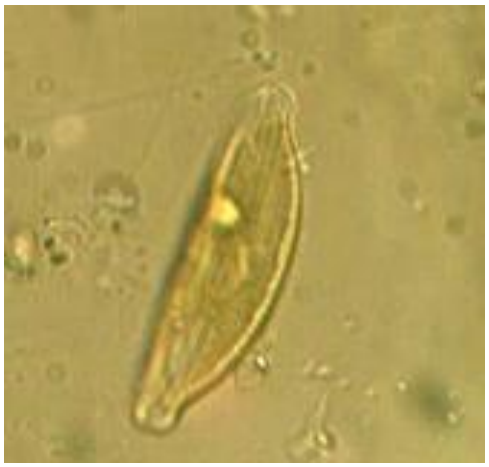


Navicula

DIATOMS
(Bacillariophyta)

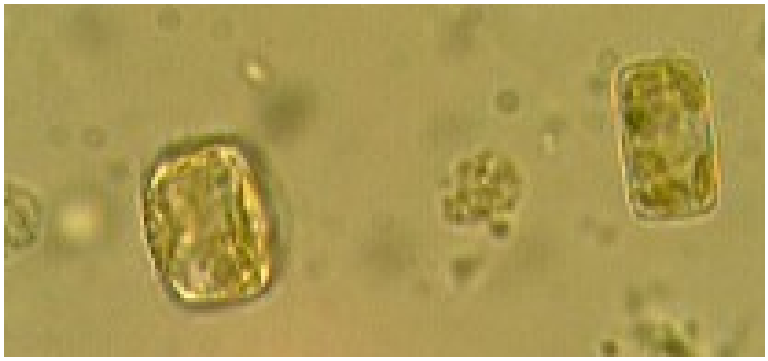


Gyrosigma



Cymbella

Cyclotella



Tabellaria

EUGLENA
(Euglenophyta)

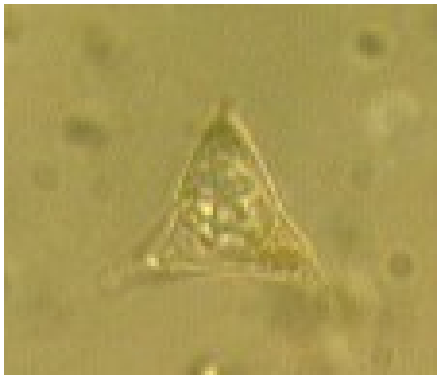
Trachelomonas

Phacus

Phacus

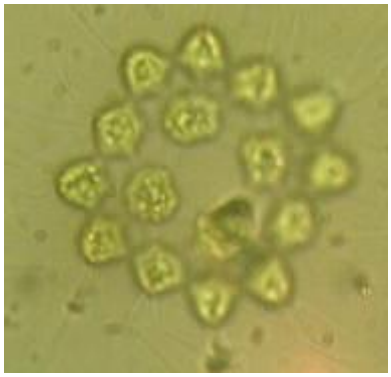
Phacus

GREEN ALGAE
(Chlorophyta)

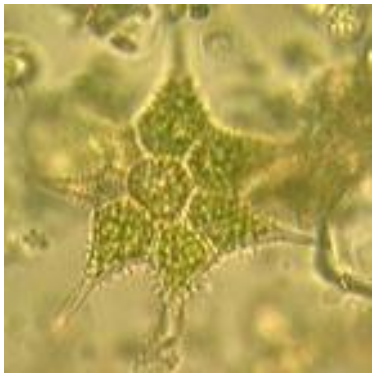


Staurastrum

Staurastrum



Pediastrum

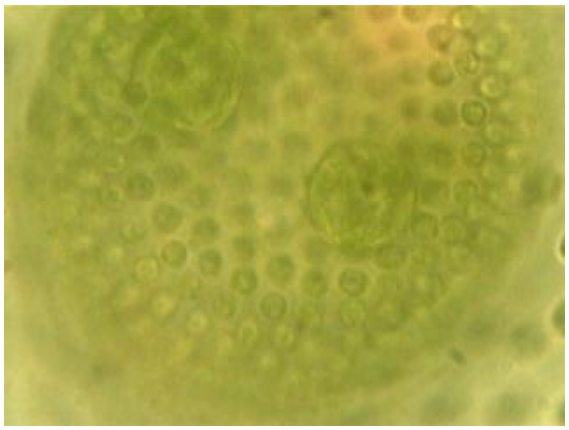


Pediastrum

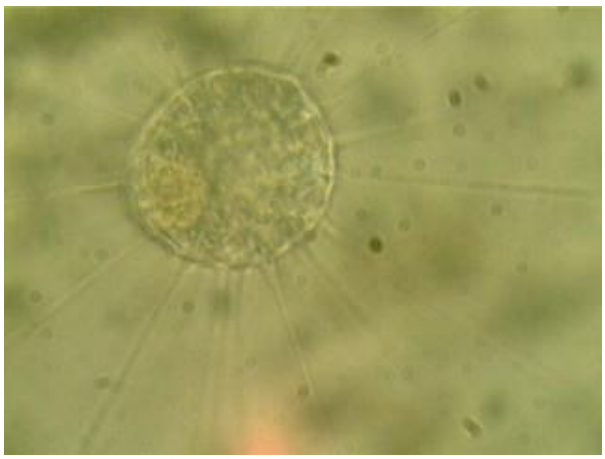
Pediastrum

GREEN ALGAE
(Chlorophyta)

Asterionella



Volvox

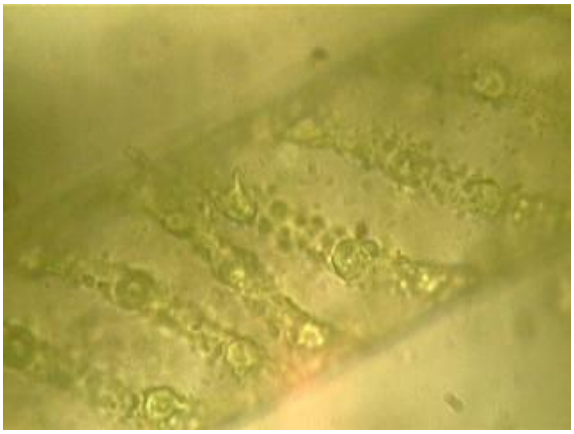


Golenkinia

Mougeotia

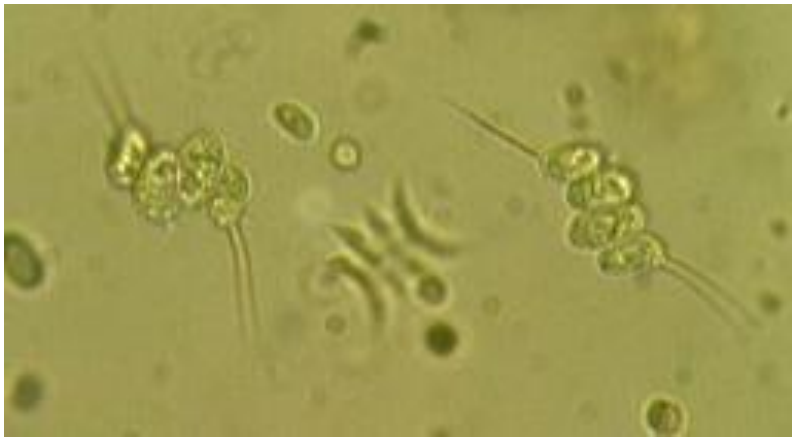
Palmellococcus

GREEN ALGAE
(Chlorophyta)

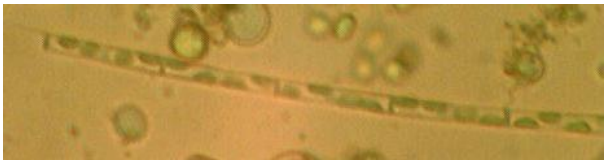


Spirogyra

Oocystis



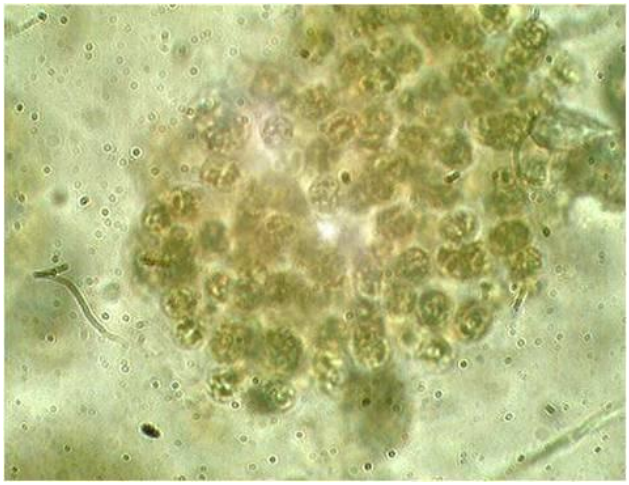
Scenedesmus



Aulacrosira

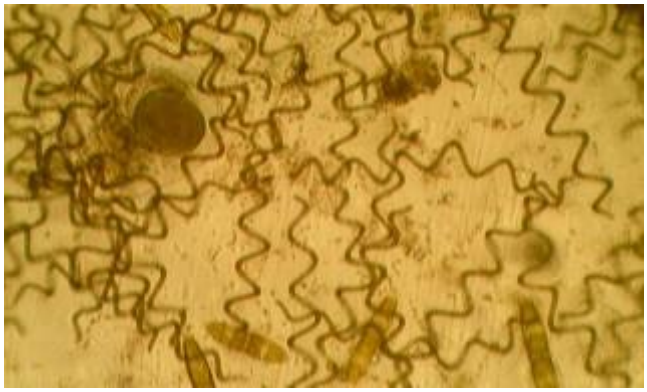
Cosmarium

BLUE-GREEN ALGAE
(Cyanophyta)

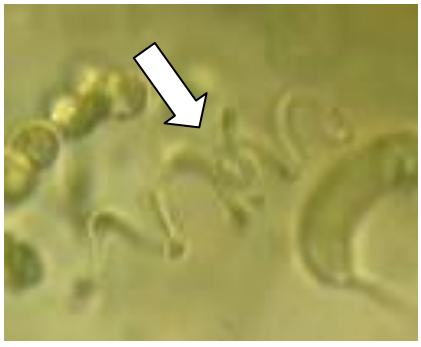


Microcystis

Anabaena



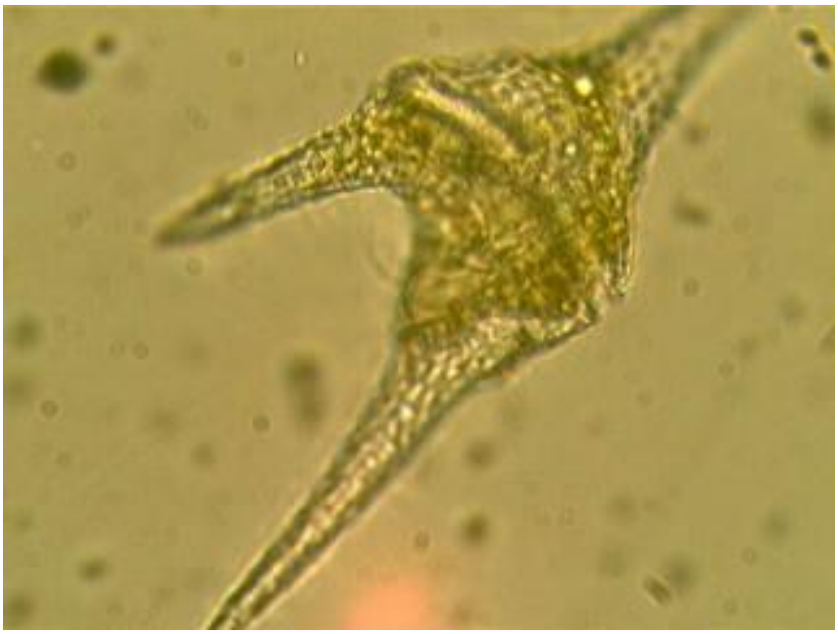
Arthrospira



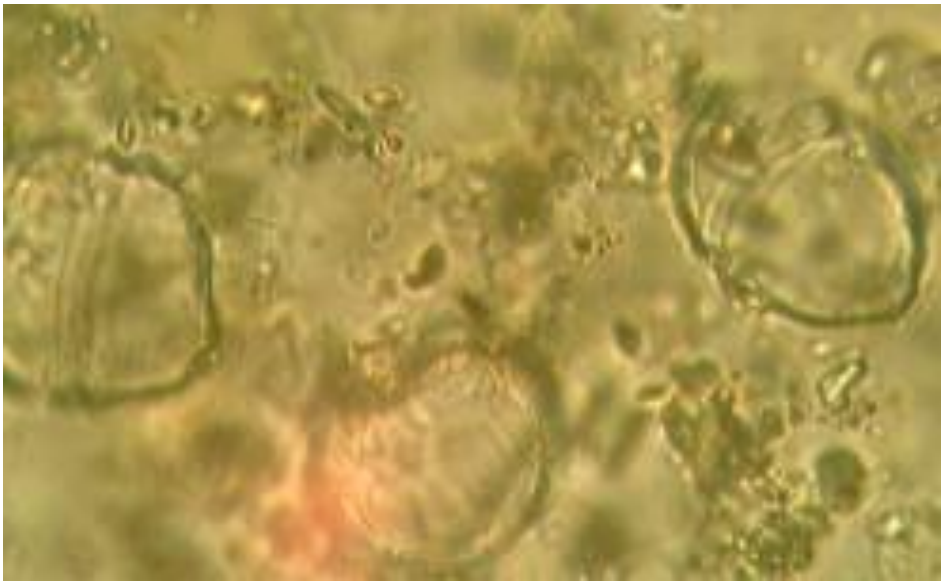
Cylindrospermopsis

Merismopedia

DINOFLAGELLATES
(Pyrrhophyta)

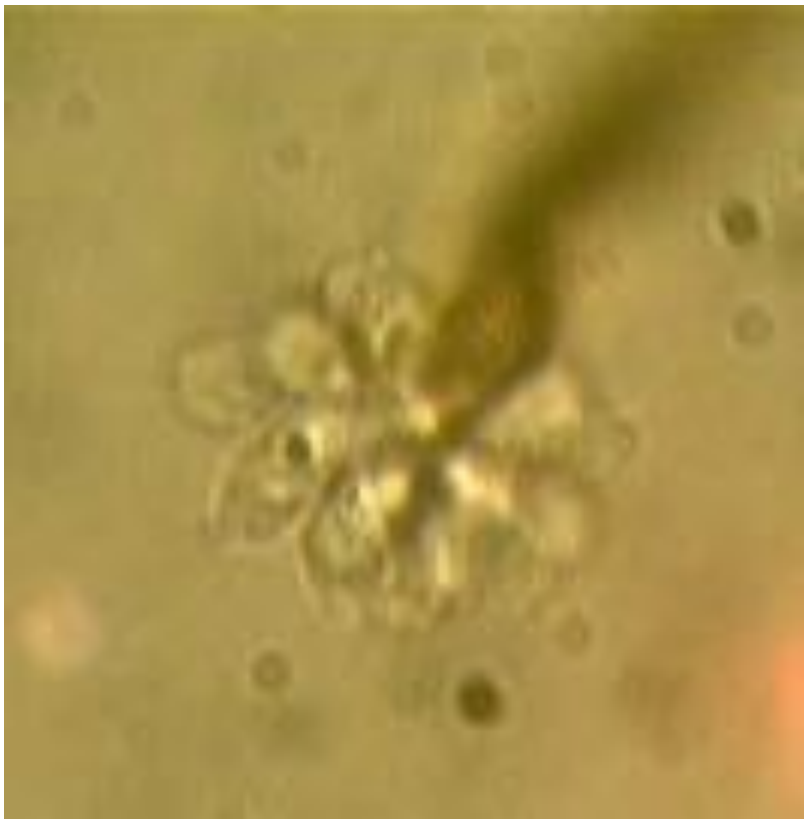


Ceratium



Peridinium

GOLDEN-BROWN ALGAE
(Chrysophyta)



Synuria

APPENDIX F

TOTAL CELLS · ML^{-1} OF ALL GENERA IN THE STUDY SITES

Cells · mL^{-1}	Genera
12,313,361	*Microcystis*
191,412	Euglenoids
178,643	*Merismopedia*
162,005	*Chlamydomonas*
133,580	*Nitzschia*
80,940	Green filaments
66,929	*Scenedesmus*
60,361	*Navicula*
42,221	*Ankistrodesmus*
4,2034	*Pandorina*
36,717	*Palmellococcus*
33,840	*Sphaerocystis*
32,026	*Anabaena*
31,150	*Tabellaria*
26,521	*Trachelomonas*
20,954	*Pyramichlamys*
20,266	*Cosmarium*
15,021	*Cocconeis*
13,886	*Volvox*
12,072	*Cylindrospermopsis*
11,277	*Desmidium*
11,259	*Dinoflagellates*
88,20	*Cymbella*
8,757	*Oocystis*
7,819	*Cyclotella*
6,755	*Aphanizomenon*
4,941	*Dictyosphaerum*
4,879	*Nodularia*
4,629	Diatoms, other
4,504	*Chodatella*
4,253	*Arthrospira*
4,253	*Rhizosolenia*
3,878	*Mesotaenium*
3,503	*Coelastrum*
3,226	*Dimorphococcus*
3,128	*Attheya*
3,128	*Aulacoseira*
3,128	*Synuria*
3,002	*Mougeotia*
2,877	*Gyrosigma*
2,252	*Cryptomonas*
1,751	*Gonium*
1,501	*Asterionella*
1,501	Green, other
1,376	*Oscillatoria*
1,126	*Straurastrum*
1,126	*Pediastrum*
626	*Actinastrum*
626	*Spirulina*
500	*Golenkinia*
250	*Eudorina*
125	*Spirogyra*
125	*Melosira*

APPENDIX G

STUDY SITES SORTED BY GENERA PREDOMINANCE

Lake Name	Number of genera	Most predominant genera	2nd most predominant genera	3rd most predominant genera
El Dorado Marsh, Sept	20	*Microcystis*	*Merismopedia*	*Sphaerocystis*
Mason Lake, June	20	*Microcystis*	*Chlamydomonas*	*Merismopedia*
Santa Ana Pond, June	20	*Microcystis*	*Pyramichlamys*	*Cocconeis*
El Dorado Pond, July	19	*Microcystis*	*Merismopedia*	*Cosmarium*
Nicholas Flat Pond, June	19	*Microcystis*	*Chlamydomonas*	*Merismopedia*
San Marcos Lake, July	18	*Microcystis*	*Nitzschia*	*Cylindrospermopsis*
Machado Lake, June	17	*Microcystis*	*Ankistrodesmus*	*Melosira*
Big Canyon Pre-Marsh, July	16	*Microcystis*	Euglenoids	*Palmellococcus*
Sepulveda Marsh, June	16	*Microcystis*	*Nodularia*	*Navicula*
El Dorado Marsh, July	15	*Microcystis*	*Merismopedia*	*Sphaerocystis*
Madrona Marsh, June	14	*Microcystis*	*Oedogonium*	*Chlamydomonas*
San Joaquin Pond 11, June	14	*Microcystis*	*Nitzschia*	*Merismopedia*
Sims Pond, June	14	*Microcystis*	*Volvox*	*Sphaerocystis*
Buena Vista Lagoon, Sept	13	Euglenoids	*Microcystis*	*Palmellococcus*
El Dorado Pond, Sept	13	*Microcystis*	Euglenoids	*Pandorina*
IRWD Pond B, June	13	*Microcystis*	*Palmellococcus*	*Pyramichlamys*
Mason Lake, Aug	13	*Microcystis*	Euglenoids	*Palmellococcus*
Newport Bay, July	13	*Microcystis*	*Palmellococcus*	*Ankistrodesmus*
San Marcos Lake, Sept	13	*Microcystis*	*Navicula*	*Sphaerocystis*
Machado Lake, Aug	12	*Microcystis*	*Trachelomonas*	*Merismopedia*
Malibu Lagoon, July	12	*Microcystis*	*Merismopedia*	*Pandorina*
Big Canyon Post	11	*Microcystis*	*Pandorina*	*Ankistrodesmus*

Marsh, July				
San Juan Creek, Sept	11	*Microcystis*	Euglenoids	*Pyramichlamys*
Buena Vista Lagoon, July	10	*Microcystis*	*Palmellococcus*	*Chlamydomonas*
Lakewood Country Club, Sept	10	*Microcystis*	Euglenoids	*Tabellaria*
San Joaquin Pond 4, Sept	10	*Microcystis*	*Nitzschia*	Euglenoids
San Joaquin Pond 7, Aug	10	*Microcystis*	*Cryptococcus*	*Aulacrosira*
Big Canyon Pre-Marsh, Aug	9	Euglenoids	*Navicula*	*Chlamydomonas*
Irvine Reg Park Pond, July	9	*Microcystis*	*Scenedesmus*	*Chlamydomonas*
Topenga Lagoon, July	9	*Attheya*	*Microcystis*	*Palmellococcus*
Ballona Marsh, June	8	*Microcystis*	Euglenoids	*Navicula*
Barbara Lake, Aug	8	*Microcystis*	*Anabaena*	Euglenoids
Big Canyon Post Marsh, Aug	8	*Microcystis*	*Synuria*	*Anabaena*
Calavera Lake, July	8	*Microcystis*	Dinoflagellates	Euglenoids
Calavera Lake, Sept	8	*Microcystis*	Euglenoids	*Pandorina*
Huntington Pond, June	8	*Microcystis*	Euglenoids	Green filaments
Nicholas Flat Pond, Aug	8	*Microcystis*	Euglenoids	*Pyramichlamys*
UCSB Campus Lagoon, Aug	8	*Microcystis*	*Gyrosigma*	*Trachelomonas*
Ventura River Mouth, Aug	8	*Microcystis*	*Asterionella*	Euglenoids
Zuma Lagoon, July	8	*Microcystis*	*Ankistrodesmus*	Euglenoids
Zuma Lagoon, Sept	8	*Microcystis*	Euglenoids	*Oocystis*
Andree Clark Bird Ref, Aug	7	*Microcystis*	Euglenoids	*Scenedesmus*
IRWD Pond B, Aug	7	*Microcystis*	Euglenoids	*Nitzschia*
IRWD Pond C, June	7	*Microcystis*	*Anabaena*	*Mougeotia*
San Mateo Lagoon, July	7	*Microcystis*	*Desmidium*	*Navicula*
Sepulveda Marsh, Sept	7	*Microcystis*	Euglenoids	*Pandorina*
Deveraux Slough, Aug	6	*Microcystis*	*Ankistrodesmus*	Euglenoids
Lakewood Country Club, July	6	*Microcystis*	*Desmidium*	Euglenoids

Ormond Bch Wetlands, Aug	6	*Microcystis*	*Ankistrodesmus*	*Trachelomonas*
Barbara Lake, July	5	*Microcystis*	*Ankistrodesmus*	*Anabaena*
San Joaquin Pond 8, Aug	5	*Microcystis*	Euglenoids	*Arthrospira*
Ventura POTW Pond, June	5	*Microcystis*	Euglenoids	*Palmellococcus*
Huntington Pond, Aug	4	*Microcystis*	Green filaments	Euglenoids
IRWD Pond C, Aug	4	*Microcystis*	*Trachelomonas*	*Navicula*
Newport Bay, Aug	4	*Microcystis*	Euglenoids	*Navicula*
Newport Valley	4	*Microcystis*	Euglenoids	*Nitzschia*
Peters Canyon Lake, July	4	*Microcystis*	*Navicula*	*Cymbella*
San Juan Creek, July	4	*Microcystis*	*Navicula*	*Cymbella*
Santa Ana Pond, Aug	4	*Microcystis*	Euglenoids	*Navicula*
Ballona Marsh, Aug	3	*Microcystis*	*Navicula*	*Cocconeis*
McGrath Lagoon, Aug	3	*Microcystis*	*Navicula*	*Cocconeis*
San Joaquin Pond 9, Aug	3	*Microcystis*	*Phacus*	Other Euglenoids
Santa Clara Lagoon, Aug	3	*Microcystis*	*Pandorina*	*Navicula*
Topenga Lagoon, Sept	3	*Microcystis*	Green filaments	*Navicula*
Ventura POTW Pond, Aug	2	*Microcystis*	*Navicula*	None
Irvine Reg. Park Pond, Aug	1	*Microcystis*	None	None

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APPENDIX H

RICHNESS (H') AND EVENNESS (J') OF GENERA AND PHYLA, WITH AND WITHOUT *MICROCYSTIS*, SORTED BY H' OF GENERA

Lake Name	# Gen-era	J Gen-era	H' Gen-era	J No Micro-cystis Genera	H' No Micro-cystis Genera	# Phy-la	J Phy-la	H' Phy-la	J No Micro-cystis Phyla	H' No Micro-cystis Phyla
Ave, all sites	9	0.28	0.61	0.69	1.48	3	0.29	0.40	0.64	0.87
Mason, June	20	69%	2.03	71%	2.08	4	82%	1.13	88%	1.22
Big Canyon Pre-Marsh, Aug	9	88%	1.92	86%	1.90	5	68%	1.10	70%	0.98
Buena Vista Lagoon, Sept	13	74%	1.91	83%	2.12	4	94%	1.31	84%	1.16
Big Canyon Post-Marsh, Jul	11	74%	1.78	75%	1.81	4	75%	1.04	86%	1.19
UCSB Lagoon Aug	8	79%	1.65	86%	1.79	3	87%	0.95	45%	0.49
Topenga Lagoon, July	9	76%	1.59	82%	1.70	1	0%	1.26	0%	1.20
Irvine Reg Park Pond, July	9	63%	1.40	69%	1.52	4	46%	0.64	72%	1.00
Zuma Lagoon, Sept	8	65%	1.36	81%	1.68	4	79%	1.10	88%	1.22
Zuma Lagoon, July	8	63%	1.23	65%	1.27	4	69%	0.96	73%	1.01
Madrona Marsh, June	14	44%	1.17	40%	1.07	4	57%	0.80	18%	0.25
Buena Vista Lagoon, July	10	49%	1.14	79%	1.82	4	65%	0.90	81%	1.13
El Dorado Marsh, July	15	42%	1.13	72%	1.95	5	34%	0.54	63%	1.01
El Dorado Pond, July	19	35%	1.04	58%	1.70	4	30%	0.42	66%	0.92
San Joaquin Pond 8, Aug	5	65%	1.04	82%	1.32	3	59%	0.65	96%	1.06
Huntington Pond, June	8	46%	0.95	83%	1.73	1	0%	0.86	0%	1.25
San Marcos Lake, July	18	32%	0.94	90%	2.15	3	69%	0.76	100%	1.29
Big Canyon Pre-Marsh, July	16	33%	0.93	84%	2.34	4	48%	0.66	83%	1.15
Nicholas Flat Pond, June	19	31%	0.90	75%	2.22	3	43%	0.47	97%	1.06
San Marcos Lake, Sept	13	35%	0.90	60%	1.71	4	48%	0.67	91%	1.26
Calavera Lake, Sept	8	43%	0.90	77%	1.59	5	48%	0.77	62%	1.00
Lakewood Golf Pond, Sept	10	37%	0.84	56%	1.17	4	51%	0.71	50%	0.69
Andree Clark Bird Ref, Aug	7	38%	0.74	50%	0.98	2	97%	0.67	100%	0.84
Big Can Post Marsh, Aug	8	34%	0.71	54%	1.11	5	19%	0.31	55%	0.89
El Dorado Marsh, Sept	20	23%	0.69	69%	2.08	4	23%	0.32	78%	1.09
Malibu Lagoon, July	12	27%	0.65	83%	1.99	4	26%	0.37	53%	0.73

Calavera Lake, July	8	31%	0.64	57%	1.19	4	28%	0.39	67%	0.93
San Mateo Lagoon, July	7	27%	0.53	53%	1.02	3	43%	0.47	76%	0.84
IRWD Pond B, June	13	20%	0.51	70%	1.80	3	27%	0.29	94%	1.03
San Juan Creek, Sept	11	20%	0.45	73%	1.68	4	27%	0.38	57%	0.79
San Joaquin Pond 11, June	14	18%	0.45	73%	1.87	4	31%	0.43	80%	1.11
El Dorado Pond, Sept	13	18%	0.45	82%	2.09	3	34%	0.38	69%	0.76
San Juan Creek, July	4	32%	0.44	61%	0.84	2	52%	0.36	100%	0.94
Newport Bay, July	13	17%	0.43	68%	1.74	4	24%	0.33	0%	0.97
Ormond Bch Wetlands, Aug	6	27%	0.43	76%	1.22	3	32%	0.35	95%	1.05
San Joaquin Pond 4, Sept	10	20%	0.43	88%	1.92	3	30%	0.33	99%	1.09
Sims Pond, June	14	16%	0.43	87%	2.30	3	25%	0.27	69%	0.76
Ventura River Mouth, Aug	8	19%	0.39	83%	1.73	4	21%	0.29	83%	1.15
Santa Ana Pond, June	20	13%	0.39	85%	2.54	4	21%	0.29	77%	1.07
Sepulveda Marsh, June	16	14%	0.38	81%	2.26	3	19%	0.21	100%	1.26
Deveraux Slough, Aug	6	21%	0.38	91%	1.63	3	29%	0.32	94%	1.03
IRWD Pond B, Aug	7	20%	0.35	60%	1.07	3	9%	0.10	88%	0.97
San Joaquin Pond 7, Aug	10	16%	0.35	77%	1.70	4	15%	0.21	44%	0.61
Lakewood Golf Pond, July	6	16%	0.29	73%	1.32	4	19%	0.27	67%	0.93
Barbara Lake, July	5	18%	0.28	73%	1.17	3	17%	0.19	97%	1.06
Machado Lake, June	17	11%	0.27	90%	2.23	4	14%	0.19	93%	1.28
Machado Lake, Aug	12	9%	0.22	56%	1.35	3	15%	0.17	88%	0.96
Topenga Lagoon, Sept	3	32%	0.22	0%	0.00	2	16%	0.11	81%	0.56
Barbara Lake, Aug	8	10%	0.20	65%	1.35	3	7%	0.07	76%	0.83
Peters Canyon Lake, July	4	14%	0.19	69%	0.96	3	16%	0.17	14%	0.15
Ballona Marsh, Aug	3	17%	0.19	58%	0.64	1	0%	0.04	0%	0.23
Mason Lake, Aug	13	7%	0.18	79%	2.03	1	0%	0.03	0%	0.05
IRWD Pond C, June	7	9%	0.18	69%	1.35	4	1%	0.01	69%	0.96
Sepulveda Marsh, Sept	7	8%	0.14	67%	1.21	4	11%	0.15	84%	1.16
Huntington Pond, Aug	4	10%	0.13	76%	1.05	1	0%	0.02	0%	0.30
Ventura POTW Pond, June	5	8%	0.11	73%	1.01	4	8%	0.10	86%	1.19
Nicholas Flat Pond, Aug	8	4%	0.08	86%	1.78	4	5%	0.07	69%	0.96
Ballona Marsh, June	8	3%	0.06	74%	1.54	3	5%	0.06	100%	1.24
Newport Bay, Aug	4	5%	0.06	75%	1.03	1	0%	0.01	0%	0.14
Ventura POTW Pond, Aug	2	6%	0.06	0%	0.00	1	0%	0.01	0%	0.01
Santa Ana Pond, Aug	4	5%	0.05	62%	0.69	2	2%	0.02	47%	0.32
Santa Clara Lagoon, Aug	3	4%	0.05	0%	0.00	2	1%	0.01	20%	0.14
Newport Valley	4	4%	0.05	77%	1.07	3	4%	0.05	0%	0.65
IRWD Pond C, Aug	4	3%	0.05	73%	1.02	4	0%	0.01	62%	0.85
San Joaquin Pond 9, Aug	3	3%	0.04	58%	0.63	3	3%	0.04	94%	1.04

McGrath Lagoon, Aug	3	3%	0.03	62%	0.68	1	0%	0.00	0%	0.26
Irvine Reg Park Pond, Aug	1	0%	0.00	74%	1.95	5	0%	0.00	64%	1.03

APPENDIX I

N:P RATIOS OF STUDY SITES

PHOSPHOROUS-LIMITED SITES	TN:TP
Ventura POTW Pond, Aug	16
Madrona Marsh, June	16
San Joaquin Pond 8, Aug	17
Buena Vista Lagoon, July	18
Calavera Lake, Sept	19
Zuma Lagoon, Sept	19
San Joaquin Pond 7, Aug	19
Sepulveda Marsh, June	21
San Joaquin Pond 11, June	21
San Joaquin Pond 4, Sept	23
Topenga Lagoon, July	24
San Marcos Lake, Sept	28
Santa Ana Pond, Aug	29
San Joaquin Pond 9, Aug	29
Calavera Lake, July	30
Andree Clark Bird Refuge, Aug	31
Barbara Lake, July	31
San Marcos Lake, July	31
Ventura POTW Pond, June	32
Lakewood Country Club, July	33
Buena Vista Lagoon, Sept	34
El Dorado Marsh, July	38
Santa Ana Pond, June	40
Barbara Lake, Aug	40
El Dorado Marsh, Sept	41
Irvine Regional Park Pond, July	43
Sepulveda Marsh, Sept	47
El Dorado Pond, Sept	48
Peters Canyon Lake, July	50
Nicholas Flat Pond, June	54
McGrath Lagoon, Aug	57

NITROGEN-LIMITED SITES	TN:TP
Santa Clara Lagoon, Aug	2
Ballona Marsh, June	2
Ballona Marsh, Aug	2
IRWD Pond C, 1 June	3
Newport Bay, Aug	3
Newport Valley	3
Machado Lake, June	5
Huntington Pond, Aug	5
Machado Lake, Aug	6
UCSB Campus Lagoon, Aug	7
Mason Lake, 2 Aug	7
Newport Bay, July	7
Big Canyon Pre-Marsh, July	7
Big Canyon Post Marsh, July	7
Big Canyon Post Marsh, Aug	8
Sims Pond, June	9
Malibu Lagoon, July	9
Big Canyon Pre-Marsh, Aug	9
Huntington Pond, June	10
Deveraux Slough, Aug	10
Mason Lake, 1 June	12
Ventura River Mouth, Aug	12
San Juan Creek, Sept	13
IRWD Pond C, 2 Aug	13
San Juan Creek, July	14
Ormond Beach Wetlands, Aug	14

Irvine Regional Park Pond, Aug	61
Zuma Lagoon, July	66
Topenga Lagoon, Sept	71
Lakewood Country Club, Sept	79
Nicholas Flat Pond, Aug	90
El Dorado Pond, July	105
IRWD Pond B, 1 June	114
IRWD Pond B, 2 Aug	141
San Mateo Lagoon, July	931

APPENDIX J

GRANTS

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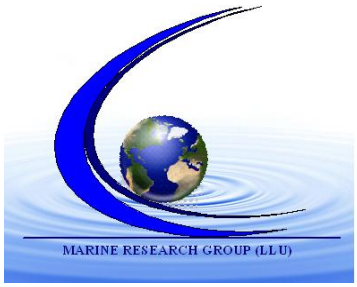

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