ASSESSMENT OF CYANOBACTERIA TOXINS AND THEIR POTENTIAL FOR RELEASE BY ALGACIDE APPLICATION IN MUSKEGON COUNTY LAKES

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samples. We also thank Victoria Webster for providing logistical and administrative support in addition to conducting outreach and education programs and providing technical input for this report.

Executive Summary

An assessment of the ability of *Cladophora* mats to sequester *E. coli* and microcystin LR and RR was conducted in the nearshore waters of Grand Traverse Bay (7 sites), Little Traverse Bay (2 sites), and Saginaw Bay (8 sites). The sampling locations were at public beach access points where *Cladophora* mats previously have been observed. The goals of this research were to determine the spatial and temporal variability of *E. coli* populations in *Cladophora* mats in these recreational waters and if cyanotoxins (microcystin LR and RR) are sequestered in the detached algae. The collection of *Cladophora* samples was coordinated with local beach monitoring programs to facilitate the comparison with ambient water bacteria concentrations. This project provided important data for the assessment of public health impacts and the development of beach management programs to address the problems associated with *Cladophora* accumulations.

Based on the results from this investigation, Saginaw Bay appears to be more heavily impacted by detached *Cladophora* than Grand Traverse/Little Traverse Bay. Mean *E. coli* concentrations in detached *Cladophora* were higher in Saginaw Bay (2,796 cfu/g dwt) than Grand Traverse/Little Traverse Bay (1,775cfu/g dwt); however, the difference was not statistically

significant (Mann-Whitney ρ=0.40). *Cladophora* deposits exhibited spatial and temporal variability in both systems. At most beaches in Grand Traverse Bay, *Cladophora* deposits were limited to small pockets at 1 location. Clinch Park had only one site with *Cladophora* on the last sampling event and two locations at the Traverse City State Park were free of detached algal accumulations. In contrast, *Cladophora* deposits in Saginaw Bay covered approximately 1 meter (m) of the shoreline at most beaches. Two locations in Saginaw Bay also had no accumulations of *Cladophor*a during the study period (White's Beach and Pinconning Park). Differences in *Cladophora* accumulation between Saginaw Bay and Grand Traverse/Little Traverse Bay may be attributed to higher total phosphorus levels in Saginaw Bay. Levels of *E. coli* in detached *Cladophora* in both systems were similar to concentrations previously reported in the Great Lakes (1,000 cfu/g dwt – 60,000 cfu/g dwt). In Saginaw Bay, the highest levels of *E. coli* in detached *Cladophora* were consistently found at beaches near the Saginaw River. Even within individual sites, locations near tributaries and drains at Wenona Beach and South Linwood Beach were significantly higher than locations further away from a point source. This relationship also was noted in Grand Traverse Bay where the location near Mitchell Creek at the Traverse City State Park had elevated *E. coli* concentrations in detached *Cladophora* compared to the other beach locations. These results suggest that *Cladophora* can trap bacteria from point sources and also be stimulated by nutrient discharges. Two locations, Pinconning Park and White's Beach, had very limited *Cladophora* growth. Both locations had *Chara* growing on the lake bottom. *Chara* is known to exhibit allelopathic activity that can limit the growth of other aquatic plants. No correlation was found between *E. coli* levels in the open water (designated beach monitoring locations) and the nearshore zone where the detached *Cladophora* samples were taken. As noted in previous studies, *Cladophora* appears to hold trapped *E. coli* and not release the entrained bacteria to the offshore water.

This investigation was the first to document the accumulation of microcystins in the detached *Cladophora* of Saginaw Bay. Total microcystins in detached *Cladophora* had a grand mean of 57 µg/g dwt for the study period. Saginaw Bay has a history of *Microcystis* blooms in the late summer months that produce both microcystin LR and RR. Since *Microcystis* has a high requirement for sunlight, cyanobacteria may become stressed when they are trapped in the detached algae mats. While accidental ingestion by humans of microcystins trapped in *Cladophora* is unlikely, these compounds can act as skin irritants. Walking through *Cladophora* accumulations to get to deeper water may provide sufficient exposure to cause irritation in sensitive individuals if microcystins are present. Although the data suggest that swimming areas (1 m depth) are not impacted by the *E. coli* accumulations in detached *Cladophora*, entrained bacteria and cyanotoxins may pose a hazard to children playing in the nearshore water and sand. Current regulations discourage beach grooming and altering the nearshore zone. The presence of elevated bacteria and microcystin levels in the nearshore environment of Saginaw Bay suggests that the current policy should be reevaluated to balance potential impacts to public health with the ecosystem services provided by coastal wetlands.

1.0 Introduction

The increasing prevalence of Harmful Algal Blooms (HABs) in freshwater arising from cultural eutrophication and the action of non-indigenous species is creating issues related to the use of water for drinking and recreational purposes. Cyanobacteria are well known for their ability to produce potent toxins, which have been responsible for animal deaths and human health problems (Chorus 1999). Toxic cyanobacteria are cosmopolitan and they have been recorded from every continent (Carmichael 1997). Of the cyanobacterial blooms tested to date, 50–75% were toxic (Codd, 1995). However not all blooms of a particular species may be toxic. In fact toxicities of blooms of the same species can vary markedly both geographically and with time (Carmichael and Gorham, 1981). Lake Associations often use a variety of algicides to control cyanobacteria blooms (Codd et al. 2005). The application of algicides has been linked to the release of cyanobacteria toxins due to death and subsequent lysis of cells (Kenefick et al. 1993; Jones and Orr 1994).

While no US standards have been developed, the World Health Organization (WHO 1999) has recommended guidelines for recreational water exposure to the cyanobacteria and their toxins:

Advisory Level	Microcystin LR
Moderate	20 µg/l
High	1,000 µg/l

West Michigan contains a number of lakes that have extensive recreational use and histories of cyanobacteria blooms. Blooms of the toxin producing genera *Microcystis, Anabaena. and Cylindrospermopsis* and the toxin, microcystin, were reported in 6 west Michigan lakes in 2006 (Rediske et al. 2008). Microcystin levels exceeding the WHO moderate and high Advisory Levels were reported during 2007 and 2008 in Bear Lake, Muskegon Lake, and Mona Lake (G. Fahnenstiel, unpublished data; R. Rediske, unpublished data). During these years, lake associations on Bear Lake and Mona Lake applied multiple treatments of algicides in an attempt to control algal blooms. Algicide treatments were not applied in Muskegon Lake and reports of algal scums accumulating along the shoreline were reported from July to the end of September in 2009. While funding for monitoring was available in 2006, the absence of an organized monitoring program in 2007 and 2008 resulted in the collection and analysis of a limited number of samples.

We conducted an assessment of the impact of algicide treatment on cyanotoxin levels in Bear Lake and the temporal variability of these compounds in Mona Lake and Muskegon Lake. The sampling locations were at open water locations and public beach access points where cyanobacteria blooms have been previously observed. This project provided data that were used to formulate public education programs and develop strategies to notify residents when excessive cyanotoxin levels are reported during bloom conditions. This type of program is critical for the future assessment of public health impacts and the development of water quality management programs to ameliorate their hazards.

2.0 Methods

2.1 Sampling methods

2.1.1 Sampling Design

Three open water sites and one public bathing beach on each lake (Figure 2.1). The beach will be sampled at the three locations used by the MDEQ for *E. coli* monitoring. GPS coordinates were taken at each station during the initial sampling survey and used as reference points for subsequent events. Integrated 1-meter water samples were collected at each location (Sutherland et al. 1992). In addition, one of the locations was collected in duplicate. We also made *in situ* measurements chlorophyll *a* and phycocyanin at each station with a YSI 6600 DataSonde. Each lake was sampled at two-week intervals during July and August 2010 (four sampling events; 96 samples total). In addition, we collected two additional sets of open water samples from the three lakes during late August and early September (n=24). If algal blooms were reported, samples of surface scum were collected in addition to the integrated water collections. When an algicide application occurred in Bear Lake, water samples were collected before and after (24 hrs and 48 hrs) after treatment. All samples were returned to the laboratory on a daily basis for further processing and storage.

2.1.2 Sampling Methods

Integrated epilimnetic (1 m) water samples (Sutherland et al. 1992) were collected for the analysis of chlorophyll *a*, microcystins, anatoxin-a, cylindrospermopsin, and phytoplankton identification. One to several integrated water samples were collected and pooled to provide a 2 L composite sample for the analyses described in Section 2.4. To collect each integrated water sample, a 1.5 m polycarbonate tube (10 cm O.D.) will be lowered to a 1 m depth. The top was sealed with a rubber stopper and then pulled up. Before the tube was pulled from the water, the bottom also was sealed with a rubber stopper. After withdrawal, contents were emptied into a 2-L amber glass bottle, and stored on ice. The samples were returned to the laboratory after each sampling event for analysis. Chlorophyll *a* and phycocyanin were measured at each location using a YSI 6600V2 DataSonde at 0.5 m depth.

2.2 Analytical Methods

Laboratory procedures have been selected based on previous use for water quality investigations, established ranges for accuracy/precision, and limited problems related to matrix interferences. A summary of analytical methods is given in Table 2.1.

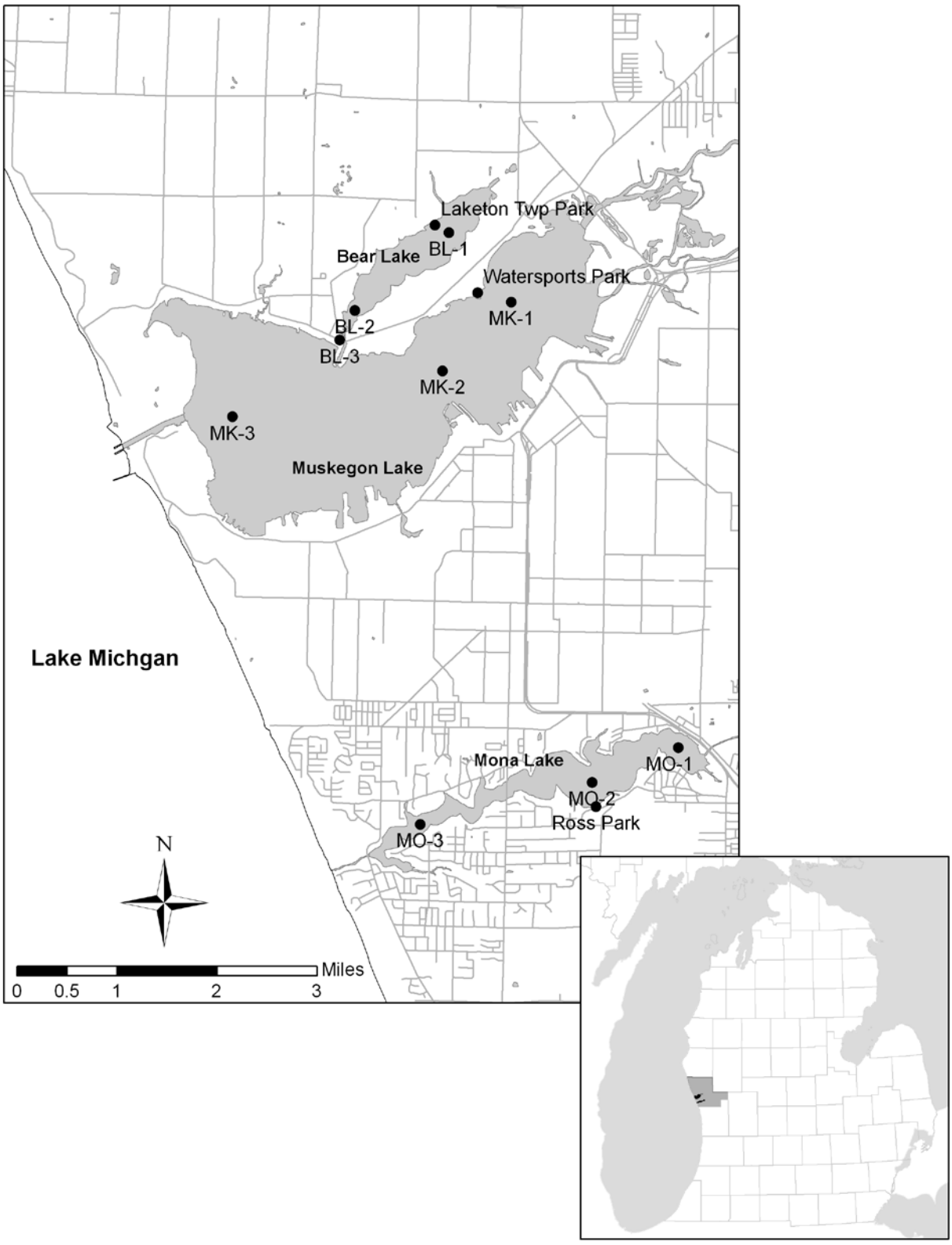

Figure 2.1. Sampling Locations on Bear Lake, Muskegon Lake, and Mona Lake.

Table 2.1. Laboratory Analytical Methods

Parameter	Preparation	Preservation	Holding Time	Methods Reference
Microcystin LR, LA, LW, LF, and RR, cylindrospermopsin and anatoxin-a by LC/MS	Filtration, methanol extraction	Freeze -10°C	6 mo	Maizels and Budde (2004)
Chlorophyll *a* and phycocyanin	*	*	*	YSI 2005
Envirologix QualiTube Kit	Boiling	Freeze -10°C	6 mo	Envirologix 2010

* Measured directly in the field

2.2.1. YSI Methods

Chlorophyll *a* and phycocyanin were measured in the field using a YSI 6600 DataSonde at each sampling station according to the methods in the instrument users manual (YSI 2005). The YSI 6600V2 DataSonde was calibrated in the lab prior to use in the field. The instrument was placed in the water at each site and the technician waited for a stable reading of the sensors. Measurements were recorded at 0.5 m depth at each station. Precision was assessed by the measurement of field duplicates at 10% of the stations. Chlorophyll was used to estimate total algal biomass and phycocyanin was used to estimate cyanobacteria density. By tracking these two indicators, the effect of algicide application on cyanobacteria and the total phytoplankton community was evaluated. Increases in the proportion of phycocyanin to chlorophyll after algicide application may indicate that cyanobacteria may be more resistant to the chemical and/or they are able to outcompete other taxa after application.

2.2.2 Sample Preparation for Microcystin and Algal Toxin Analysis

Water samples for the analyses for microcystin were prepared according to methods outlined by Lawerence et al. (2001). A 100-500 mL aliquot was filtered through a Whatman GF/C glass fiber filter. The filter then was be placed in a 5 mL centrifuge tube and lyophilized for 8 hours. The residue on filter was extracted with three successive aliquots of methanol followed by sonication. The methanol extract was evaporated to dryness under nitrogen and redissolved on 1 mL of 50% methanol in water. This mixture was passed through a 1 g SPE C-18 cartridge (Baker) that had been preconditioned with 10 mL of methanol followed by 10 mL of water. The cartridge then was washed with 5 mL of 25% methanol in water and this fraction was discarded. The microcystins were eluted with 4 mL of 100% methanol in water. This fraction was collected and the volume reduced to 2 mL for LC/MS analysis.

For the sampling events conducted before and after algicide application, dissolved microcystin LR and RR was measured by concentrating the toxins on C18 solid phase extraction cartridges followed by methanol elution. The filtrate was collected from the initial filtration was passed through a 1 g SPE C-18 cartridge (Baker) that had been preconditioned with 10 mL of methanol followed by 10 mL of water. The cartridge then was washed with 5 mL of 25% methanol in water and this fraction was discarded. The microcystins was eluted from the column with 4 mL of 100% methanol in water. This fraction was collected and the volume reduced to 2 mL.

2.2.3 Sample Preparation for Microcystin Analysis

Water samples for the analyses of microcystin were prepared according to methods outlined by Lawerence et al. (2001). A 100-500 mL aliquot was filtered through a Whatman GF/C glass fiber filter. The filter then was placed in a 5 mL centrifuge tube and lyophilized for 8 hours. The residue on filter was extracted with three successive aliquots of methanol followed by sonication. The methanol extract was evaporated to dryness under nitrogen and redissolved on 1 mL of 50% methanol in water. This mixture was passed through a 1 gram (g) SPE C-18 cartridge (Baker) that had been preconditioned with 10 mL of methanol followed by 10 mL of water. The cartridge then was washed with 5 mL of 25% methanol in water and the eluted fraction was discarded. The microcystins then were eluted with 4 mL of 100% methanol in water. This fraction was collected and the volume reduced to 2 mL. The volume was adjusted back to 3 mL with methanol and split into three 1 mL aliquots for HPLC/MS analysis.

2.4.4 Microcystin LR, YR, RR, Anatoxin-a, and Cylindrospermopsin by LC/MS

The concentrations of microcystin LR and RR, cylindrospermopsin and anatoxin-a were determined by liquid chromatography-tandem mass spectrometry using a Thermo Surveyor MSQ Single Quadrupole Mass Selective Detector and Thermo Spectrasystem HPLC system according to a modified method described by Maizels and Budde (2004). Nodularin was added to the extracts and used as an internal standard. Compounds were separated on a Phenomenex Gemini C-18 column at 50^{o}C. The mobile phase was a binary gradient of water and methanol, both containing 0.1% formic acid. The initial gradient was started at 95% water and 5% methanol, followed by a step change to 50% water and 50% methanol at 3 minutes, with a linear gradient from 5 to 20 minutes to 5% water and 95% methanol. Instrument detection limits for these toxins were determined to be near 20 picograms on-column. For calibration, a series of 6 solutions were prepared with the internal standard at 1000 pg/µL and the analytes in the range of 1 to 500 nn/mL in final volumes of 1 mL of 90:10 water:methanol.

2.4.5 Microcystin LR Analysis by the Envirologix QualiTube Kit

Samples for the Envirologix QualiTube Test Kit analysis of microcystin were conducted one a portion of the integrated 1 m sample (100 mL). Briefly, a 25 mL sample was heated to boiling in a microwave for 3 min to extract intercellular toxins. The extract was cooled, centrifuged, and the sample was ready for QualitTube analysis. The test kit is based on an Enzyme-Linked Immunosorbent Assay (ELISA) method that offers the analyst the ability to qualitatively and/or quantitatively measure microcystins in water. For the qualitative option, a blank, two standards (3 µg/L and 0.5 µg/L microcystin LR), and the samples were reacted with Microcystin Enzyme Conjugate for 20 min at room temperature in the test tubes provided in the kit. The test tubes contain the immunosorbent material on the inner surface. After incubation, the test tubes are triple rinsed with DI water and shaken to remove excess water. The enzyme substrate then was added to the tube and incubated for an additional 10 min. After the second incubation, the tubes were visually read to determine the presence/absence of color and the relative concentration range (Figure 2.2).

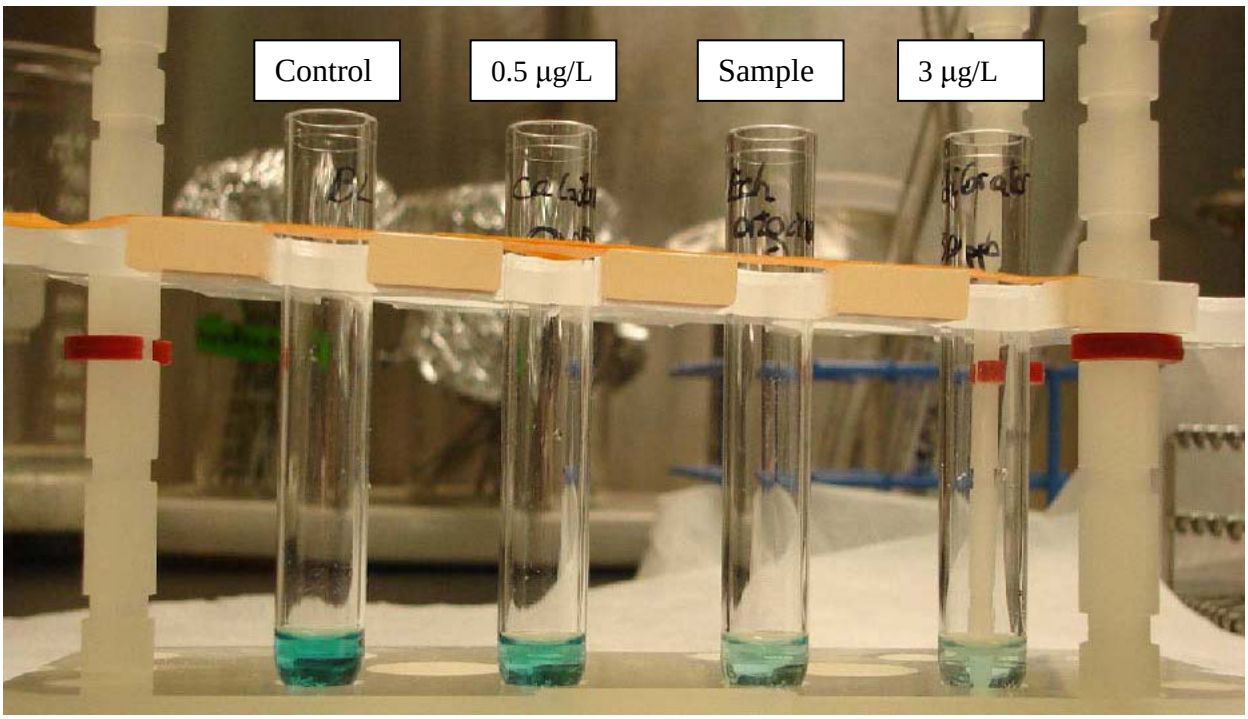

Figure 2.2. The Envirologix QualiTube Kit

Since the color changes were subtle, the quantitative option was used for the investigative samples. The substrate from each tube was transferred to a StatFax 3200 Plate Reader and the absorbance was measured at 450nm. Microcystin LR calibration standards of 0.5 µg/L, 1.0 µg/L and 3.0 µg/L were measured along with a blank with each batch of 15 samples.

2.2 Statistical Methods

Statistical analyses were performed using SigmaPlot 11.0 (Systat Software, Inc). Paired T-tests were used if the data were normally distributed. The Mann–Whitney U test was used for non-normal data.

3.0 Results

The results of the sampling and analyses for Bear Lake, Muskegon Lake and Mona Lake are presented in Sections 3.1, 3.2, and 3.3, respectively. An evaluation of the Envirologix QualiTube Test Kit is provided in Section 3.4.

3.1 Bear Lake Cyanobacteria Results

3.1.1. Bear Lake Algicide Application

Algicide (chelated copper sulfate) was applied in Bear Lake on June 6, 2010 at 11:00 AM. The results of the pre and post algicide monitoring are summarized in Table 3.1.1. Phycocyanin (Figure 3.1.1) and Chlorophyll *a* (Figure 3.1.2) data show a decrease in concentration 24 hrs and 48 hrs after application at all locations except BL-3. The degree of decrease was not significantly different from post application levels for phycocyanin (p=0.61; p=0.37, respectively) and chlorophyll a (p=0.31; p=0.19, respectively). Phycocyanin results showed that cyanobacteria cell densities were significantly higher 14 days after algicide application (p=0.008). Chlorophyll a results also were significantly higher (p=0.047) after 14 days.

Total intercellular microcystins (Figure 3.1.3) declined significantly from post application levels at both 24 hr (p=0.12) and 48 hr (p=0.005) time intervals. Cyanotoxin concentrations remained significantly lower 13 days after application (p=0.008) and there was no significant difference between total intercellular microcystins at the 48 hr and 14 day time intervals (p=0.23). Dissolved total microcystins increased after algicide application (Figure 3.3.4) and there was no significant difference between 24 hr and 48 hr time intervals (p=0.09).

Table 3.1.1 Bear Lake Algicide Application Results 2010. (PC=Phycocyanin, IC=Intercellular, MC=Microcystin, DS=Dissolved, NA=Not Analyzed.)

Site	Date	PC (cells/ml)	Chlorophyll *a* (µg/L)	IC MC-LR µg/l	IC MC-RR µg/l	IC MC-YR µg/l	Total IC MCs µg/l	DIS MC-LR µg/l	DS MC-RR µg/l	DS MC-YR µg/l	Total DS MCs µg/l
BL-1	7/6/2006	17256	15.7	0.92	1.01	0.26	2.19	< 0.02	< 0.02	< 0.02	< 0.02
BL-2	7/6/2006	16169	14.2	1.00	0.98	0.22	2.20	< 0.02	< 0.02	< 0.02	< 0.02
BL-3	7/6/2006	13107	11.5	0.78	0.78	0.18	1.74	< 0.02	< 0.02	< 0.02	< 0.02
Laketon Beach	7/6/2010	18765	15.0	1.14	1.50	0.10	2.74	< 0.02	< 0.02	< 0.02	< 0.02
BL-1	7/7/2010	13860	12.6	0.42	0.47	0.10	0.99	0.11	0.15	0.05	0.31
BL-2	7/7/2010	15622	13.9	0.45	0.54	0.12	1.11	0.16	0.17	0.05	0.38
BL-3	7/7/2006	14165	13.0	0.49	0.56	0.14	1.19	0.18	0.14	0.05	0.37
Laketon Beach	7/7/2010	11401	7.5	0.48	0.66	0.10	1.24	0.15	0.17	0.05	0.37
BL-1	7/8/2006	14982	12.8	0.23	0.26	0.05	0.54	0.12	0.18	0.06	0.36
BL-2	7/8/2006	15120	11.8	0.25	0.29	0.05	0.59	0.24	0.14	0.06	0.44
BL-3	7/8/2006	15996	12.9	0.20	0.28	0.04	0.52	0.16	0.16	0.06	0.38
Laketon Beach	7/8/2006	16476	10.6	0.18	0.23	0.03	0.44	0.16	0.16	0.06	0.38
BL-1	7/20/2006	20894	17.4	0.16	0.25	0.03	0.44	NA	NA	NA	NA
BL-2	7/20/2006	22286	20.6	0.31	0.31	0.05	0.67	NA	NA	NA	NA
BL-3	7/20/2006	21010	18.8	0.33	0.47	0.07	0.87	NA	NA	NA	NA
Laketon Beach	7/20/2006	23941	17.6	0.22	0.47	0.05	0.74	NA	NA	NA	NA

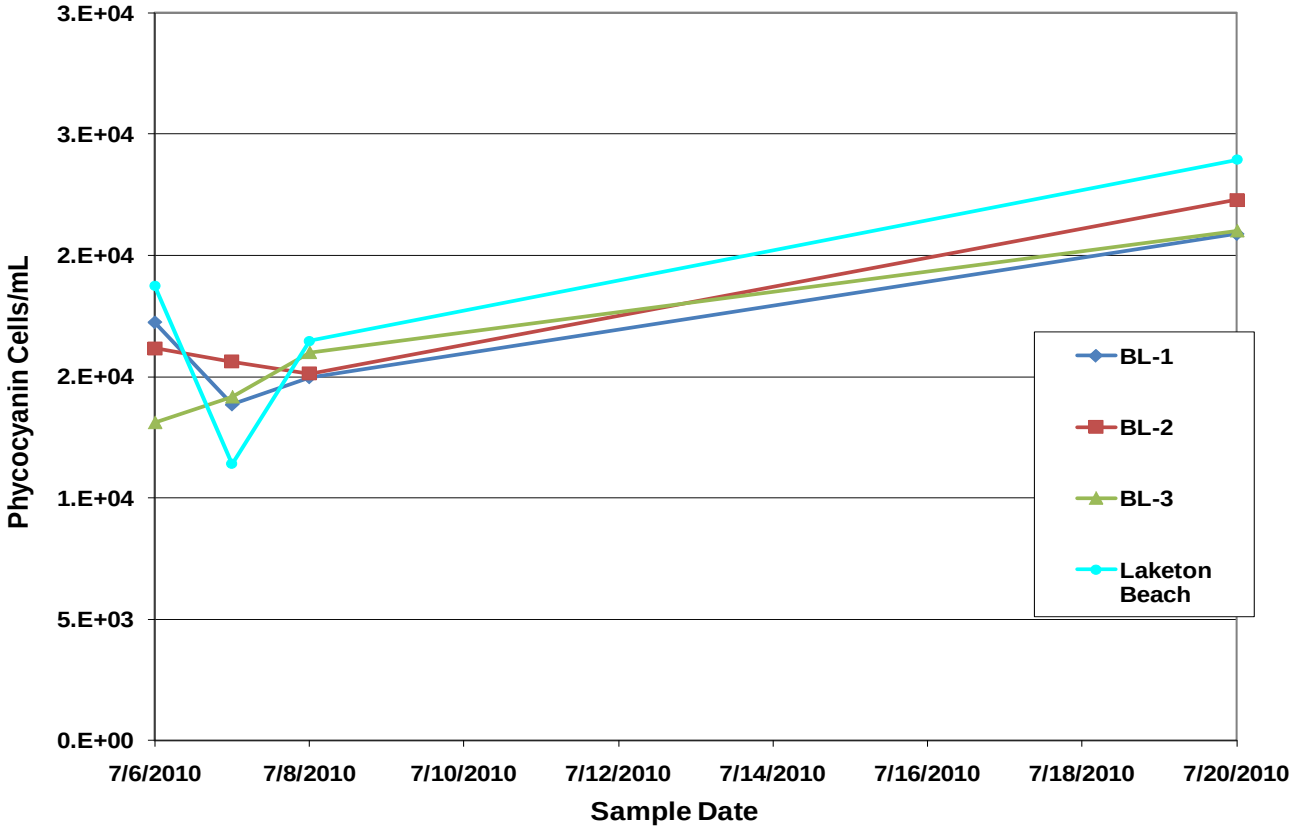

Figure 3.1.1. Phycocyanin Results for Bear Lake Pre and Post Algicide Application 2010.

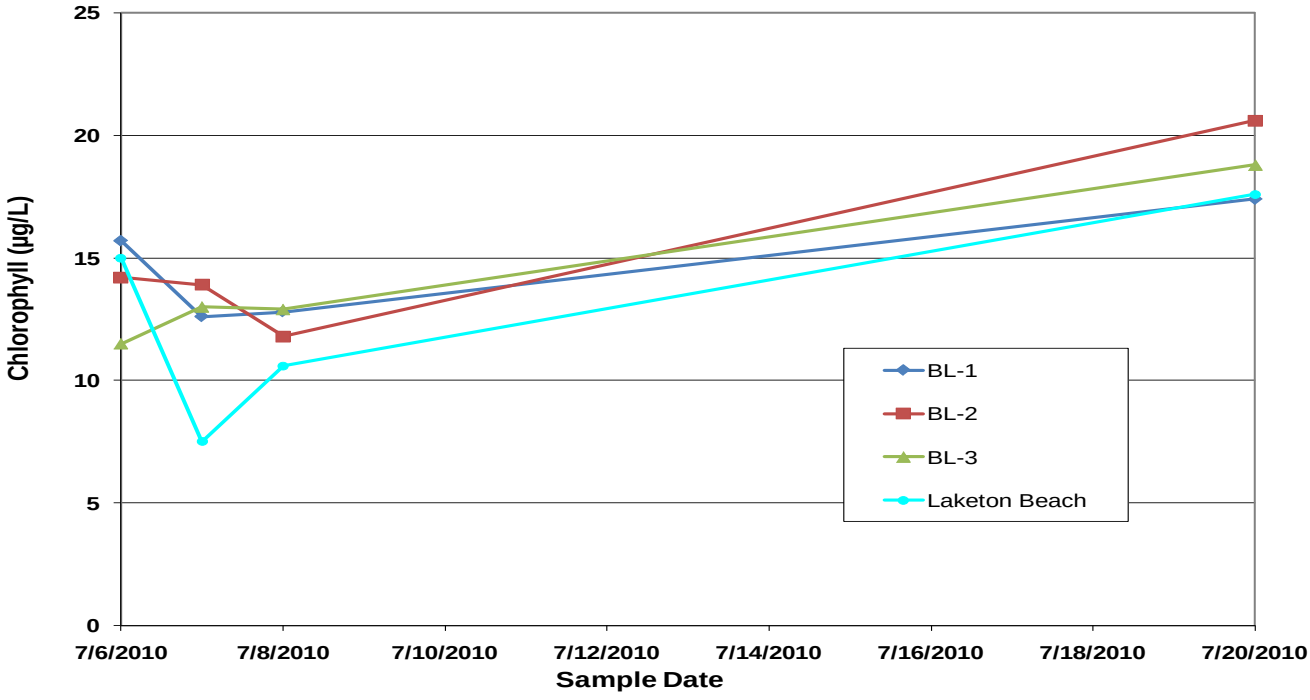

Figure 3.1.2. Chlorophyll *a* Results for Bear Lake Pre and Post Algicide Application 2010.

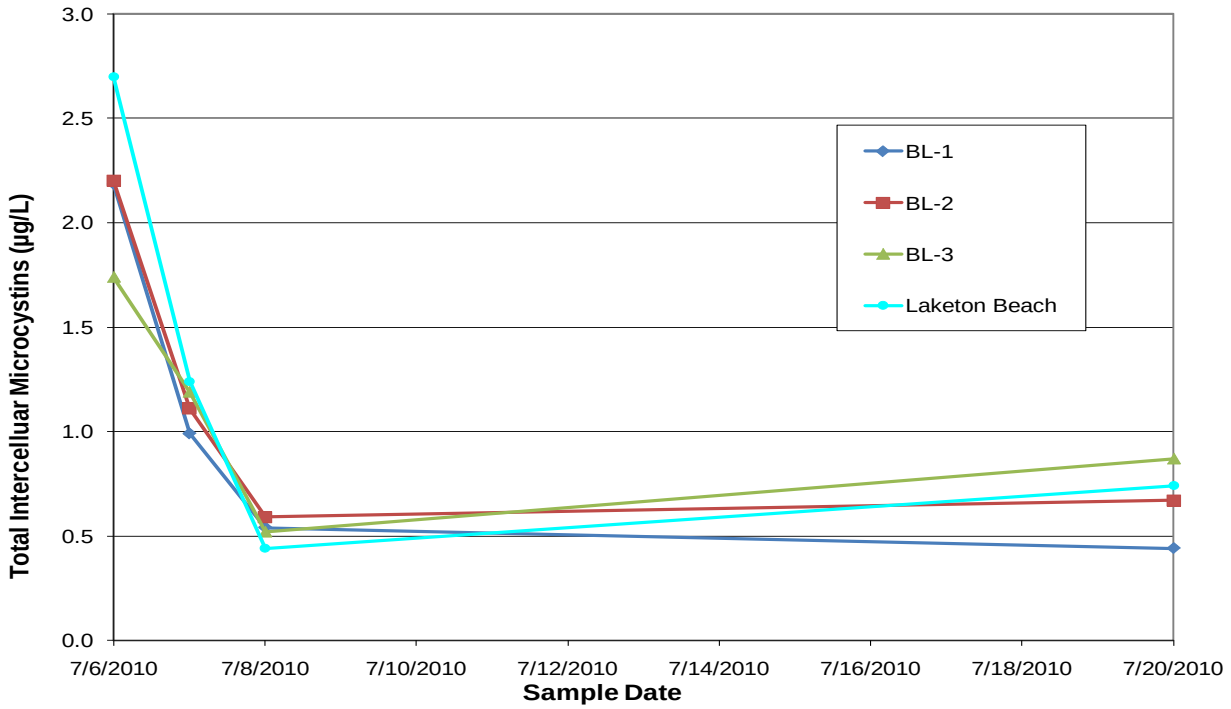

Figure 3.1.3. Total Intercellular Microcystin Results for Bear Lake Pre and Post Algicide Application 2010.

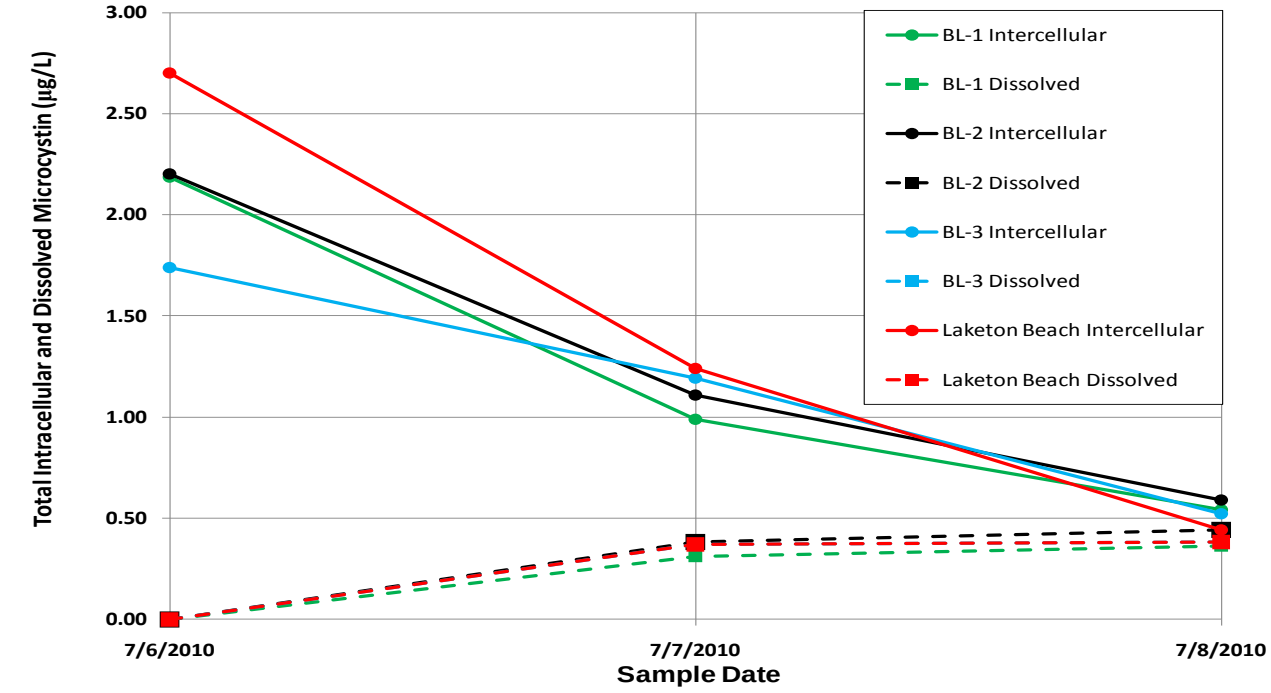

Figure 3.1.4. Total Intercellular and Dissolved Microcystin Results for Bear Lake Pre and Post Algicide Application 2011.

3.1.2. Bear Lake Summer Data

The results of the monitoring data for Bear Lake are summarized in Table 3.1.2. Mean phycocyanin results were 19,755±826 cells/mL and ranged from 11,401-27,409 cells/mL. Cyanobacteria density increased after algicide application and peaked on August 16 (Figure 3.1.5). Chlorophyll a concentrations averaged 17±0.8 µg/L and ranged from 7.5-25.4 µg/L. Chlorophyll *a* concentrations also increased after algicide application and peaked on August 3 (Figure 3.1.6). Total microcystin concentrations averaged 1.22±0.10 µg/L and ranged from 0.10-2.74 µg/L. Mean total microcystin concentrations consisted of microcystin LR (mean 0.47 µg/L) and microcystin RR (0.65 µg/L). Maximum total microcystin concentrations were observed on July 6 followed by a decline after algicide application (Figure 3.1.7). Total microcystin concentrations increased to a second maxima on August 16, followed by a decline. Cylindrospermopsin and anatoxin *a* were not detected at a detection limit of 1 µg/L.

Table 3.1.2. Bear Lake Monitoring Data 2010. (PC=Phycocyanin, IC=Intercellular, MC=Microcystin, DS=Dissolved, AN A=Anatoxin *a*, CYN=Cylindrospermopsin, NA=Not Analyzed.)

Site	Date	PC (cells/ml)	Chlorophyll *a* (µg/L)	IC MC-LR µg/l	IC MC-RR µg/l	IC MC-YR µg/l	Total IC MCs µg/l	DIS MC-LR µg/l	DS MC-RR µg/l	DS MC-YR µg/l	Total DS MCs µg/l	AN A µg/l	CYN µg/l
BL-1	7/6/2006	17256	15.7	0.92	1.01	0.26	2.19	< 0.02	< 0.02	< 0.02	< 0.02	<1.0	<1.0
BL-2	7/6/2006	16169	14.2	1.00	0.98	0.22	2.20	< 0.02	< 0.02	< 0.02	< 0.02	<1.0	<1.0
BL-3	7/6/2006	13107	11.5	0.78	0.78	0.18	1.74	< 0.02	< 0.02	< 0.02	< 0.02	<1.0	<1.0
Laketon Beach	7/6/2010	18765	15.0	1.14	1.50	0.10	2.74	< 0.02	< 0.02	< 0.02	< 0.02	<1.0	<1.0
BL-1	7/7/2010	13860	12.6	0.42	0.47	0.10	0.99	0.11	0.15	0.05	0.31	<1.0	<1.0
BL-2	7/7/2010	15622	13.9	0.45	0.54	0.12	1.11	0.16	0.17	0.05	0.38	<1.0	<1.0
BL-3	7/7/2006	14165	13.0	0.49	0.56	0.14	1.19	0.18	0.14	0.05	0.37	<1.0	<1.0
Laketon Beach	7/7/2010	11401	7.5	0.48	0.66	0.10	1.24	0.15	0.17	0.05	0.37	<1.0	<1.0
BL-1	7/8/2006	14982	12.8	0.23	0.26	0.05	0.54	0.12	0.18	0.06	0.36	<1.0	<1.0
BL-2	7/8/2006	15120	11.8	0.25	0.29	0.05	0.59	0.24	0.14	0.06	0.44	<1.0	<1.0
BL-3	7/8/2006	15996	12.9	0.20	0.28	0.04	0.52	0.16	0.16	0.06	0.38	<1.0	<1.0
Laketon Beach	7/8/2006	16476	10.6	0.18	0.23	0.03	0.44	0.16	0.16	0.06	0.38	<1.0	<1.0
BL-1	7/20/2006	20894	17.4	0.16	0.25	0.03	0.44	NA	NA	NA	NA	<1.0	<1.0
BL-2	7/20/2006	22286	20.6	0.31	0.31	0.05	0.67	NA	NA	NA	NA	<1.0	<1.0
BL-3	7/20/2006	21010	18.8	0.33	0.47	0.07	0.87	NA	NA	NA	NA	<1.0	<1.0
Laketon Beach	7/20/2006	23941	17.6	0.22	0.47	0.05	0.74	NA	NA	NA	NA	<1.0	<1.0
BL-1	8/3/2010	23188	19.8	0.53	0.68	0.12	1.33	NA	NA	NA	NA	<1.0	<1.0
BL-2	8/3/2010	24900	22.1	0.34	0.78	0.08	1.2	NA	NA	NA	NA	<1.0	<1.0
BL-3	8/3/2010	26100	24.2	0.36	0.56	0.07	0.99	NA	NA	NA	NA	<1.0	<1.0
Laketon Beach	8/3/2010	24954	22.4	0.33	0.57	0.06	0.96	NA	NA	NA	NA	<1.0	<1.0
BL-1	8/16/2006	25451	22.5	0.45	1.21	0.11	1.77	NA	NA	NA	NA	<1.0	<1.0
BL-2	8/16/2006	27409	21.9	0.6	1.24	0.12	1.96	NA	NA	NA	NA	<1.0	<1.0
BL-3	8/16/2006	26999	20.4	0.52	1.02	0.11	1.65	NA	NA	NA	NA	<1.0	<1.0
Laketon Beach	8/16/2006	15952	25.4	0.85	0.89	0.06	1.8	NA	NA	NA	NA	<1.0	<1.0
BL-1	8/30/2006	17508	19.2	0.29	0.54	0.05	0.88	NA	NA	NA	NA	<1.0	<1.0
BL-2	8/30/2006	21209	13.1	0.43	0.73	0.08	1.24	NA	NA	NA	NA	<1.0	<1.0
BL-3	8/30/2006	24208	21.0	0.52	0.83	0.09	1.44	NA	NA	NA	NA	<1.0	<1.0
Laketon Beach	8/30/2006	19544	11.0	0.70	0.82	0.06	1.58	NA	NA	NA	NA	<1.0	<1.0
BL-1	9/9/2006	22114	18.6	0.62	0.59	0.1	1.31	NA	NA	NA	NA	<1.0	<1.0
BL-2	9/9/2006	24513	19.8	0.35	0.47	0.04	0.86	NA	NA	NA	NA	<1.0	<1.0
BL-3	9/9/2006	18102	21.5	0.29	0.5	0.06	0.85	NA	NA	NA	NA	<1.0	<1.0
Laketon Beach	9/9/2006	18956	18.0	0.41	0.45	0.04	0.90	NA	NA	NA	NA	<1.0	<1.0
Mean		19755	17	0.47	0.65	0.09	1.22	NA	NA	NA	NA	<1.0	<1.0
SE		826	0.8	0.04	0.06	0.01	0.10	NA	NA	NA	NA	x	x
Maximum		27409	25.4	1.14	1.50	0.26	2.74	NA	NA	NA	NA	x	x
Minimum		11401	7.5	0.16	0.23	0.03	0.44	NA	NA	NA	NA	x	x

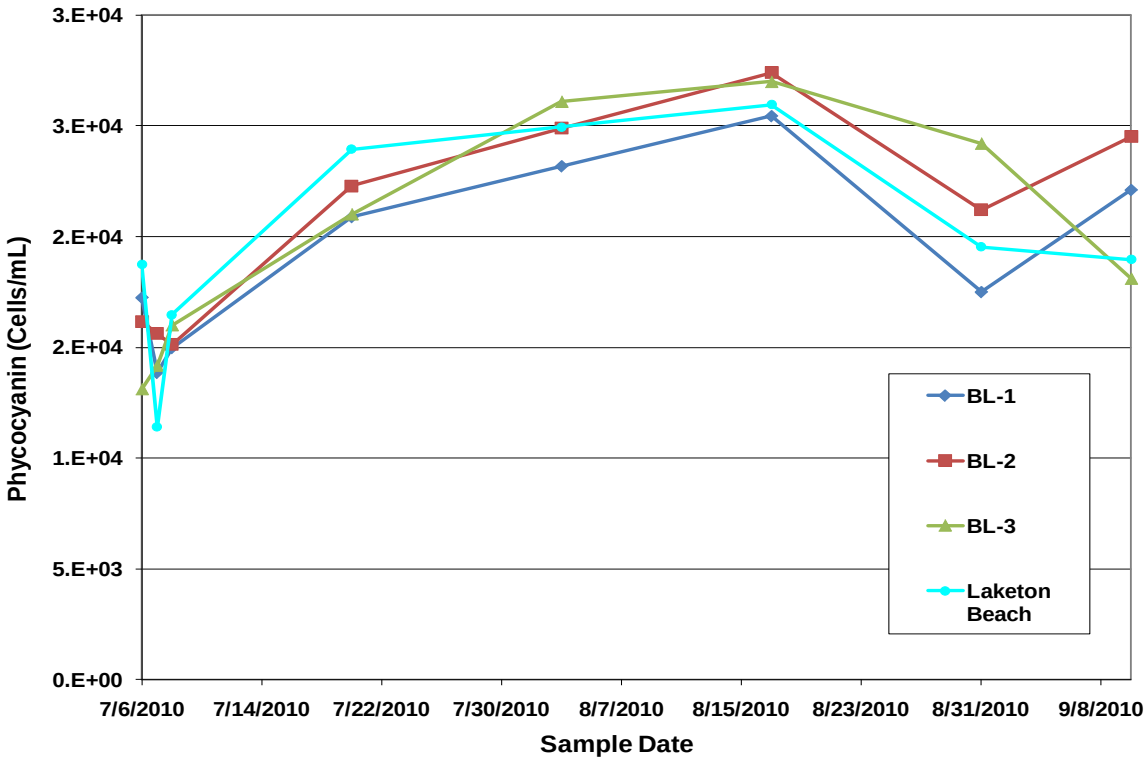

Figure 3.1.5. Phycocyanin Results for Bear Lake 2010.

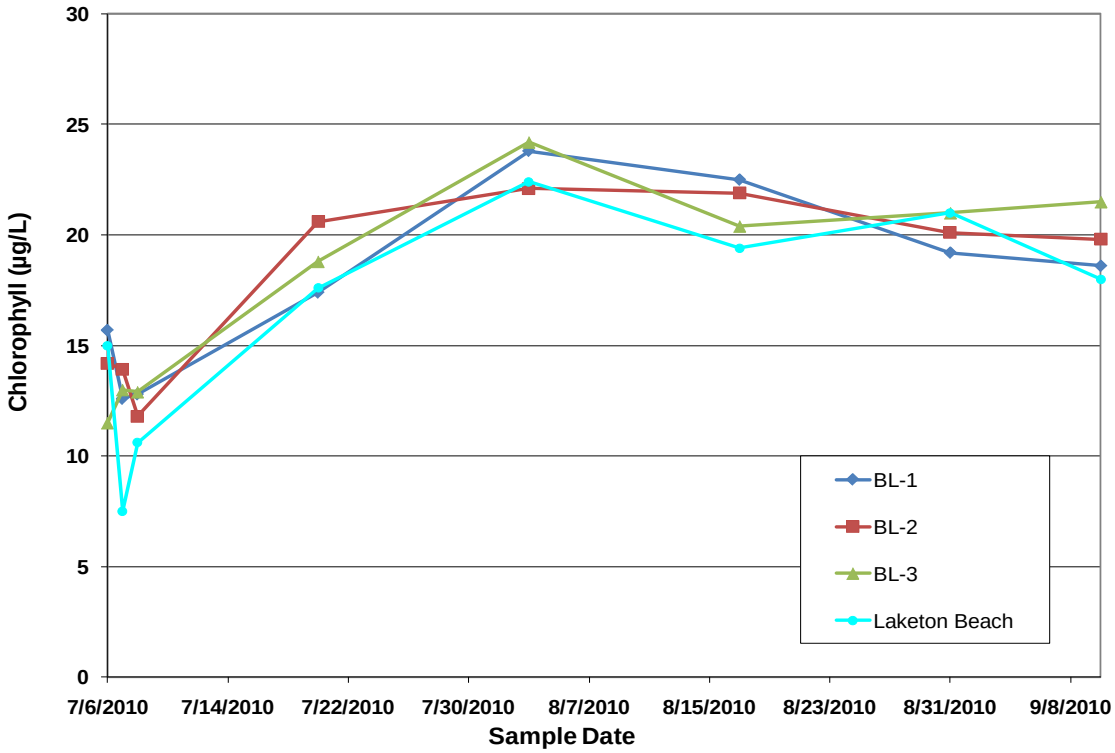

Figure 3.1.6. Chlorophyll *a* Results for Bear Lake 2010.

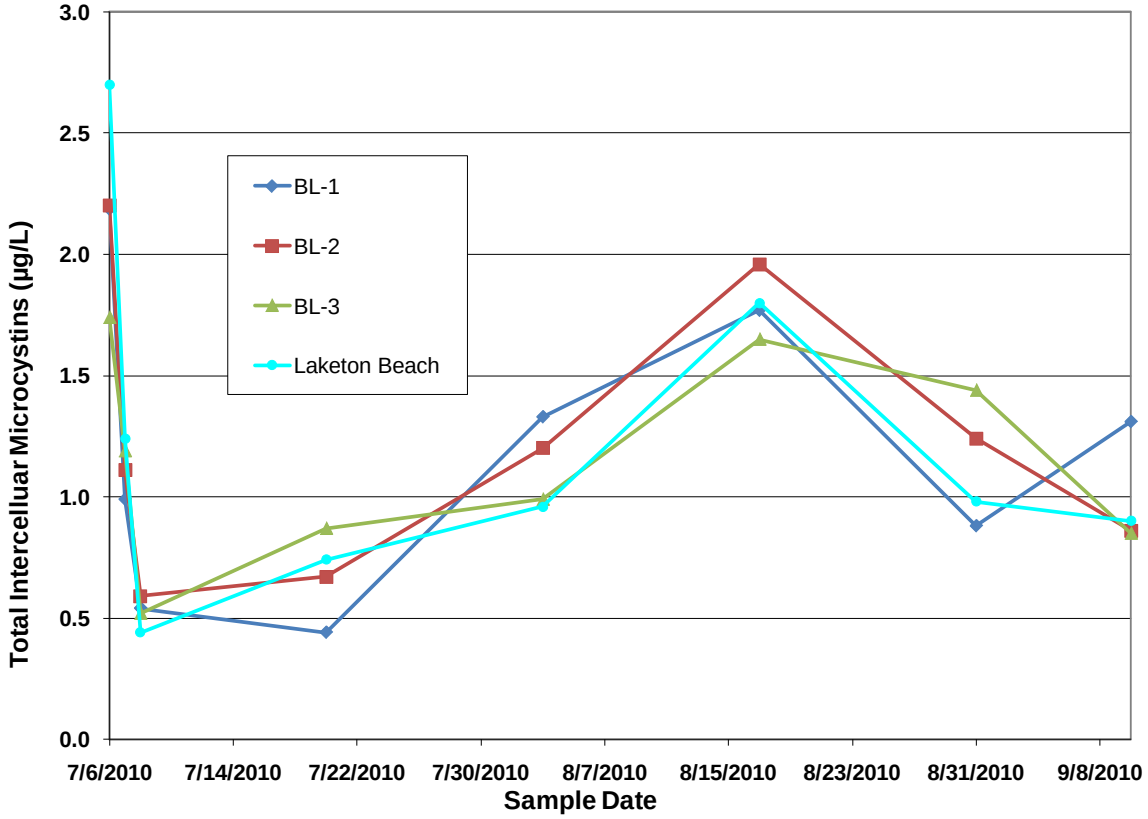

Figure 3.1.7. Total Intercellular Microcystin Results for Beat Lake 2010.

3.2 Muskegon Lake Monitoring Results

3.2.1. Muskegon Lake Summer Data

The results of the monitoring data for Muskegon Lake are summarized in Table 3.2.2. Mean phycocyanin results were 19,755±826 cells/mL and ranged from 11,401-27,409 cells/mL. Cyanobacteria density increased after algicide application and peaked on August 16 (Figure 3.1.5). Chlorophyll a concentrations averaged 17±0.8 µg/L and ranged from 7.5-25.4 µg/L. Chlorophyll *a* concentrations also increased after algicide application and peaked on August 3 (Figure 3.1.6). Total microcystin concentrations averaged 1.22±0.10 µg/L and ranged from 0.10-2.74 µg/L. Mean total microcystin concentrations consisted of microcystin LR (mean 0.47 µg/L) and microcystin RR (0.65 µg/L). Maximum total microcystin concentrations were observed on July 6 followed by a decline after algicide application (Figure 3.1.7). Total microcystin concentrations increased to a second maxima on August 16, followed by a decline. Cylindrospermopsin and anatoxin *a* were not detected at a detection limit of 1 µg/L.

Table 3.2.1 Muskegon Lake Monitoring Data 2010. (PC=Phycocyanin, IC=Intercellular, MC=Microcystin, DS=Dissolved, AN A=Anatoxin *a*, CYN=Cylindrospermopsin, NA=Not Analyzed.)

Site	Date	PC (cells/ml)	Chlorophyll *a* (µg/L)	IC MC-LR µg/l	IC MC-RR µg/l	IC MC-YR µg/l	Total IC MCs µg/l	AN A µg/l	CYN µg/l
MK-1	7/5/2006	1319	6.6	0.03	0.01	0.01	0.05	<1.0	<1.0
MK-2	7/5/2006	970	7.0	0.06	0.01	0.01	0.07	<1.0	<1.0
MK-3	7/5/2006	1017	5.3	0.04	0.01	0.01	0.06	<1.0	<1.0
Watersports Park	7/7/2010	4268	4.9	0.03	< 0.02	< 0.02	0.03	<1.0	<1.0
MK-1	7/19/2006	3962	18.4	0.14	0.04	0.03	0.21	<1.0	<1.0
MK-2	7/19/2006	3512	14.7	0.26	0.07	0.05	0.38	<1.0	<1.0
MK-3	7/19/2006	2083	5.5	0.30	0.11	0.04	0.45	<1.0	<1.0
Watersports Park	7/19/2006	42531	14.2	0.78	0.32	0.11	1.21	<1.0	<1.0
MK-1	8/3/2010	5300	6.2	0.50	0.20	0.04	0.74	<1.0	<1.0
MK-2	8/3/2010	6600	10.1	0.73	0.29	0.05	1.07	<1.0	<1.0
MK-3	8/3/2010	3560	8.8	0.19	0.17	0.02	0.38	<1.0	<1.0
Watersports Park	8/3/2010	4550	10.5	1.14	0.45	0.09	1.68	<1.0	<1.0
MK-1	8/16/2006	8365	9.8	0.53	0.27	0.11	0.91	<1.0	<1.0
MK-2	8/16/2006	6936	7.9	0.78	0.50	0.13	1.41	<1.0	<1.0
MK-3	8/16/2006	4713	5.5	0.27	0.21	0.03	0.51	<1.0	<1.0
Watersports Park	8/16/2006	3908	10.4	0.35	0.39	0.06	0.80	<1.0	<1.0
MK-1	8/30/2006	5514	10.8	2.34	1.55	0.44	4.33	<1.0	<1.0
MK-2	8/30/2006	3160	13.6	1.49	0.75	0.23	2.47	<1.0	<1.0
MK-3	8/30/2006	4185	10.9	0.49	0.23	0.06	0.78	<1.0	<1.0
Watersports Park	8/30/2010	4332	11.5	1.24	0.35	0.09	1.68	<1.0	<1.0
MK-1	9/9/2006	4310	4.2	1.51	0.64	0.22	2.37	<1.0	<1.0
MK-2	9/9/2006	6410	6.3	1.12	0.66	0.25	2.03	<1.0	<1.0
MK-3	9/9/2006	2508	6.5	0.24	0.34	0.06	0.64	<1.0	<1.0
Watersports Park	9/9/2010	4109	5.4	1.02	0.33	0.09	1.44	<1.0	<1.0
Mean		2714	6.0	0.53	0.17	0.05	0.75	<1.0	<1.0
SE		411	0.18	0.15	0.05	0.01	0.20	x	x
Maximum		4109	6.6	1.02	0.33	0.09	1.44	x	x
Minimum		1319	5.4	0.03	0.01	0.01	0.05	x	x

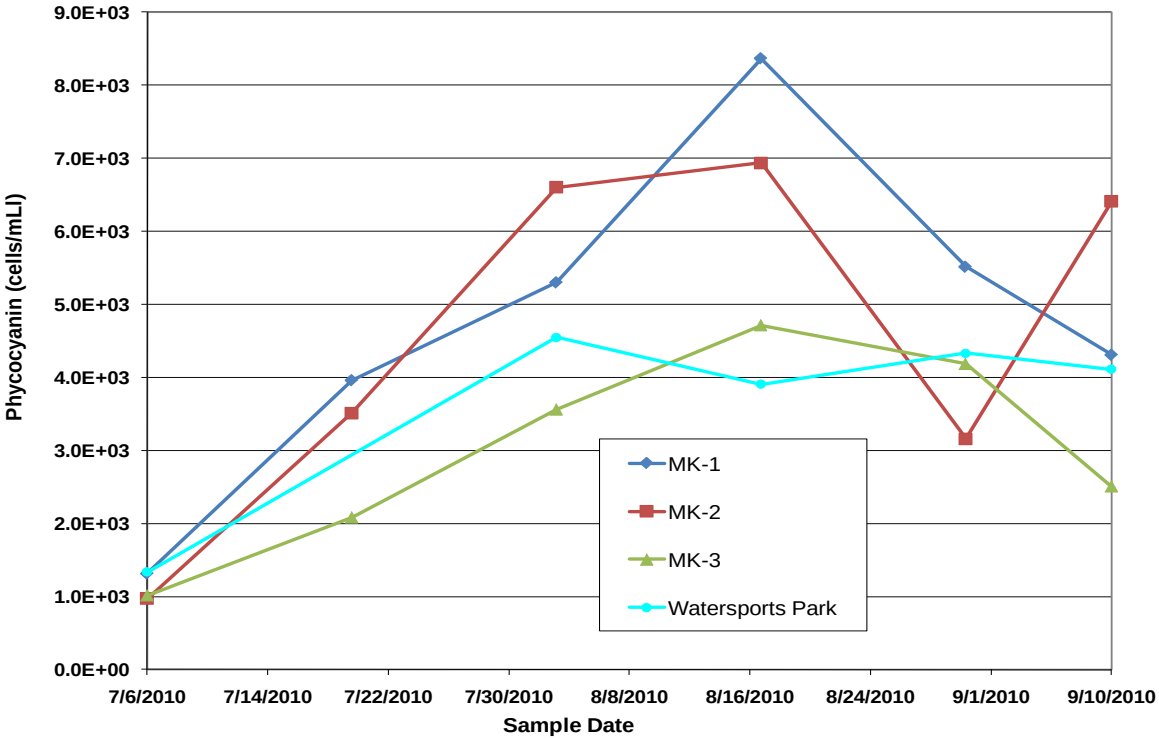

Figure 3.2.1. Phycocyanin Results for Muskegon Lake 2010.

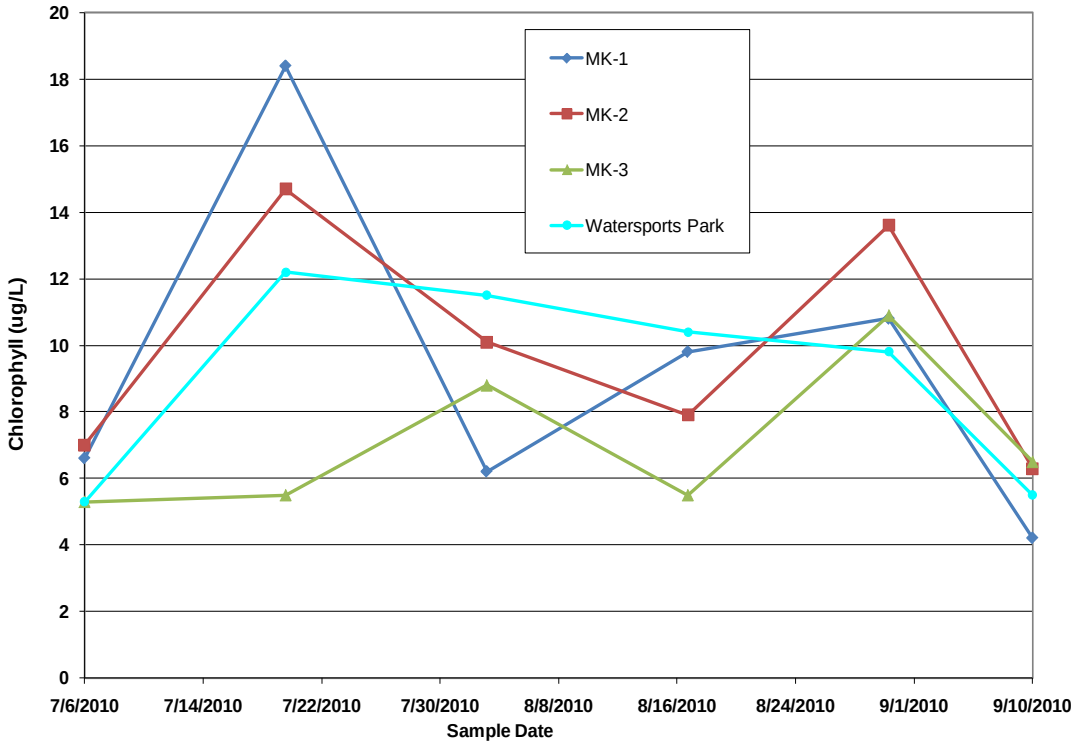

Figure 3.2.2. Chlorophyll *a* Results for Muskegon Lake 2010.

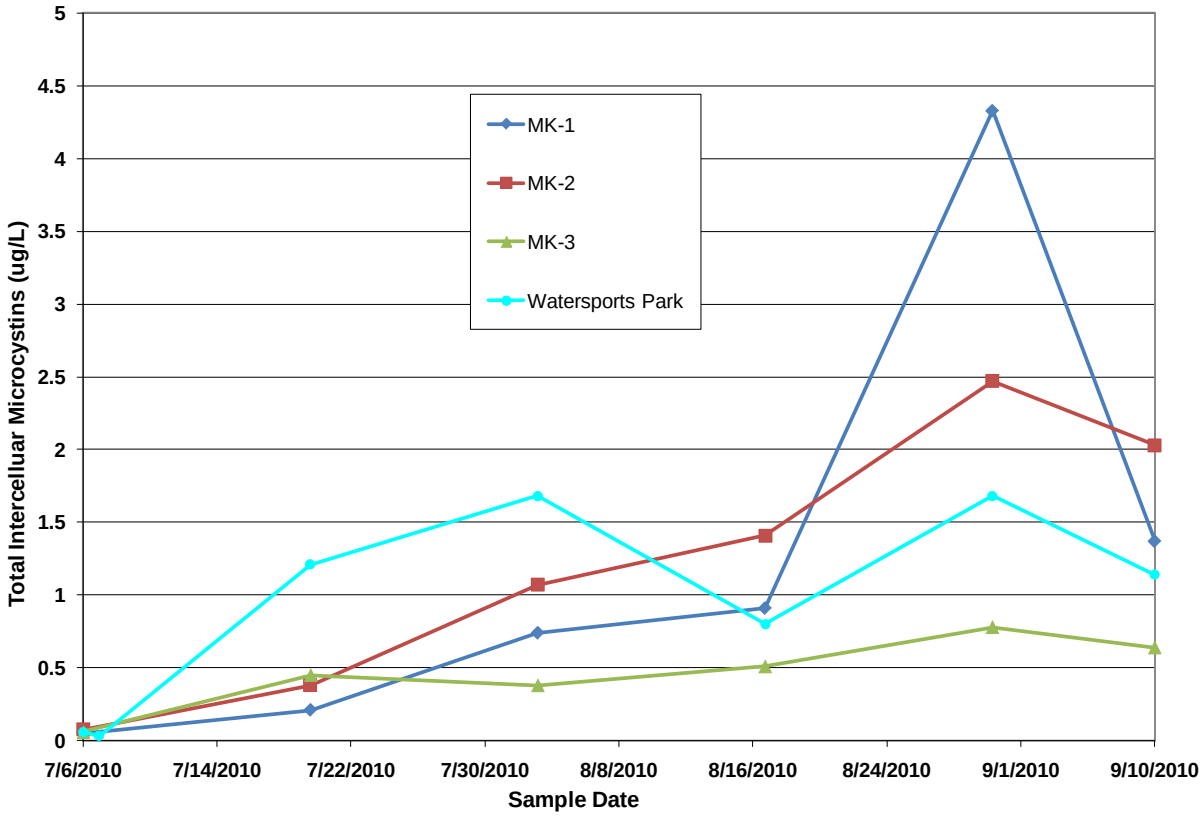

Figure 3.2.3. Total Microcystin Results for Muskegon Lake 2010.

3.3 Saginaw Bay and Grand Traverse Bay/Little Traverse Bay Comparisons

A summary of the project data is presented in Table 3.3.1. Mean temperatures for Saginaw Bay were higher than Grand Traverse/Little Traverse Bay (23°C and 20°C, respectively). Microcystins in detached *Cladophora* were absent in Grand Traverse/Little Traverse Bay. In Saginaw Bay, the grand mean of all locations with detached *Cladophora* was 57 µg/g dwt for the study period. Mean concentrations of *E. coli* in water were significantly higher (Mann-Whitney ρ=0.003) in Grand Traverse/Little Traverse Bay (34 cfu/100 mL) than Saginaw Bay (18 cfu/100 mL). In contrast, mean *E. coli* concentrations in detached *Cladophora* were higher in Saginaw Bay (2,796 cfu/g dwt) than Grand Traverse/Little Traverse Bay (1,775 cfu/g dwt); however, the difference was not statistically significant (Mann-Whitney ρ=0.40). The mean *Cladophora* extent score for Grand Traverse/Little Traverse Bay was 1, indicating that detached algae were found in small pockets. The mean *Cladophora* extent score for Saginaw Bay was 3 indicating that detached algae were found in 1 m^2 deposits.

Table 3.3.1. Summary of Water Quality and *Cladophora* Data for Grand Traverse/Little Traverse Bay and Saginaw Bay 2008.

Parameter	Saginaw Bay	Grand Traverse/Little Traverse Bay
Mean Temperature	23°C	20°C
Mean Total Microcystins	57 µg/g dwt	<0.1 µg/g dwt
Cladophora Extent Score	3	1
Mean *E coli* in Water	18 cfu/100 mL	34 cfu/100 mL
Mean *E coli* in *Cladophora*	2,796 cfu/g dwt	1,775 cfu/g dwt

(*Cladophora* Extent: 0 = None Present; 1 = Present in isolated pockets at one location; 2 = Present in isolated pockets at all locations; 3 = Deposits 1 m^2 in area; 4= Deposits 1-5 m^2 in area; 5 = Deposits > 5 m^2 in area.)

4.0 Discussion

The presence of *Cladophora* has been associated with high levels of *E. coli* and species of potentially pathogenic enteric bacteria in beach sand and swimming waters of the Great Lakes (Whitman et al. 2003; Ishii et al. 2006; Olapade et al. 2006). Enteric bacteria can grow within detached *Cladophora* under certain conditions (Byappanahalli et al. 2005). In addition, detached *Cladophora* deposits can potentially harbor and enhance the survival of pathogenic bacteria released into the environment through point and nonpoint sources (Byappanahalli et al. 2005). Recently, *Clostridium* was found to grow in detached *Cladophora* in the Great Lakes (Byappanahalli and Whitman 2009). Algal species, including *Cladophora*, have been reported to provide nutrients and to protect attached bacteria from environmental stresses, desiccation, predation, and harmful UV radiation (Byappanahalli et al. 2005). In this manner, *Cladophora* mats may play a significant role as a source and sink for pathogens in the nearshore environment. Based on the results from this investigation, Saginaw Bay appears to be more heavily impacted by detached *Cladophora* than Grand Traverse/Little Traverse Bay. Eutrophication of lakes has been associated with increases in *Cladophora* mat production and accumulation (Stevenson et al. 2006; Herbst, 1969), but water transparency also has been suggested as an important causative factor (Barbiero et al. 2006; Bootsma, 2007). Elevated levels of nutrients in Saginaw Bay have been reported to stimulate blooms of cyanobacteria in Saginaw Bay and also influence the nearshore environment (Fahnenstiel et al. 2008; Millie et al. 2008). Differences in *Cladophora* accumulation between the Lake Huron and Lake Michigan sites may be attributed to higher total phosphorus levels (18 µg/l vs. 5 µg/l; MDNRE 2006). Levels of *E. coli* in detached *Cladophora* in both systems were similar to concentrations previously reported in the Great Lakes (3 $\log_{10}$ - 5 $\log_{10}$; Engelbert et al. 2008).

In Saginaw Bay, the highest levels of *E. coli* in detached *Cladophora* were consistently found at locations near the Saginaw River (Wenona Beach and the Bay City Recreation Area). Even within individual sites, locations near tributaries and drains at Wenona Beach and South Linwood Beach were significantly higher than locations further away form a point source. This relationship also was noted in Grand Traverse Bay where the location near Mitchell Creek at the Traverse City State Park had elevated *E. coli* concentrations in detached *Cladophora* compared to the other two beach stations. At Traverse City State Park, *Cladophora* deposits were present only at the location near Mitchell Creek. These results suggest that *Cladophora* can trap bacteria from point sources and also be stimulated by nutrient discharges. Two locations, Pinconning Park and White's Beach had very limited *Cladophora* growth. Both locations had *Chara* growing on the lake bottom. *Chara* is known to exhibit allelopathic activity that can limit the growth of other aquatic plants (Berger and Schagerl 2004). No correlation was found between

E. coli levels in the open water (designated beach monitoring locations) and the nearshore zone where the detached *Cladophora* samples were taken. As noted in previous studies, *Cladophora* appears to hold trapped *E. coli* and not release the entrained bacteria to the offshore water (Byappanahalli et al. 2003; Engelbert et al. 2008).

This investigation was the first to document the accumulation of microcystins in the detached *Cladophora* of Saginaw Bay. Saginaw Bay has a history of *Microcystis* blooms in the late summer months that produce both microcystin LR and RR (Fahnenstiel et al. 2008; Millie et al. 2008). Since *Microcystis* has a high requirement for sunlight, cyanobacteria may become stressed when they become trapped in the detached algae deposits. Stress has been implicated in initiating cyanotoxin production (Codd 1995). While accidental ingestion by humans of microcystins trapped in *Cladophora* is unlikely, these compounds can act as skin irritants (Bell and Codd 1994). Walking through *Cladophora* accumulations to get to deeper water may provide sufficient exposure to cause irritation in sensitive individuals if microcystins are present.

While all of the data suggest that swimming areas (1 m depth) are not impacted by *E. coli* accumulations in detached *Cladophora*, entrained bacteria and cyanotoxins may pose a hazard to children playing in the nearshore water and sand. While current regulations discourage beach grooming, the presence of elevated bacteria and microcystin levels in the nearshore environment of Saginaw Bay suggests that the current policy should balance potential impacts to public health with the ecosystem services provided by coastal wetlands.

5.0 Conclusions

An assessment of the ability of *Cladophora* mats to sequester *E. coli* and microcystin LR and RR was conducted in nearshore waters of Grand Traverse Bay (7 sites), Little Traverse Bay (2 sites), and Saginaw Bay (8 sites). The sampling locations were at public beach access points where *Cladophora* mats previously have been observed. The goals of this research were to determine the spatial and temporal variability of *E. coli* populations in *Cladophora* mats in these recreational waters and if cyanotoxins (microcystin LR and RR) were sequestered in the detached algae. The collection of *Cladophora* samples was coordinated with local beach monitoring programs to facilitate the comparison with ambient water bacteria concentrations. This project provided important data for the assessment of public health impacts and the development of beach management programs to address the problems associated with *Cladophora* accumulations.

Based on the results from this investigation, Saginaw Bay appears to be more heavily impacted by detached *Cladophora* than Grand Traverse/Little Traverse Bay. Mean *E. coli* concentrations in detached *Cladophora* were higher in Saginaw Bay (2,796 cfu/g dwt) than Grand Traverse/Little Traverse Bay (1,775cfu/g dwt); however, the difference was not statistically significant (Mann-Whitney ρ=0.40). The mean *Cladophora* extent score for Grand Traverse/Little Traverse Bay was 1, indicating that detached alga was consistently found in small pockets at a single location. The mean *Cladophora* extent score for Saginaw Bay was 3, indicating that detached alga was found in 1 m^2 deposits at all locations. Differences in *Cladophora* accumulation between Saginaw Bay and Grand Traverse/Little Traverse Bay may be attributed to higher total phosphorus levels in Saginaw Bay. Levels of *E. coli* in detached

Cladophora in both systems were similar to concentrations previously reported in the Great Lakes (1,000 cfu/g dwt – 60,000 cfu/g dwt). In Saginaw Bay, the highest levels of *E. coli* in detached *Cladophora* were consistently found at locations near the Saginaw River (Wenona Beach and the Bay City Recreation Area). Even within individual sites, locations near tributaries and drains at Wenona Beach and South Linwood Beach were significantly higher than locations further away form a point source. This relationship also was noted in Grand Traverse Bay where the location near Mitchell Creek at the Traverse City State Park had elevated *E. coli* concentrations in detached *Cladophora* compared to the other two beach stations where no detached algae was found. These results suggest that *Cladophora* can trap bacteria from point sources and also be stimulated by nutrient discharges. Two locations, Pinconning Park and White's Beach, had very limited *Cladophora* growth. Both locations had *Chara* growing on the lake bottom. *Chara* is known to exhibit allelopathic activity that can limit the growth of other aquatic plants. No correlation was found between *E. coli* levels in the open water (designated beach monitoring locations) and the nearshore zone where the detached *Cladophora* samples were taken. As noted in previous studies, *Cladophora* appears to hold trapped *E. coli* and does not release the entrained bacteria to the offshore water.

This investigation was the first to document the accumulation of microcystins in the detached *Cladophora* of Saginaw Bay. Total microcystins in detached *Cladophora* had a grand mean of 57 µg/g dwt for the study period. Saginaw Bay has a history of *Microcystis* blooms in the late summer months that produce both microcystin LR and RR. Since *Microcystis* has a high requirement for sunlight, cyanobacteria may become stressed when they become trapped in the detached algae mats. While accidental ingestion by humans of microcystins trapped in *Cladophora* is unlikely, these compounds can act as skin irritants. Walking through *Cladophora* accumulations to get to deeper water may provide sufficient exposure to cause irritation in sensitive individuals if microcystins are present. While all of the data suggest that swimming areas (1 m depth) are not impacted by *E. coli* accumulations in detached *Cladophora*, entrained bacteria and cyanotoxins may pose a hazard to children playing in the nearshore water and sand. Current regulations discourage beach grooming and altering the nearshore zone. The presence of elevated bacteria and microcystin levels in the nearshore environment of Saginaw Bay suggest that the current policy should be reevaluated to balance potential impacts to public health with the ecosystem services provided by coastal wetlands.

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