Detection and quantification of *Microcystis* spp. and microcystin-LR in Western Lake Erie during the summer of 2007

Hui Wang, Cyndee L. Gruden, Thomas B. Bridgeman and Justin D. Chaffin

ABSTRACT

Microcystis spp. blooms have occurred annually in western Lake Erie since about 1995. *Microcystis* produce a group of toxins known as microcystins which can be harmful to livestock and to humans. In this study, surface water samples were collected from six sites during six sampling events from July to October in 2007. In situ environmental data (e.g. pH, temperature) and laboratory analyses (e.g. nutrients) were carried out to characterize the six sites. The *Microcystis* spp. density ranged from 10^2 to 10^7 cells/ml. Microcystin-LR concentration of 20 of all 36 samples were below the detection limit (0.15–5 ppb), while the microcystin-LR concentration in the 16 remaining samples ranged from 0.5 to 3×10^3 μg per gram dry weight. The aim of this research was to investigate the relationships between sampling location, environmental parameters, *Microcystis* spp. concentration, and microcystin-LR concentration. The results suggest that temperature, nutrient concentration, turbidity, and wind speed and direction ($P < 0.05$) are factors which affected *Microcystis* spp. density. Sampling site 8M, located 13 m from the Maumee River, provided an advantage for *Microcystis* spp. growth, presumably due to intermediate water depth (5.5 m) combined with impact from the river. No relationship was found between *Microcystis* spp. density and microcystin-LR concentration. Temperature, nutrient concentration and DO ($P < 0.05$) were associated with the production of microcystin-LR.

Key words | ELISA, fluorescence microscope, Lake Erie, microcystin-LR, *Microcystis* spp.

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INTRODUCTION

Microcystis spp. is a common genus of cyanobacteria, sometimes called blue green algae, it often grows in shallow eutrophic lakes. Massive growth of *Microcystis* spp. in eutrophic freshwater can have a significant impact on the water quality resulting in diminished recreational use of water bodies and requiring additional treatment for source (drinking) water. Microcystins are a family of hepatotoxins produced by several species of cyanobacteria including *Microcystis* spp., *Anabaena* and *Oscillatoria* (Hudder *et al.* 2007). The most common variant of microcystins is microcystin-LR. Long-term exposure to low levels of microcystins has been implicated in liver tumor promotion (Humpage & Falconer 1999). Specifically, it was reported that the incidence of primary liver cancer in certain areas of China was related to the presence of microcystins in drinking water (Yu 1995). The World Health Organization (WHO 1998) has proposed a guide value of 1 μg/l for microcystin-LR in drinking water, which is intended to be safe for life-long consumption.

Lake Erie is the eleventh largest lake in the world by surface area. Algal blooms occurred in Lake Erie during the 1960s, the U.S. and Canada signed the Great Lakes Water Quality Agreement to limit phosphorus inputs to the Great Lakes in 1972, including Lake Erie (Dolan 1993). Lower phosphorus input reduced the amount of algae and the blooms had disappeared by the late 1980s

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(Nicholls *et al.* 1977). However, toxic *Microcystis* spp. returned to western Lake Erie in 1995 (Brittain *et al.* 2000). Many municipalities along the shoreline in both Michigan and Ohio (OH) obtain drinking water from western Lake Erie. Therefore, the detection, quantification and prediction of *Microcystis* spp. and the presence of microcystins in western Lake Erie are fundamental for sound water management and good water quality.

The light conditions, typically dictated by turbidity, were found to promote *Microcystis* spp. dominance over other cyanobacteria. *Microcystis* spp. species can form blooms in nutrient-rich water bodies. Weather conditions such as wind speed, wind direction and heavy rain are the possible factors affection *Microcystis* spp. abundance. Several factors have been confirmed to impact toxins such as pH, temperature, turbidity and nutrient concentration. Most of the studies that have been performed to date have focused on the effect of nutrient concentration.

Factors affecting *Microcystis* spp. growth and microcystin-LR production have been studied by many researchers. High nutrient loading and weather conditions have been considered important factors that promote growth of *Microcystis* spp. (Wicks & Thiel 1990; Vezie *et al.* 2002). For example, *Microcystis* spp. occurrence was associated with surface water temperature greater than 20°C (Johnston & Jacoby 2003). In addition, wind speed and direction were reported to impact *Microcystis* presence (Ha *et al.* 2000). Light conditions, typically dictated by turbidity associated with surface run-off, were found to promote *Microcystis* spp. dominance over other cyanobacteria (Shapiro 1990). In addition, several factors have been confirmed to impact the presence of toxins associated with *Microcystis* spp. such as pH, temperature, turbidity and nutrient concentration (Wicks & Thiel 1990; Kotak *et al.* 1994; Hesse & Kohl 2001; Ame *et al.* 2003). Previous studies present several possible explanations for *Mycrocystis* spp. bloom formation and the presence of associated toxins. This suggests that these relationships may be site specific since geographical constraints and weather conditions will vary by location.

The aim of this research was to investigate the potential relationships between sampling location, environmental parameters, *Microcystis* spp. concentration, and microcystin-LR concentration using field samples collected in Lake Erie in 2007. Few studies to date have incorporated spatial and temporal variation of *Microcystis* spp. and microcystin-LR as well as a suite of environmental parameters (e.g. pH, dissolved oxygen, temperature, nutrients) collected during one season. This study is of particular interest to the western Lake Erie basin where algal bloom occurrence seems to be on the rise and is of great concern to local stakeholders. In order to study *Microcystis* spp. and related toxins, fluorescent microscopy and antibody-based methods were used. In addition, the potential relationships between sampling site, environmental factors, *Microcystis* spp. density and microcystin-LR concentration were investigated using a multiple linear regression model.

MATERIALS AND METHODS

Field sampling

Between July and October of 2007, surface water samples were collected approximately every two weeks from six fixed locations in the western Lake Erie (MB20, MB18, 8M, 7M, GR1 and 4P) (Figure 1). Table 1 describes the information of these six sampling locations. These locations were selected based on an historical pattern of sampling. They vary in both water depth and distance from the mouth of Maumee River. Sites 4P and GR1 are the deepest and furthest, sites 8M and 7M are closer to the mouth of river and shallower sites. MB18 and MB20 are located in Maumee Bay and shallowest. At each sampling site, two surface water samples were collected in 250 ml field sample bottles for every sampling event, one for *Microcystis* spp. density measurements and the other for microcystin-LR analysis. Samples for *Microcystis* spp. analysis were preserved with 10% buffered formalin. One ml formalin was added in every 10 ml water sample and kept at 4°C in the dark until processing. The water samples collected for microcystin-LR analysis were frozen at −20°C until processing. A total of six sampling events occurred including July 10th, July 24th, August 2nd, August 27th, September 18th and October 8th.

Field measurements

Measurement of some environmental parameters (see Table 2) was performed in the field. pH, temperature, chlorophyll a, turbidity, conductivity, dissolved oxygen (DO)

Figure 1 | Sampling locations in Maumee Bay and western Lake Erie, 2007.

concentration, total dissolved solids were measured using a YSI 6600 monitoring sonde (Yellow Springs Instruments, Yellow Springs, OH), except on July 10th and July 24th. Secchi depth (an indicator of turbidity), wind speed and wind direction were also measured using a Pocket Weather Meter (Kestrel 1000, Boothwyn, PA) every sampling site. Water samples collected at 1 m depth were sent to National Center for Water Quality Research at Hiedelburg University (Tiffin, OH) for nutrient analysis. The nutrient analysis for the water samples included NH_4^+, NO_3^-, NO_2^-, SRP (soluble reactive phosphorus), TP (total phosphorus), TKN (total Kjeldahl nitrogen).

Table 1 | Information on sampling sites

Sampling site	Latitude (°)	Longitude (°)	Distance to the Mouth of Maumee River (km)	Water depth (m)
MB20	41.715	−83.456	2	2
MB18	41.742	−83.402	7	1.5
8M	41.789	−83.356	13	5.5
7M	41.733	−83.297	14	5.7
GR1	41.821	−83.186	26	8.5
4P	41.750	−83.103	30	9.5

Table 2 | Range of environmental factors measured during this study

Environmental factor	Range	Unit
Temperature	19.04–26.83	°C
Conductivity	210–540	μs/cm
TDS	136.5–351	g/l
Chlorophyll a	3.085–82.137	μg/l
DO	6.42–12.92	mg/l
pH	7.76–8.94	
Secchi depth	30–220	cm
Wind speed	1.0–6.0	m/s
NH_4^+	0.011–0.139	mg/l
Cl^-	8.1–39.6	mg/l
SO_4^{2-}	16.7–57.5	mg/l
NO_2^-	0–0.12	mg/l
NO_3^-	0–5.52	mg/l
SRP	0.001–0.105	mg/l
TP	0.011–0.33	mg/l
TKN	0.165–1.227	mg/l

Method for *Microcystis* spp. analysis

In an effort to isolate *Microcystis* spp. from other less buoyant microorganisms, the water sample was settled in a 250 ml graduated cylinder (Thermo Fisher Science Inc, Waltham, MA). Presumably, the diatoms and other organisms settled while *Microcystis* spp. remained floating on the top of graduated cylinder after 48 hours at room temperature. A defined surface volume (3 ml), presumably containing all of *Microcystis* spp. at the surface, was then transferred to a 15 ml centrifuge tube. Because of high *Microcystis* biovolume, this volume was diluted to 15 ml with laboratory grade water. The diluted sample was vortexed (Scientific Industries, Inc., Bohemia, NY) for 30 seconds to create a homogeneous sample. A 1 ml subsample was transferred to a 1.5 ml microcentrifuge tube and sonicated (Branson UITrasonics, Danbury, CT) for 3 minutes to minimize clumping of *Microcystis* spp. colonies. After sonication, 1 ml sample was filtered through 0.22 micron, diameter of 25 mm, black polycarbonate filter (Thermo Fisher Science Inc, Waltham, MA), which was backed by the 25 mm glass-fiber pre-filter (Thermo Fisher Science Inc, Waltham, MA). After filters were dried, they were mounted on a glass microscopic slide (Thermo Fisher Science Inc, Waltham, MA) with immersion oil and a cover slip (Thermo Fisher Science Inc, Waltham, MA). *Microcystis* spp. detection was conducted using a fluorescence microscope (Olympus BX51, Japan) equipped with the digital camera connected to a personal computer. *Microcystis* spp. cells were observed with a magnification of 400X and triplicates were observed.

Method for microcystin-LR analysis

Microcystin-LR concentration of each sample was determined using an antibody-based enzyme-linked immunosorbent assay (ELISA) (Microcystins/Nodularins (ADDA) ELISA Kit, Abraxis, USA). The microcystins ELISA kit was stored in the refrigerator (4°C) before use, and the plates were read using a microtiter plate reader (microplate manager 5.2, Amersham Biosciences Corp., Piscataway, NJ). Because the detection range of this assay was 0.15 to 5 ppb, samples were diluted (or concentrated) to the appropriate range. The ELISA procedure, microcystin extraction before ELISA testing and the evaluation of microcystin concentration were carried out in accordance with the protocol from Abraxis BioScience Inc. (Product No. 520011, Los Angeles, CA).

Method for statistical analysis

The factors affecting *Microcystis* spp. density/microcystin-LR concentration were investigated by linear regression model using SAS (Statistical Analysis System). The natural log-transformed values of *Microcystis* density and the square root of microcystin-LR concentration were used to follow the normal distribution. The correlation between natural log of *Microcystis* density/square root of microcystin-LR concentration and environmental factors was first checked. When P value was less than 0.05, the factor was considered to be highly correlated. These highly correlated factors were then selected to be involved in the multiple linear regression model.

$$\text{Log}\, Y_1 = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_4 + \ldots\ldots + \varepsilon_1$$

Y_1: *Microcystis* spp. density;
a_0: Intercept;
a_1, a_2, a_3, a_4…: Coefficient;

X_1, X_2, X_3, X_4…: Environmental factor;
ε_1: Error term.

$$Y_2 \wedge 0.5 = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + \ldots\ldots + \varepsilon_2$$

Y_2: Microcystin-LR concentration;
b_0: Intercept;
b_1, b_2, b_3, b_4…: Coefficient;
X_1, X_2, X_3, X_4, X_5…: Environmental factor;
ε_2: Error term.

RESULTS

Six sites were sampled during six different sampling events resulting in the collection of 36 surface water samples from July to October in 2007. In addition to providing the results of the sampling events, this section highlights the potential impact of sampling date, sampling location, and environmental factors on *Microcystis* spp. density and microcystin-LR concentration.

Microcystis spp. density

The *Microcystis* spp. density was determined for all 36 surface water samples collected. The density ranged from 10^2 to 10^7 cells/ml. For this work, *Microcystis* spp. densities less than 5×10^2 cells/ml were considered low, while *Microcystis* spp. densities above 10^4 cells/ml were considered high. Figure 2 shows natural Log of *Microcystis* spp. density measured at six sampling sites varying with the distance to the mouth of Maumee River during summer 2007. The highest *Microcystis* spp. density (1.03×10^7 cells/ml) was measured at site 8M (13 km) on October 8th. The lowest *Microcystis* spp. density (56 cells/ml) occurred at site 7M on August 27th. Although there did not appear to be an obvious relationship between *Microcystis* spp. density and sampling date, *Microcystis* spp. density was generally low during the sampling date of August 27th (3 of 6 sampling sites) and September 18th (4 of 6 sampling sites), and high during the sampling dates of July 10th (4 of 6 sampling sites) and August 2nd (5 of 6 sampling sites).

Low *Microcystis* spp. density was measured at site 7M (14 km) (3 of 6 sampling events), site MB20 (2 km) (3 of 6 sampling events), site 4P (30 km) (2 of 6 sampling events), site MB18 (7 km) (2 of 6 sampling events) and site GR1 (26 km) (1 of 6 sampling events). Site 8M (13 km) is the only location where low *Microcystis* spp. density was not measured during any sampling event, and high *Microcystis* spp. density was found during 4 of 6 sampling events at this site. In addition, site MB18 (7 km) is another location where high *Microcystis* spp. density occurred generally (3 of 6 sampling events).

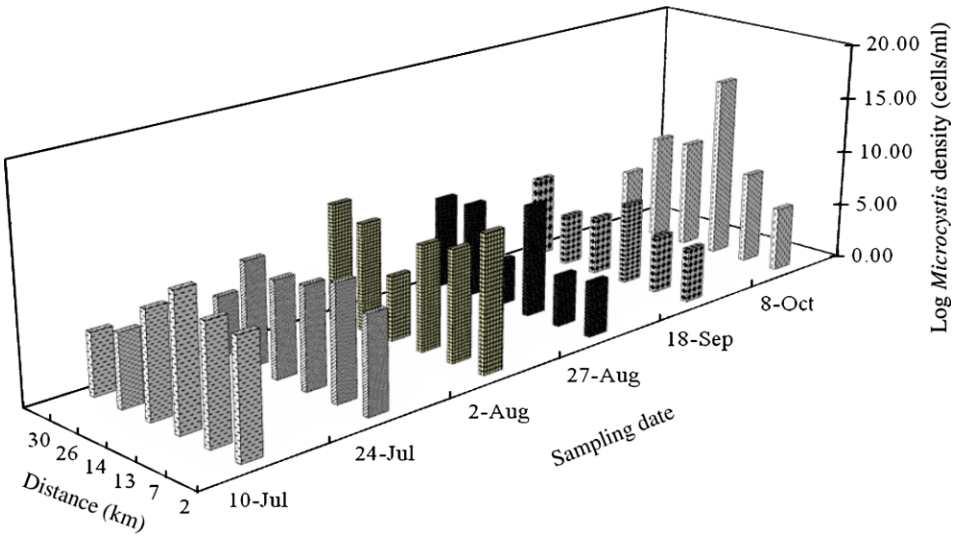

Figure 2 | *Microcystis* density measured during summer 2007.

Microcystin-LR concentration

Microcystin-LR concentration in 20 of 36 samples was below the detection range (0.15–5 ppb) of the ELISA kit used in this study. The corresponding undetectable toxin concentrations were not shown in Figure 3. The toxin concentrations detected in 16 samples were wide ranging 0.5 to 3,000 μg/gdw (gram dry weight), while 13 of 16 toxin concentrations ranged from 10 to 100 μg/gdw. Figure 3 shows the variation of natural Log of microcystin-LR concentration with sampling dates and the distance to the mouth of Maumee River. The highest (2.86×10^3 μg/gdw) and the lowest (0.41 μg/gdw) microcystin-LR concentrations were found on October 8th at site 8M (13 km) and site MB20 (2 km), respectively. July 24th and October 8th are two dates when microcystin-LR was detected at most of the sampling sites. Microcystin-LR was measured at 5 of 6 sampling locations on July 24th, ranging from 40 to 70 μg/gdw. Microcystin-LR was detected at 4 of 6 sampling sites on October 8th, ranging widely from 0.5–3×10^3 μg/gdw. Microcystin-LR concentration generally was below the detection limit during the sampling date of July 10th (5 of 6 sampling sites), August 2nd (4 of 6 sampling sites), August 27th (4 of 6 sampling sites) and September 18th (4 of 6 sampling sites). No obvious relationship between microcystin-LR concentration and sampling date was observed.

There is no apparent trend regarding microcystin-LR concentration with sampling location. However, microcystin-LR was generally presented at site MB18 (7 km) (4 of 6 sampling events) and site 8M (13 km) (4 of 6 sampling events). Notably, these two sites are the locations where high *Microcystis* spp. density also occurred. Microcystin-LR was not detected at site MB20 (2 km) and at site 4P (30 km) in 4 of 6 sampling events, or at site GR1 (26 km) in 5 of 6 sampling events.

The relationship between *Microcystis* density and microcystin-LR concentration

Microcystin-LR concentration was below the detection limit in 20 of 36 samples, while the corresponding *Microcystis* spp. density ranged from 100 to 2×10^5 cells/ml. Among the remaining 16 samples, the highest microcystin-LR content (2.86×10^3 μg/gdw) corresponded to the highest *Microcystis* spp. density of 1.03×10^7 cells/ml, they were determined to be outliers using a QQ-plot. Because outliers might influence the result of analysis, outliers were not involved in the regression analysis to investigate the relationship between *Microcystis* spp. density of the remaining samples and their corresponding microcystin-LR concentration (Figure 4). As indicated in this figure, microcystin-LR concentration of 2 different samples was approximately 20 μg/gdw, while their corresponding *Microcystis* spp. densities varied significantly. The microcystin-LR content of 6 samples ranged 40–50 μg/gdw, while the corresponding *Microcystis* spp. density ranged 3×10^3 to 3×10^4 cells/ml. The regression equation showed that positive correlation existed in microcystin-LR concentration and *Microcystis* spp. density, but this relationship is not strong.

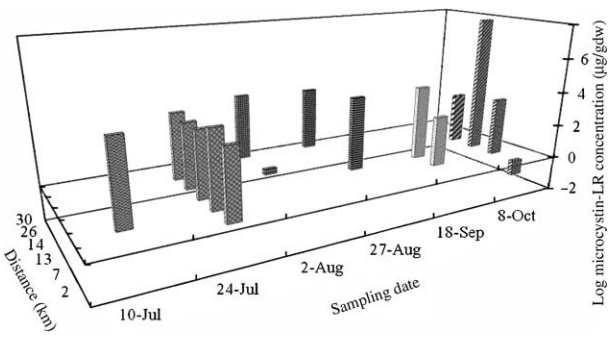

Figure 3 | Microcystin-LR concentration measured during summer 2007.

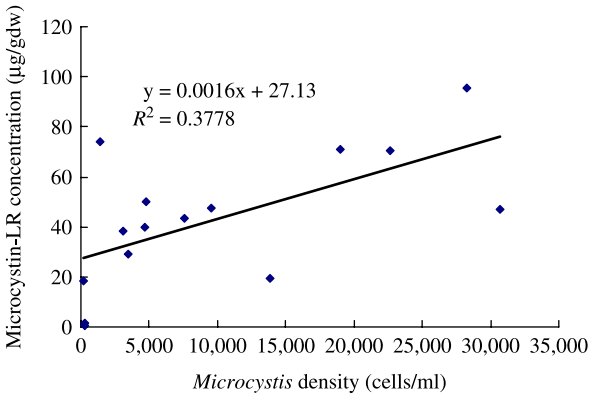

Figure 4 | *Microcystis* spp. density versus microcystin-LR concentration.

The relationship between *Microcystis* spp. density and environmental factors

Statistical analysis for *Microcystis* spp. density and environmental factors was processed by SAS (Statistical Analysis System) software. The environmental parameters having a *P* value less than 0.05 are listed in Table 3, other environmental factors with *P* value more than 0.05 was omitted. Smaller *P* value represents more significant correlation between *Microcystis* spp. density and environmental factors. Generally, when *P* value is less than 0.05, the correlation is considered significant. According to the results, temperature and NO_2^- (nitrite) were significantly correlative with the *Microcystis* spp. density. Positively correlation existed between temperature and *Microcystis* spp. density, and nitrite was negatively correlated with *Microcystis* spp. density.

The relationship between microcystin-LR concentration and environmental factors

Similar to *Microcystis* spp. density, SAS (Statistical Analysis System), and the multiple linear regression model was used for the statistical analysis of microcystin-LR content and environmental factors. The samples that did not contain microcystin-LR (or below detection limit) were excluded from this statistical analysis. The environmental factors having *P* value less than 0.05 are shown in Table 4. Temperature and DO were two important environmental factors affecting microcystin-LR concentration. Temperature was positively correlated with microcystin-LR concentration, while DO was negatively correlated with microcystin-LR concentration.

DISCUSSION

Microcystis spp. density ranged from 100 to 10^7 cells/ml during summer 2007. On the basis of real-time PCR analysis of the samples at 1 m depth in August 2003 and August 2004 in western Lake Erie, the abundance of *Microcystis* spp. varied from 20 to 4×10^6 cells/ml (Ouellette *et al.* 2006). During the summer period of 1995–1997 in western Lake Erie, *Microcystis* spp. density obtained through light microscope was reported to vary between 2×10^5 and 3×10^7 cells/ml (Ouellette *et al.* 2006). Thus, the results obtained in this study were comparable to previous results obtained in western Lake Erie.

Table 3 | Results of statistical analysis for *Microcystis* spp. density and environmental factors

Environmental factor	Coefficient	*p* value	Intercept
Temperature	0.575	0.006	0.133
NO_2^-	−35.789	0.0003	

Table 4 | Results of statistical analysis for microcystin-LR content and environmental factors

Environmental factor	Coefficient	*p* value	Intercept
Temperature	0.969	0.0066	0.649
DO	−1.464	0.0013	

Microcystis density

The highest density of *Microcystis* spp. of 10^7 cells/ml during summer 2007 was observed at site 8M (13 km) on October 8th. It was a special case that the highest *Microcystis* spp. density was measured in October. Some possibilities may explain this. The growth physiology and growth rates of bloom-forming cyanobacterial genera are optimal around 22°C or higher (Robarts & Zohary 1987). Wicks & Thiel (1990) reported a positive correlation of temperature with *Microcystis* levels. Our results agree with their results. The highest *Microcystis* spp. density was preceded by a heavy rainfall event lasting more than one week, reinforcing the importance of nutrient availability for *Microcystis* spp. growth. Total phosphorus (TP) (0.073 mg/l) and NO_3^- (1.73 mg/l) were high on September 18th. There existed a time lag between the *Microcystis* spp. Growth and nutrient concentration deposition.

High *Microcystis* spp. density occurred at site 8M (13 km) most frequently (4 of 6 sampling events) as compared to the other 5 sampling locations, which suggested that *Microcystis* spp. had the more competitive growth condition at this site than other locations. Secchi depth was 100–150 cm in this site and deep enough (around 5.5 m) to limit the light penetration to the substrate, so the light was available to the upper position of lake water. At this site, *Microcysti* spp. possesses a competitive advantage over other algae, because it can regulate its

buoyancy to remain in the euphotic zone, whereas most of other algae cannot (Humphries & Lyne 1988).

The second largest *Microcystis* spp. density of 1.66×10^5 cells/ml was measured at site MB20 (2 km) on August 2nd. This site is close to the mouth of Maumee River, and influenced by Maumee River discharge. Maumee River is highly nutrient enriched from agriculture runoff, so at this site high nutrient concentration promote the abundance of *Microcystis* spp. Temperature also has been considered as important environmental factors that influence *Microcystis* spp. Dominance (Robarts & Zohary 1987). The highest temperature of 30°C in all sampling events occurred on this date, which provided the growth advantage for the growth of *Microcystis* spp.

At site MB18 (7 km) and MB20 (2 km), which are near to the mouth of Maumee River, the water had high turbidity. Secchi depth at site MB18 (7 km) was generally less than 100 cm and was around 40 cm at site MB20 (2 km), resulting in a narrow euphotic zone. However, the shallow depth (1–2 m) allows the light to penetrate to the bottom of the lake. High *Microcystis* spp. densities generally are not expected to be measured at these two sites. However, high *Microcystis* spp. density occurred at site MB18 (7 km) during 3 of 6 sampling events and at site MB20 (2 km) during 2 of 6 sampling events. The high *Microcystis* spp. densities at these two sites during these sampling events are likely attributed to the high temperature (higher than 25°C) and high nutrient concentration. In addition, the strong wind speed and wind direction could have caused this high *Microcystis* spp. density at these sites located downwind. For example, the weather was not calm on July 24th, the wind direction of north east with the wind speed of 5.7 m/s was measured at site MB18 (7 km), and this site was downwind, which resulted in the accumulation of floating *Microcystis* spp.

September 18th is the date when low *Microcystis* spp. density occurred at 4 of 6 sampling sites. In this case, temperature and DO may have affected *Microcystis* spp. density since the lowest temperature (around 20°) of all the sampling events occurred on that date. The DO ranged from 6.5 to 7.5 mg/l on that date (September 18th), which was lower than the more than 8 mg/l typically measured at all six sampling sites (5 of 6 sampling events). DO has previously been shown to be correlated with *Microcystis* spp. growth (Wicks *et al.* 1990).

At the location farthest from the mouth of Maumee River (site 4P (30 km)), waters were generally clearer than other locations, secchi depth ranged from 180 cm – 220 cm in 4 of 6 events. Under these conditions, there was no competitive growth advantage for *Microcystis* spp., explaining our findings of no high *Microcystis* spp. densities at this site during six sampling events.

Strong winds will lead to the mixing of lake water. Prior studies suggested that when the wind speed is more than 4 m/s, the mixing of lake water occurs (Ha *et al.* 2000). Since the samples for this study were collected from the surface of lake at all sampling sites, the data collected were not reflective of the *Microcystis* density throughout the water column.

Microcystin-LR concentration

Microcystin-LR is the most common microcystin in western Lake Erie. In our study, only the concentration of microcystin-LR was measured. In the 16 samples where it was detected, microcystin-LR ranged from 0.5–3,000 μg/gdw. According to Figure 4, no obvious correlation was found between the density of *Microcystis* and toxin concentration. Therefore, the toxin content probably does not relate to *Microcystis* density. And, this result is consistent with prior studies which have shown that the abundance of *Microcystis* did not correspond to a high toxicity (Lehman *et al.* 2008).

The maximum toxin concentration was found at the same sampling location (site 8M (13 km)) and on the same date (October 8th) as the highest *Microcystis* density. The highest toxin concentration was 30 fold higher than the second highest toxin concentration. Laboratory studies with toxic *Microcysti* spp. strains have shown the influence of environmental conditions such as light and pH on growth of toxin production (Ohkubo *et al.* 1991). Light availability and high pH (8.42) on October 8th at site 8M (13 km) promoted the production of microcystin. High nutrient concentration may result in high toxin level as well. Although nutrient concentration at site 8M (13 km) is not highest in all sampling locations, nutrient concentration here may be high enough for the production of microcystin. Additionally, the production of microcystin has also been found to be coupled to the cell cycle of *Microcystis* spp.,

with a maximal production at the late logarithmic and early stationary phases where dissolved oxygen (DO) levels can also be low (Ohtake *et al.* 1989; Wicks *et al.* 1990). This sample may have coincided with the *Microcystis* spp. growth phase characterized by the highest growth rate and cell density (Paerl *et al.* 2001). Orr & Jones (1998) also investigated toxin production by *Microcystis* spp., finding cell division rates were correlated with toxin production.

The production of microcystin by *Microcystis* spp. is under complex genetic and ecological control (Dyble 2006). Dyble (2006) has reported that populations of *Microcystis* in western Lake Erie were composed both toxic and nontoxic strains. Multiple *Microcystis* colonies were isolated from western Lake Erie, only 25% contained *mcy* B genes, which can be correlated to the number of toxic *Microcystis* spp. cells present. So, the genetic structure of the population might be another factor affecting the toxicity of *Microcystis* spp.

CONCLUSION

Our data suggests that site 8M, located an intermediate distance from the mouth of Maumee River, provided an advantage for *Microcystis* spp. growth, presumably due to water depth combined with impact from the Maumee River run-off. Our results generally agreed with the previous studies that the abundance of *Microcystis* spp. was influenced by environmental factors, such as nutrient concentration, temperature, wind speed and wind direction. Statistical analysis confirmed that higher temperature and lower nitrite concentration ($p < 0.05$) were associated with the *Microcystis* spp. abundance. However, the limitations of the data set reduced the reliability of statistical results.

No obvious relationship was found between the density of *Microcystis* spp. (cells/ml) and toxin concentration (μg/gdw). The toxicity of samples from *Microcystis* spp. blooms varied with regard to sampling location and date of collection. The variation in the concentration of microcystin-LR observed in this study is likely due to environmental factors (e.g. temperature, nutrient concentration), to the variant of microcystin produced, and to the physiological condition of the cells. The statistical results from this study suggested that higher temperature and lower DO concentration resulted in high microcystin-LR concentration ($p < 0.05$). As with the *Microcystis* data, the small data set available impacts the reliability of statistical result.

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REFERENCES

Ame, M. V., Pilar Diaz, M. & Wunderlin, D. A. 2003 Occurrence of toxic cyanobacterial blooms in San Rogue Reservoir: a field and chemometric study. *Environ. Toxicol.* **18**, 192–201.

Brittain, S. M., Wang, J., Babcock-Jackson, C., Armichael, W. W., Rinehart, K. L. & Culver, D. A. 2000 Isolation and characterization of microcystins, cyclic heptapeptide hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. *J. Great Lake Res.* **26**, 241–249.

Dolan, D. M. 1993 Point source loadings of phosphorus to Lake Erie: 1986–1990. *J. Great Lakes Res.* **19**, 212–223.

Dyble, J. 2006 Genetic and environmental factors influencing *Microcystis* bloom toxicity. http://www.glerl.noaa.gov/res/Task_rpts/2006/aisdyble06-1.html (accessed 20 June 2008).

Ha, K., Kim, H. W., Jeong, K. S. & Joo, G. J. 2000 Vertical distribution of *Microcystis* population in the regulated Nakdong River, Korea. *Limonology* **1**, 225–230.

Hesse, K. & Kohl, J. G. 2001 Effects of light and nutrient supply on growth and microcystin concent of different stains of *Microcystis* aeruginosa. In: Chorus, I. (ed.) *Cyanotoxins*. Springer, Berlin, pp. 104–115.

Hudder, A., Song, W., Shea, K. E. & Walsh, J. P. 2007 Toxicogenomic evaluation of microcystin-LR treated with ultrasonic irradiation. *Toxicol. Appl. Pharmacol.* **220**, 357–364.

Humpage, R. A. & Falconer, R. I. 1999 Microcystin-LR and liver tumor promotion: effects on cytokinesis, ploidy, and apoptosis in cultured hepatocytes. *Environ. Toxicol.* **14**, 61–75.

Humphries, S. E. & Lyne, V. D. 1988 Cyanophyte blooms: the role of cell buoyancy. *Limnol. Oceanogr.* **33**, 79–91.

Johnston, R. B. & Jacoby, M. J. 2003 Cyanobaterial toxicity and migration in a mestrophic lake in western Washington, USA. *Hydrobiologia* **495**, 79–91.

Kotak, B., Serediak, M., Prepas, E., Hrudey, S., Semalulu, S. & Coppock, R. 1994 Cyanobacterial liver toxins in the aquatic

environment: implications for fish health Lake. *Reserv. Manage.* **9**, 90.

Lehman, P. W., Boyer, G., Satchwell, M. & Waller, S. 2008 The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in San Francisco Estuary. *Hydrobiologia* **600**, 187–204.

Nicholls, K. H., Standen, D. W., Hopkins, G. J. & Carney, E. C. 1977 Declines in the nearshore phytoplankton of Lake Erie's western basin since 1971. *J. Great Lakes Res.* **3**, 72–78.

Ohkubo, N., Yagi, O. & Okada, M. 1991 Effect of temperature and illumination on the growth of blue-green alga *Microcystis* viridis. *Limnology* **52**, 255–261.

Ohtake, A., Shirai, M., Aida, T., Mori, N., Harada, K.-I., Matsuura, K. & Nakano, M. 1989 Toxicity of *Microcystis* species isolated from natural blooms and purification of the toxin. *Appl. Environ. Microbiol.* **55**, 3202–3207.

Orr, P. T. & Jones, G. J. 1998 Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis* aeruginosa cultures. *Limnology* **43**, 1604–1614.

Ouellette, J. A. A., Handy, S. M. & Wilhelm, S. W. 2006 Toxic Microcystis is widespread in Lake Erie: PCR detection of toxin genes and molecular characterization of associated cyanobacterial communities. *Microb. Ecol.* **51**, 154–165.

Paerl, H. W., Fulton, R. S., Moisander, P. H. & Dyble, J. 2001 Harmful freshwater algal blooms with an emphasis on cyanobacteria. *Sci. World* **1**, 76–113.

Robarts, R. D. & Zohary, T. 1987 Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *Mar. Freshw. Res.* **21**, 391–399.

Shapiro, J. 1990 Current beliefs regarding dominance of blue-greens: the case for the importance of CO_2 and pH. *Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie* **24**, 38–54.

Vezie, C., Rapala, J., Vaitomaa, J., Seitsonen, J. & Sivonen, K. 2002 Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis* strains and intracellular microcystin concentrations. *Microb. Ecol.* **43**, 443–454.

World Health Organization 1998 Guidelines for drinking water quality 2nd edition. Addendum to Vol. 2 Health Criteria and other supporting information. Geneva, Switzerland: World Health Organization, pp. 95–110.

Wicks, R. J. & Thiel, P. G. 1990 Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis* aeruginosa in a hypertropic African reservoir. *Environ. Sci. Technol.* **24**, 1413–1418.

Yu, S. Z. 1995 Primary prevention of hepatocellular carcinoma. *J. Gastroenterol. Hepatol.* **10**, 74–82.

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