Impacts of *Microcystis* on algal biodiversity and use of new technology to remove *Microcystis* and dissolved nutrients

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Abstract

Cyanobacteria often dominate eutrophic lakes, outcompeting green algae that are required by fish and zooplankton. 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 phosphate restriction assay on the algae in Mason Lake (Irvine, CA, USA) and found that the threshold needed for algal growth there was $0.02 \text{ mg L}^{-1} \text{ PO}_4$. 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 phosphate after just one 10 min 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

Southern California coastal lentic waters are important habitats for endangered birds that migrate throughout the Pacific flyway. These sites are often contaminated by *Microcystis*, a cyanobacterium known for producing carcinogenic toxins (Boyer 2007). Phosphate (PO_4) and nitrate (NO_3) are the major nutrients needed for growth of this type of algae (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 (Boyer 2007). 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 odour 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 1999), 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 caused by hypovolemic shock, and chronic exposure has been shown to enhance the growth of hepatic and colonic precancerous lesions, suggesting that microcystins act as tumour 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.

As 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; Rocke & Friend 1991). 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 algal 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. Tatrai *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. New technology exists which removes dissolved nutrients and filters out colonial cyanobacterial colonies in small lakes and ponds. However, its effectiveness in mitigating eutrophication has not been determined.

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^{TM} technology in decreasing PO_4 and removing toxic algae. If cyanobacterial cell numbers can be decreased in small lakes and ponds, overall algal biodiversity may 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 millilitres of modified BG-11 medium containing f/2 vitamin solution and silica (Canadian Phycological Culture Centre, Waterloo, ON, Canada) was added to 10, 150 mL beakers. Five beakers were inoculated with 5 mL of each of the following strains of algae (Canadian Phycological Culture Centre): 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, USA) attached to a five gang valve (Penn Plax, Hauppauge, NY, USA). Each flask was covered with non-absorbent cotton to maintain sterility. Continuous lighting was provided by 1 daytime fluorescent and one cool white bulb, and room temperature was regulated between 20 and 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 5 weeks. Total concentration of cells mL^{-1} was calculated using the equation.

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

Statistical analyses

We subjected each of the four biodiversity measures (richness and evenness of phyla and genera) to a 2×6 (condition $\times$ time) mixed analysis of variance model (Mertler & Vannatta 2004), treating condition (two levels; with and without *Microcystis*) as a between-subjects factor and time (six 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 approximately 0.06 generally regarded moderate and ≥ 0.14 large (Cohen 1988). As 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, IL, USA, 2004), with $\alpha = 0.05$.

Phosphate restriction assay at Mason Lake

Ten millilitre 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 3 K for 4 min 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. Algal 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 to 0.10 mg L^{-1} was created, as was a control tube. The tubes were tightly capped and gently inverted until the algal 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 colour in each tube was recorded once a week for 6 weeks. We used curve-fitting regression analysis in SPSS to evaluate the influence of PO$_4$ dilution on algal growth.

Blue Pro^{TM} pilot study at Mason Lake

Mason Lake, in Mason Regional Park (Irvine, CA, USA), is a 37 000 m^2 freshwater lake, approximately 20 years old, and very eutrophic. The park is owned by the County government and is intended for public fishing and toy sailboat regattas. A temporary Blue Pro^{TM} treatment facility was rented for 3 weeks for a pilot study at Mason Lake (Fig. 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 centre 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 towards the top of the tank (Fig. 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

Fig. 1. Temporary Blue Pro^{TM} 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.

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.

Sample acquisition

Lake water was evaluated before and after treatment. A grab sample (labelled 'influent') was obtained directly from Mason Lake in April, 2008. The Blue Pro™ technician provided two more samples. One sample (labelled 'Phase 1') was obtained after the first pass through the Blue Pro™ system (after treatment with alum), and one sample (labelled '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 & 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 millilitres
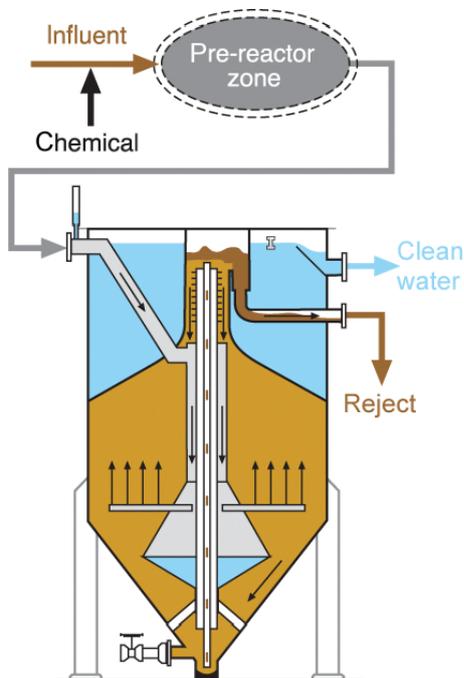

Fig. 2. Internal diagram of Blue Pro™ sand tower (BlueH2O.com).

of lake water from each sample was added to a chamber and allowed to settle for 8 h. 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 per mL calculated.

Chlorophyll assay technique

A filtration flask was assembled with a 50 mm perforated glass funnel in the top. A glass fibre 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 Kontes Tissue Grinder (Kimble & Chase, Vineland, NJ, USA). 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, and 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 3 k for 5 min. 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 & Humphrey 1975) were used for determining chlorophylls in a mixed phytoplankton assemblage in which both chlorophyll-*b* and chlorophyll-*c* containing organisms are present,

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

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

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

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

$$\text{OD}_{664} = \text{Abs}_{664} - \text{Abs}_{750} \quad (5)$$

The values were multiplied by volume of acetone extract and divided by the volume filtered (all in millilitres). 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 2×6 (condition $\times$ 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 1 and Fig. 3. In the control batch (without *Microcystis*), mean phyla and genera richness (H') and evenness (J') changed negligibly (1.1–4.4%) during the 5 weeks 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.

Phosphate restriction assay at Mason Lake

Algal growth was seen throughout the 6 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. 4), confirming the PO_4 limitation of algal growth for Mason Lake water.

Blue Pro™ pilot study at Mason Lake
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 2. The experiment was not repeated to obtain multiple samples. Therefore, statistical analysis could not be conducted.

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), Chloro-

Table 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 days period

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

Results from 2×6 (condition $\times$ time) mixed ANOVAS, with P-values and adjusted partial eta-squared (η^2) effect sizes provided.
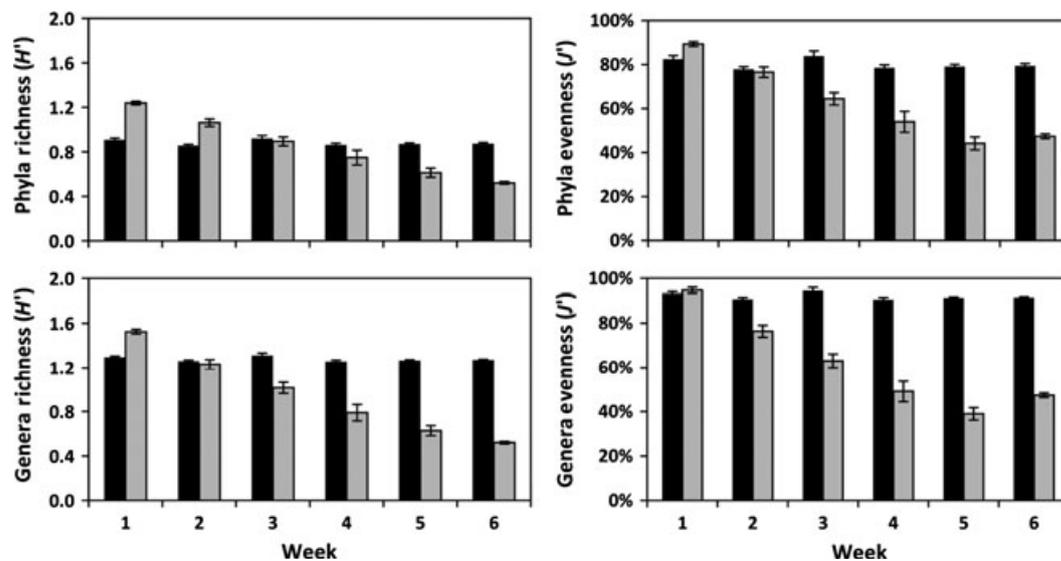
Fig. 3. Richness and evenness of phyla and genera. Dark bars are control batches, light bars are experimental batches. Vertical black lines are standard deviation. Significance is described in Table 1.
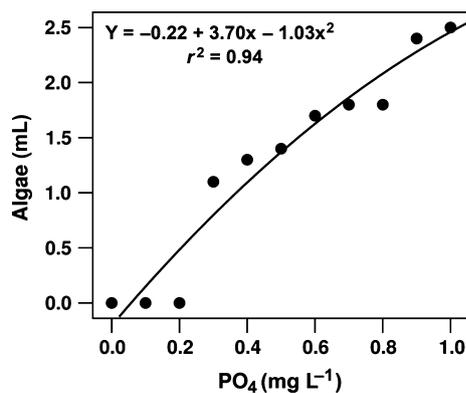
Fig. 4. Growth of algae with increasing PO_4 levels.

Table 2. Changes in cells and nutrients after Blue Pro^{TM} 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%

phyll-*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 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 towards the end of the 5-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 outcompete 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 5-week experiment. As Cyanophyta outcompete 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, which has had extensive algal problems for years, resulting in poor visibility, malodourous water, and a slimy surface film. Most of the fishes have died, the surface of the lake is covered with a thick film with the odour 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 because of 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 treatments at Mason Lake included copper sulphate, 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). As the use of copper sulphate is restricted because of its toxicity, an alternative is the use of alum (aluminium), 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 which we performed on the algal communities of Mason Lake demonstrated that a PO_4 threshold of 0.02 mg L^{-1} was needed for growth. This is a much lower threshold than 0.2 mg L^{-1} found by Shen and Song (2007), and lower than 0.1 mg L^{-1} found by Tatrai *et al.* (2005) and Oh *et al.* (2000). However, Xavier *et al.* (2007) also found a PO_4 threshold of 0.02 mg L^{-1} was needed for growth of nuisance algae in two man made urban lakes in Portugal. As Mason Lake is 37 000 m^2, we estimated it would take approximately 3 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. As the system removed 97% of the *Microcystis* colonies, toxin levels will also decrease, because the toxins are embedded in the cell walls (Carmichael & An 1999). There were 27 μg 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 μg 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, because no variances or trends could be determined. Further research with this technology is needed to evaluate its effectiveness in eliminating PO_4 loads from urban run-off in ponds, small lakes, and river mouths.

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 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 in the *Microcystis* colonies. However, because 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.

ACKNOWLEDGEMENTS

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