Toxic Algae Blooms in Minnesota
Brief Overview for LCCMR,
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Outline

- Introduction to toxic algae blooms AKA “Harmful Algal Blooms (HAB)” in Minnesota
- Blue-green algae – Why so abundant?
- MN history with HAB & toxins
- Case examples: HAB & dog deaths
- Some findings from MN studies
- Summary –
    a) What we know
    b) What we don’t know (possible research)

Blue-greens – one of several forms of algae found in MN lakes and rivers.

Why are they so common & what promotes their growth?

Preferences

- **<u>Nutrient-rich, high phosphorus</u>**
- **Prosper in very warm water >75-80 F**
- **Calm sunny conditions**
- **All of above commonly found in shallow lakes**

Attributes

- **If nitrogen in short supply - get N from atmosphere**
- **Buoyant**
- **Resistant to grazing by zooplankton**

Blue-green blooms: many different forms & colors

(Little Rock, Benton Co.)

Blue-green algal toxins

Three classes of toxins (all highly toxic)
- **Hepatotoxins (liver)**
- **Neurotoxins (nervous system)**
- **Dermatoxins (skin)**

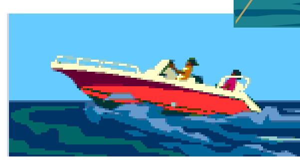
Hepatotoxins	Neurotoxins	Dermatoxins
Microcystin (MC)	Anatoxin-a	Lyngbyatoxin
Cylindrospermopsin	Anatoxin-a(s)	
Nodularin	Saxitoxin	

- **MC most commonly measured & studied**

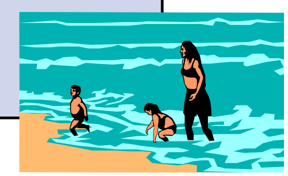
- **Common blue-greens that can produce one or more of these toxins: Anabaena, Aphanizomenon, and Microcystis**

Ingestion	Inhalation	Skin Contact
Swimming	Water skiing	Swimming
Water skiing	Tubing	Wading
Tubing	Boating	Water skiing
Drinking	Irrigation	Tubing
Fish (?)		









- **Recreational water exposures most common in Minnesota;**
- **However, we do not have a human health recreational standard;**
- **MDH has developed a drinking water guidance value for MC for source water;**

MN History With HAB

- **Late 1800's - First accounts of algal toxicity in MN;**
- **1990's - Increasing concern and reports world wide;**
- **2004 -Three dog deaths: Fish (Mora) and Benton (Lincoln) Lakes prompted more attention on this issue in MN.**
- **2005 -MPCA joined MDNR, MDH and the Minnesota Veterinary Medicine Association (MVMA) to form the Minnesota Blue- green Algal Toxicity Workgroup**
- **2006 MPCA conducted systematic study of MC in 12 eutrophic lakes in Blue Earth & McLeod**
- **2007 - Five suspected dog deaths**

  - National lake study: measured MC in 50 random lakes

  - & 35 southern MN lakes
- **2009 – Published research findings on work-to date**
- **2012 – National study - 150 random lakes;**
- **2014 – Published article on all MN MC data; 3 reported dog deaths**
- **2015 – <u>3 reported dog deaths thus far, 1 human health incident</u>**

Collaboration & Public Outreach

Collaboration with MDH, MDNR, MVMA, & U of M Vet Hospital

Outreach included press releases in papers, radio and TV

Updated web site for MDH & MPCA

MPCA – technical studies, publish findings, & lead public outreach

MDH – lead in contacting veterinarians & developed posters for beach managers to use;

Goal: enhance awareness & coordinate public outreach

Case study #1 Lake Benton, Lincoln County: September 2004

1. **Sept. 24-25th - Two dog deaths over weekend;**
2. **Sept. 27th - Marshall MPCA notified about dog deaths in Lake Benton & investigated Sept. 28th;**
3. **Water cleared but Microcystin at 100 ppb; Saxitoxin ~0.2 ppb**

Case Study #2. Little Rock Lake, Benton County: July 2007

- **Response to numerous WQ & odor complaints;**
- **July 25, 2007 sampling & observations by MPCA;**
- **Strong wind from S piled algae N & W shores.**
- **Sampled multiple sites**
- **Benton County closed beach for ~ 4 weeks;**

July 2007 samples –

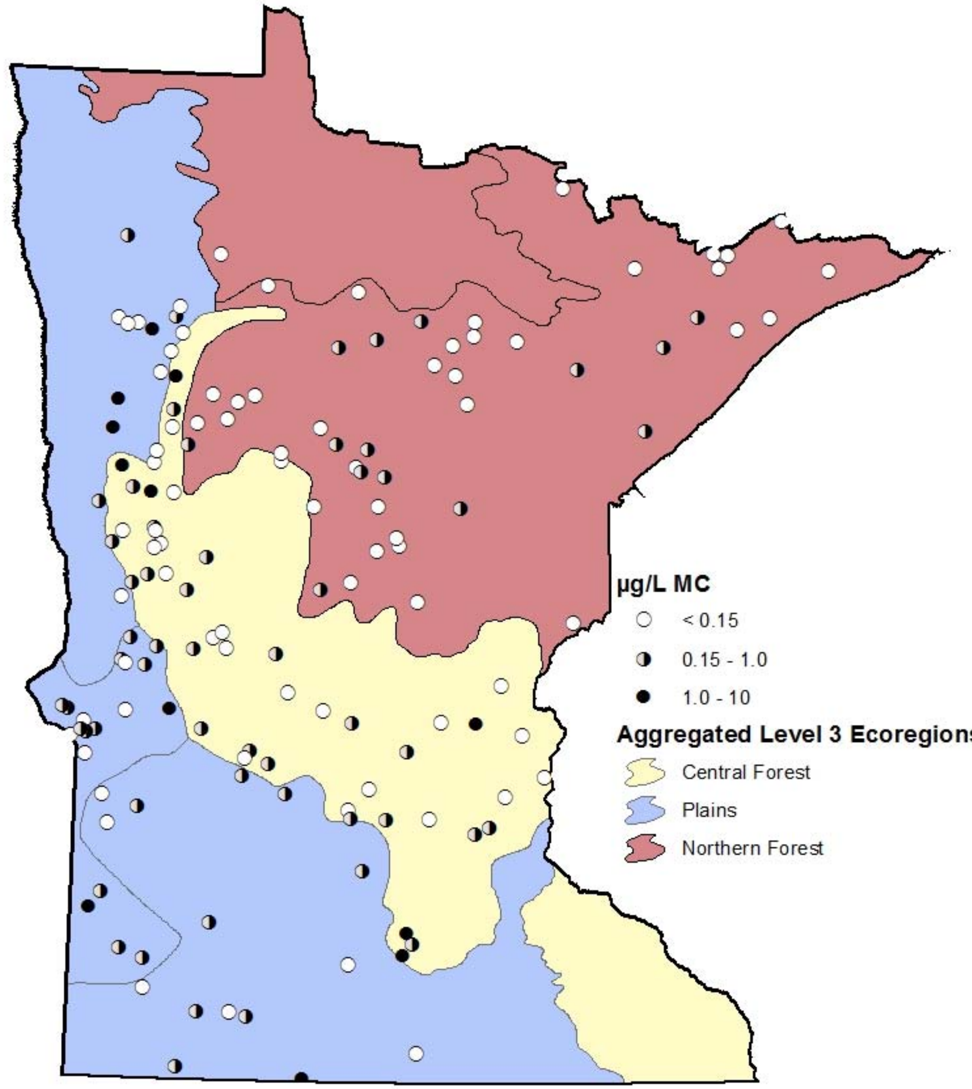
Chl-a: 120-130 ppb
Microcystin 20 to >80,000 ppb
Saxitoxin 0.03-0.04 ppt

Microcystin subject of several studies

2012 National Lakes Assessment

- **Part of a national randomized lake study**
- **50-lakes per ecoregion:**

	MC detect	Max.MC
• **North**	**29%**	**0.6 ppb**
• **Central**	**54%**	**2.2 ppb**
• **South**	**60%**	**8.2 ppb**



Findings from MN studies.

- Blue-green blooms not limited to late summer;
- High microcystin may be found at any time during open water season;
- Dog and animal deaths in MN have occurred as early as June and as late as September to October;
- The likelihood of encountering high MC increases as the intensity/magnitude of algal bloom increases;
- Samples at drinking water intakes indicate low MC & no measurable MC in finished water;

Summary: *What we know*

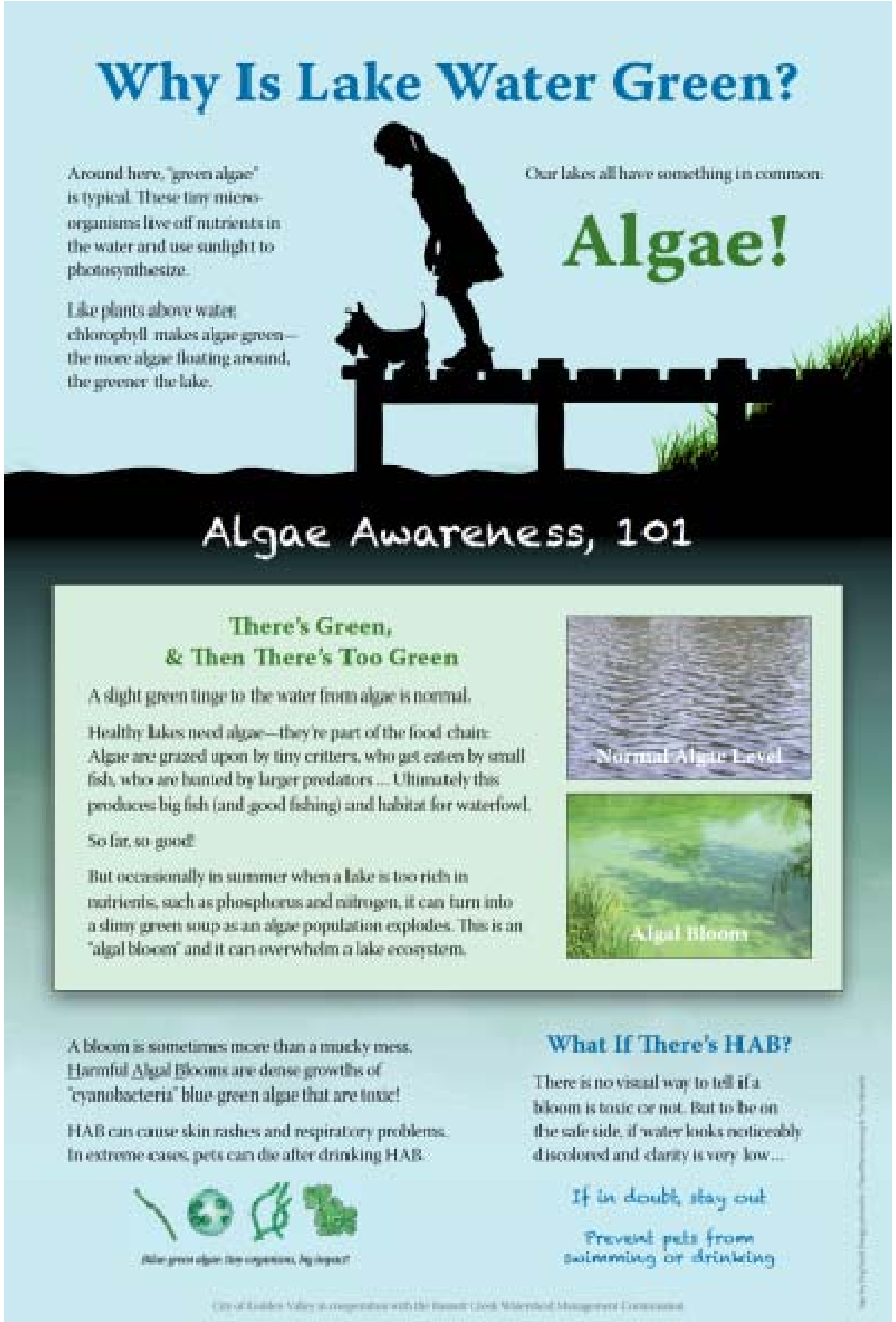
- **Blue-greens: one of several forms of algae in MN lakes;**
- **Blue-greens present in all MN lakes;**
- **Severe blooms most frequently in lakes with high nutrients, warm water, low wind and abundant sunlight;**
- **Several blue-green forms produce toxins, which can cause death for animals that consume water with toxins;**
- **People coming in contact with water can get gastrointestinal illness, skin rashes from contact & respiratory problems when inhaling fine particles;**
- **Most commonly measured toxin *microcystin*, is commonly found when lakes with blooms are sampled**
- **MPCA collaborates with MDH, MVMA & MDNR to address this issue and develop public awareness;**

HAB Challenges

- **Dog illness - Non-specific symptoms, lack of diagnostic tests & very rapid decline in health;**
- **Changing environmental conditions between exposure, reporting, and site investigation (blooms one day, gone the next)**
- **Limited ability to test for toxins (e.g. anatoxin) in waterbodies (can't be testing everywhere, conditions change rapidly)**
- **Some recent dog deaths 2014 & 2015 have occurred in lakes & rivers without severe (obvious) blooms;**
- **Ensuring adequate and balanced public outreach**

Research needs or what we need to know

- **Improve ability to predict when & where blue-green blooms will occur; [“early warning”]**
- **What conditions favor the production of toxins?**
- **Can these factors be controlled or moderated?**
- **Do toxins persists after bloom subsides? When is the water safe?**
- **Viable short-term remedies for reducing blue-green blooms? If so, impact on toxin production/release?**
- **What are safe levels of microcystin for contact recreation? [*MPCA & MDH underway*]**
- **What are safe levels for source water for drinking & MC concentrations at intakes [*MDH work underway*]**
- **Health-related issues with consuming fish from lakes with HAB [*MPCA & MDH underway*]**



Summer 2014 presentation to Bassett Creek Watershed

City of Golden Valley & Bassett Creek developed this poster for use in District lakes.

Summary

1. **Interest in HAB remains high in MN & across US;**

2. **Collaboration with MDH, DNR, veterinarians aided public outreach & spurred useful discussions.**

2005 Work group poster

Any questions?

Microcystin in Minnesota Lakes

Steven Heiskary and Matt Lindon

Where Do We Find It and What Does It Relate To?

Blue-green algae, more appropriately referred to as cyanobacteria, are a common component of the algal community in lakes and rivers in Minnesota and elsewhere in the world. It has been long known that certain forms of blue-greens have the ability to produce toxins and these toxins have been implicated in animal deaths and human-health related problems. These toxins, which include anatoxin, saxitoxin, microcystin (MC), and a more recently described toxin, cylindrospermopsin, vary in their toxicity. Of these, MC is the most commonly measured in many studies.

While there has long been concern regarding blue-greens and the production of toxins (Carmichael 1977), recent literature suggests there are numerous efforts in countries such as, Australia (Brookes and Bruch 2004), Germany (Chorus et al. 2001), and the U.S. (Graham et al. 2004) intended to improve understanding of this issue, the factors that lead to toxicity and the ability to manage the blooms that cause the toxicity. In this article we will share findings from several Minnesota studies, describe the range of MC in Minnesota lakes and some of the characteristics of these lakes that may be associated with elevated MC.

Blue-green algal toxicity is not a new issue in Minnesota. Several articles dating back to the late 1800s document several incidences of blue-green algal blooms that have led to cattle, horse, and dog deaths (Lindon and Heiskary 2009). Studies conducted in response to these incidents associated toxicity with the blue-green genera: *Anabaena*, *Aphanizomenon*, *Coelosphaerium*, *Lyngbya*, and *Microcystis*.

In recent years (2004 to 2008) several dog deaths, potential human health impacts, and reports of very severe nuisance blooms prompted renewed interest in blue-green algal toxicity in Minnesota. In 2005, the Minnesota Pollution Control Agency (MPCA) joined with the Department of Natural Resources (MDNR), Department of Health (MDH), and the Minnesota Veterinary Medicine Association (MVMA) to form the Minnesota Blue-green Algal Toxicity Workgroup, for the express purpose of increasing awareness of blue-green algal toxicity within agencies, the veterinarian community, and the public. Fact sheets, posters, and news releases were developed as a result of workgroup efforts. The workgroup also determined that Minnesota had minimal information on the magnitude and frequency of occurrence of MC in Minnesota waters.

Several studies were undertaken to assess the extent and magnitude of MC in Minnesota lakes and to improve our understanding of factors associated with elevated MC (Figure 1). The three efforts specifically addressed in this article include:

- A targeted study of 12 eutrophic lakes in south central Minnesota in 2006 (Figure 1) allowed us to examine spatial and temporal variation in MC and the various limnological and physical factors that may contribute to observed variation in MC.
- The 2007 National Lakes Assessment Project (NLAP) study provided an opportunity to assess MC in 50 randomly selected lakes as a part of this nation-wide statistically-based project

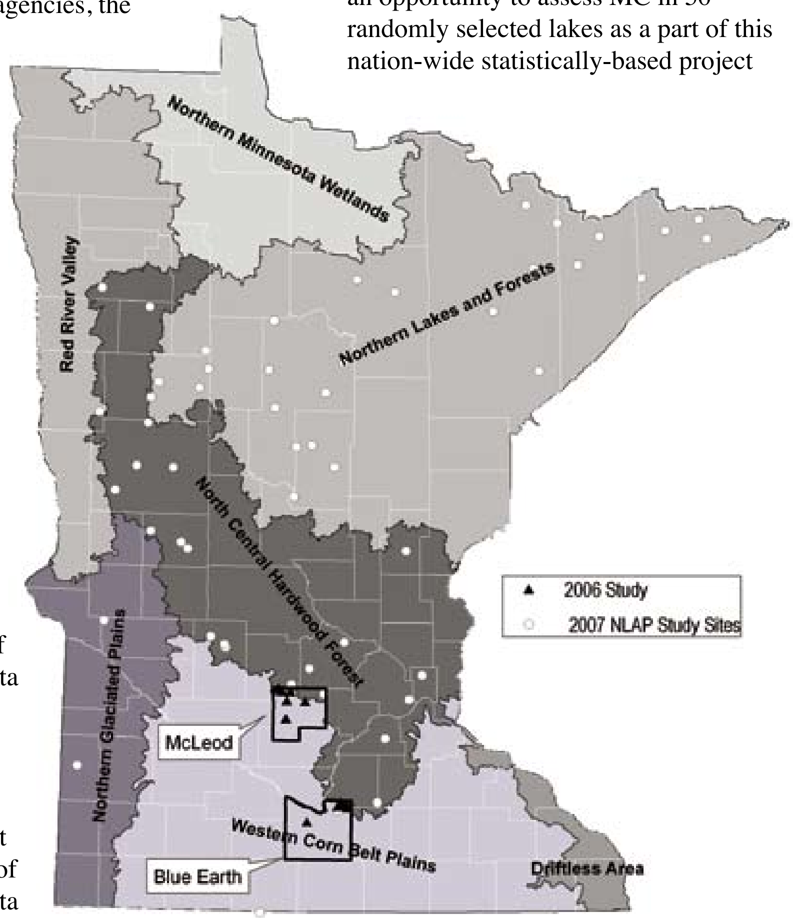


Figure 1. Study lake locations and ecoregion map; 2006 study and 2007 National Lake Assessment Project (NLAP) sites noted.

(Figure 1). Details on NLAP may be found at: http://www.pca.state.mn.us/water/nlap.html.

- Incident-based monitoring, in 2004 to 2007, in response to citizen concerns regarding severe nuisance blooms, fish kills, dog deaths, and/or potential human-health problems provided additional insight on the magnitude and frequency of MC. As a part of "incident" investigations MPCA typically collected MC and supporting water quality data (Figure 2).

Background

Sample Collection and Study Descriptions

In the 2006 study, mid-lake sites were selected based on pre-existing sampling sites, typically located near the site of maximum depth. Near-shore sites were located near a downwind shoreline area that allowed for accumulation of algae and often resulted in a distinct algal scum on the surface of the water. Mid-lake sites were constant among monthly sample events, whereas near-shore sites varied dependant on the wind direction, intensity, and presence of an algal bloom. In the 2007 NLAP study, MC samples were collected on one occasion in July or August from a mid-lake (index) site and from a random near-shore site on each lake. As per NLAP protocol, random sites were established in the office prior to going into the field so there was no subjectivity in their selection.

MC Sample Analysis and WHO Guidelines

MDH analyzed MC using a bench-top Abraxis ELISA method, with a method detection limit (MDL) of 0.15 μg/L total MC (for purposes of statistical analysis a non-detect substitution of 0.075 μg/L was used). MC samples underwent a triple freezing cell lysis procedure. World Health Organization (WHO) risk guideline categories, established for recreational waters and drinking water, provide a basis for placing the MC data in perspective and describing relative risk. The guidelines are detailed in WHO (2003). The categories used are: <1 μg/L very low risk, 1- 10 μg/L low risk, 10 - 20 μg/L moderate risk, 20 – 2000 μg/L high risk, and > 2,000 μg/L very high risk. The four categories from 1 to >2,000 μg/L were drawn directly from the WHO guidelines. The very low risk category was added to include measurements that were very near the MDL for MC and below the 1 μg/L drinking water guideline for MC LR, one of the most common and toxic MC compounds.

Figure 2. A fish kill that resulted from a severe blue-green algal bloom in Lake Benton, Lincoln County, Minnesota in September 2004. Two dogs that came in contact with the water died as well during this event.

Results

2006 Study: MC Patterns and Relationships

MC exhibited no consistent seasonal pattern at either the mid-lake or nears-shore sites. Silver and Hook Lakes exhibited the highest pelagic concentrations and had the only concentrations that fell in the moderate risk level (Figure 3a). In contrast, 7 of 12 lakes were below the low risk threshold concentration (10 μg/L) for the entire summer at the mid-lake site. High to very high risk concentrations were noted at near-shore sites on three lakes: Madison, Hook, and George (Figure 3b). Some of the highest concentrations were from samples collected in May and June, which was not anticipated.

MC was highly variable within and among lakes (Figures 3a and b). About 30 percent of MC results were less than one μg/L. One μg/L and greater concentrations were unevenly distributed up to 8,400 μg/L. Elevated MC occurred more often at near-shore sites with scums as compared to those without surface scums (Figure 4). Though it was common for mid-lake sites to have distinct green coloration and high Chlorophyll-*a*, surface scums were limited to the near-shore sites (Figure 5). Even at the near-shore sites, distinct surface scums were not that common.

MC and Other Environmental Factors

Spearman's rank correlation (R_s) analysis indicated MC was positively related to pH, percent of MC-producing blue-greens (e.g. *Microcystis* and *Anabaena*), and Chlorophyll-*a*. Inverse relationships were found with alkalinity, Secchi depth and specific conductance (Lindon and Heiskary 2009). Parameters highly correlated with MC were typically related to algal biomass (Chlorophyll-*a*) or algal productivity.

The relationship between MC and these parameters was generally not linear; rather the relationships were best described based on comparison of "classes" or "groups" of measurements through cross-tabulation (Lindon and Heiskary 2009). For example, combining MC and Chlorophyll-*a* classes, which approximate varying degrees of "bloom intensity," provides a basis for describing the "risk" of encountering specified levels of MC as a function of bloom intensity (details on conducting cross-tabulation in Lindon and Heiskary 2009). Based on Figure 3, moderate risk MC was not encountered until blooms exceeded

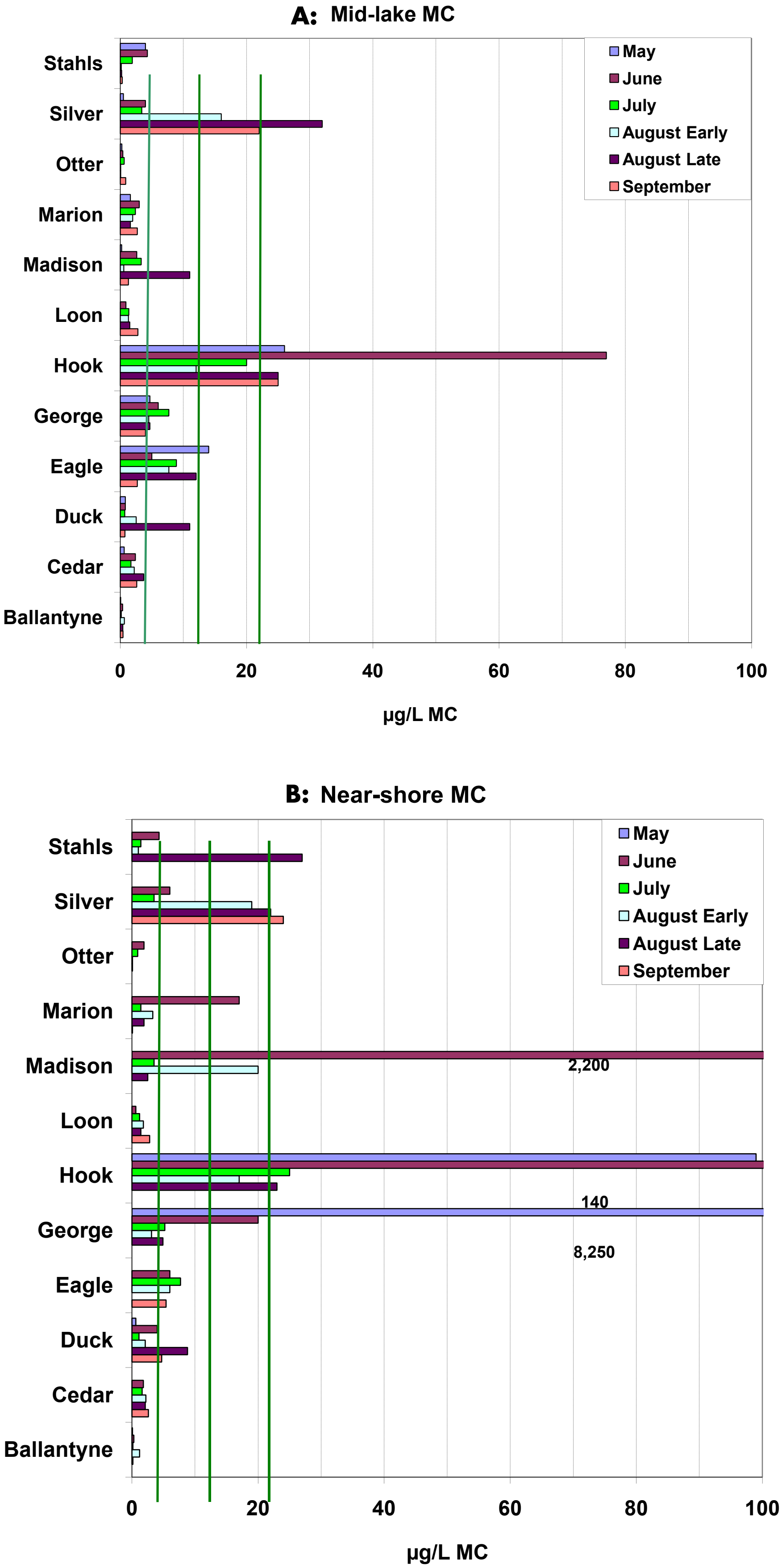


Figure 3. Microcystin (MC) concentrations by lake and date for 2006 study. WHO (2003) risk guidelines noted for very low risk (<1.0 µg/L), low risk (1-10 µg/L), moderate risk (10-20 µg/L) and high risk (20-2000 µg/L) noted by heavy green lines. MC concentrations beyond scale noted on graph: (a) mid-lake sites and (b) near shore sites.

30 µg/L ("severe nuisance blooms"). As blooms exceeded 30-50 µg/L the frequency of moderate to high risk MC increased to ~30 percent. All high risk MC concentrations were associated with Chlorophyll-*a* > 30 µg/L.

Similar analyses were conducted for MC and Secchi and MC and pH (Figure 6). Similar to MC and bloom intensity there is somewhat of a threshold affect; as Secchi declines below 0.5 m there is an increased risk of MC concentrations in the moderate to high risk categories. Over 90 percent of the pH values >9.0 were associated with severe nuisance bloom levels (Chlorophyll-*a* >30 µg/L). With the exception of one sample, all lakes with moderate to high risk MC had a pH of 9.0 or greater (Lindon and Heiskary 2009).

MC Comparison Among Studies

A comparison of the 2006 study of 12 eutrophic lakes with the NLAP and incident-based samples provide further perspective on MC concentrations in Minnesota lakes (Figure 7). Based on this comparison it is evident that the distributions of these three data sets are significantly different, which comes as no surprise given the population of lakes sampled and focus of each study.

The 2006 eutrophic lakes exhibit a larger range and more extreme MC concentrations as compared with the NLAP data (Figure 7). Both data sets reveal higher MC concentrations at near-shore sites as compared to mid-lake index sites. About 85 percent of the 2006 mid-lake MC samples were considered very low to low risk (Figure 7). Likewise, a high percentage of the near-shore samples were in these categories as well. The only very high risk measures were found at the near-shore sites. The incident-based sampling yielded some extremely high event-based values (Figure 8), which again is not too surprising given the intensity (magnitude) of the blue-green blooms sampled and the subjective nature of site selection.

Discussion

The 2006 MC study, which focused on eutrophic to hypereutrophic lakes in south-central Minnesota, was designed to answer several questions to advance knowledge on the extent, magnitude, and frequency of MC in Minnesota lakes.

Figure 4. Typical near-shore green coloration but no scum.

Figure 5. Typical near-shore green coloration with scum formation.

Answers or observations relative to these questions help frame the following discussion.

The likelihood of encountering measurable MC at a mid-lake site in a eutrophic lake is quite high based on the 2006 study (Figure 3a). When combined with the near-shore samples 94 percent of the MC measurements were above the MDL. Near-shore samples exhibited a larger range, higher average, and much higher maximum concentration (8,400 μg/L) as compared to the maximum concentration of mid-lake samples (69 μg/L) (Figure 3b). In the NLAP study near-shore samples exhibited higher MC as compared to mid-lake samples as well (Figure 4).

WHO guidelines provided a basis for evaluating the relative risk of the MC in the 2006 study. Eighty percent of all MC concentrations were in the WHO low risk category for recreational waters (82 percent mid-lake and 72percent near-shore). The remainder of the mid-lake samples was in the moderate to high risk category (Figure 3a). Only two near-shore samples were in the very high risk category (Figure 3a).

Seasonal patterns in MC, similar to typical patterns observed for Chlorophyll-*a* and nuisance algal blooms were anticipated. However, based on the 2006 study, there was no distinct seasonality to MC concentrations. This was due, in part, to two lakes that exhibited very high MC in May and June at near-shore sites. The incident-based sampling and dog deaths (2004-2007) also indicate that elevated MC may occur at any time in the summer. In 2007, incident-based sampling was initiated in June in response to complaints of very severe algal blooms and a documented dog death and in 2004, two dog deaths occurred in late September and early October.

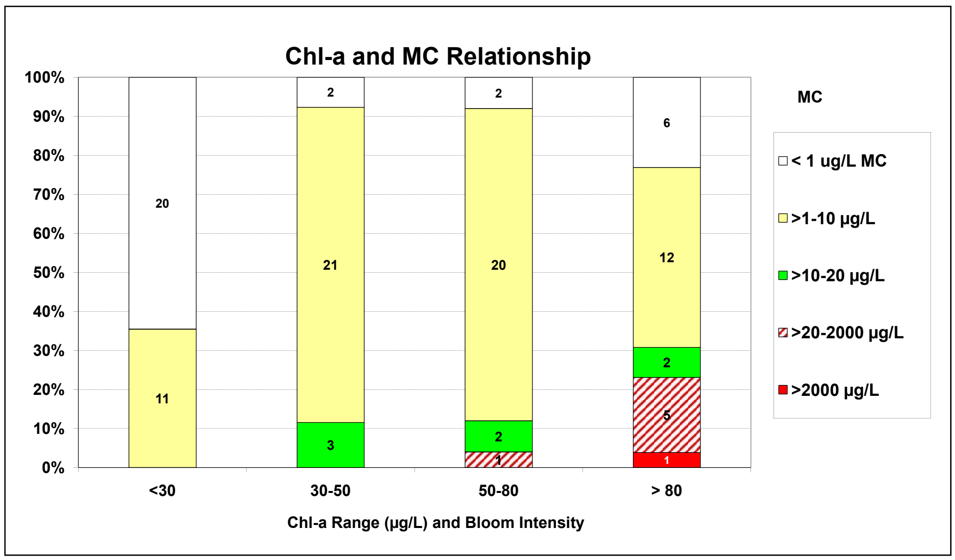


Figure 6. Cross tabulation of microcystin (MC) relative to chlorophyll-a classes. Comparison based on 108 pairs of data from 2006 study. Classes reflect different degrees of "bloom intensity."

Relatively distinct relationships were observed among MC and Chlorophyll-*a*, pH and Secchi in the 2006 study. When Chlorophyll-*a* remained <30 μg/L MC was in the very low to low risk categories. As Chlorophyll-*a* increased to >30-50 μg/L the risk of moderate to high MC increased to 30 percent. As for Secchi, high risk MC was found only when Secchi was 0.5 m or less. Because surface blooms of MCP blue-greens, such as *Anabaena* and *Microcystis,* routinely result in very low transparency this relationship makes intuitive sense (Figure 9). This is in contrast to the non-MCP *Aphanizomenon,* which forms large "rafts" that float at the surface that often allow for higher transparency (Figure 10).

Based on MC measurements and supporting data collected in the various studies, we have several observations to share on MC in Minnesota lakes.

1. MC is measurable across wide (trophic and spatial) range of Minnesota lakes. It is not limited to the highly eutrophic lakes of southern Minnesota but may be found in mildly eutrophic to even mesotrophic lakes in central and northern Minnesota as indicated in the NLAP study.

2. MC concentrations are often higher at near-shore sites as compared to mid-lake sites. Further, the likelihood of moderate to very high risk MC was found to be greater at sites with a distinct surface scum. These results are consistent with observations by Graham et al. (2004) who note that MC in scums may be much greater than at mid-lake locations.
3. Elevated MC may be found at anytime from late spring/early summer through late summer/early fall.
4. Last, these studies, as with most MC studies, do not allow for precise prediction as to which blue-green algal blooms will produce MC in the moderate to very high risk range. However, the collective results suggest that Minnesota's current recommendation to the public to avoid contact with very severe nuisance blooms is sound advice. Very severe nuisance blooms (Chlorophyll-*a* >30 µg/L) are readily recognizable by the public. It was also found that moderate to high risk MC was associated with high pH (≥9.0) and low Secchi (<0.5 m) – two parameters that are easy to measure. This suggests that a combination of visual examination of the water to assess bloom intensity (e.g., subjective assessments of physical appearance and recreational suitability), combined with measurement of all or some of the three variables (Chlorophyll-*a*, Secchi and pH) can allow resource managers, to estimate the potential risk of encountering moderate to high risk MC. Finally, three independent data sets indicate MC is measurable across a wide range of Minnesota lakes and ecoregions. Further, these data (Figure 7) provide a good basis for evaluating MC data from future monitoring

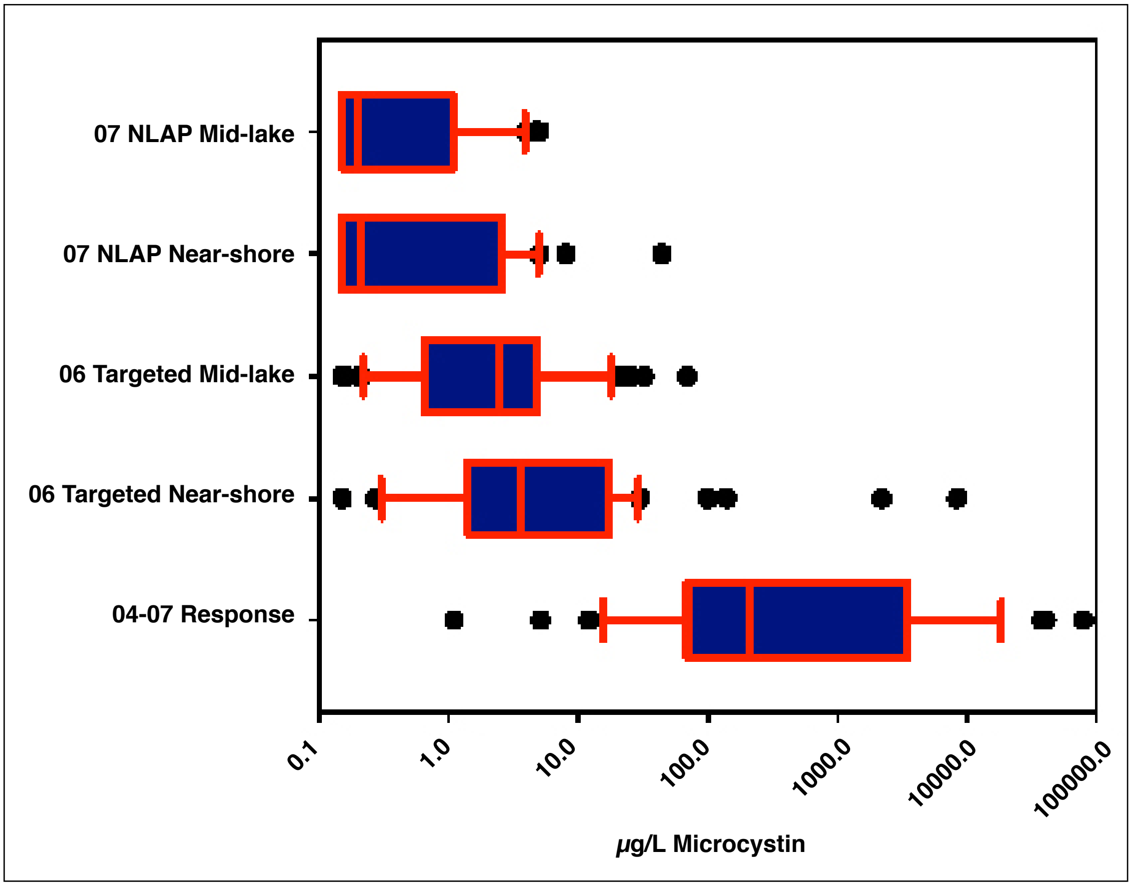


Figure 7. Comparison of MC box and whisker plots by study and site location for: 2006 study (mid-lake and near-shore), 2007 NLAP study (mid-lake and near-shore), and incident-based sampling.

Figure 8. Example of very severe nuisance blooms that occurred on Little Rock Lake in Benton County Minnesota in July 2007. Corresponding MC concentrations were in the "very high risk" range.

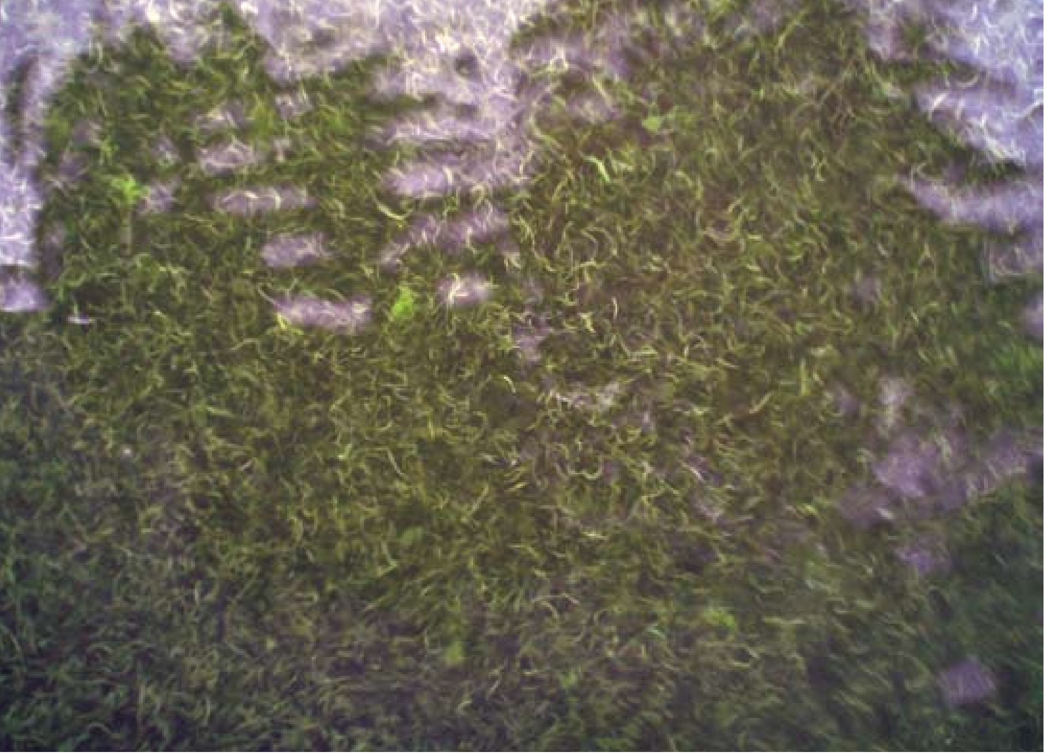


Figure 9. Example of an Aphanizomenon-dominated bloom.

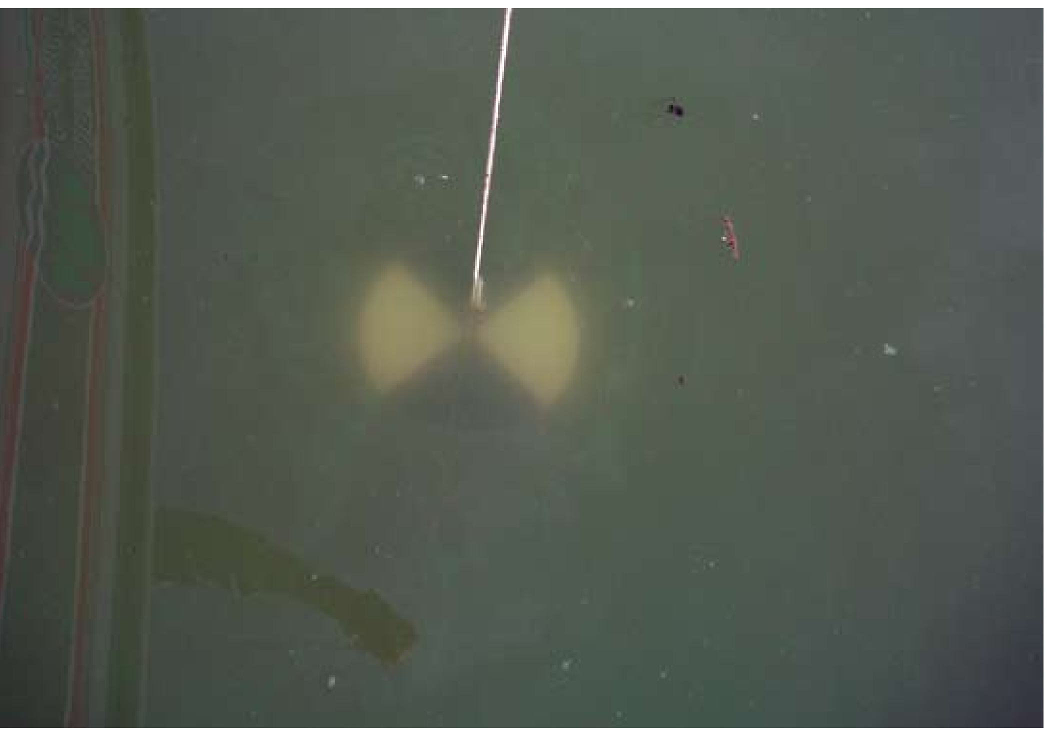


Figure 10. Example of a blue-green bloom not dominated by Aphanizomenon. Note the very low transparency.

efforts in Minnesota and may be provide a useful yardstick for studies conducted elsewhere.

References

Brookes, J. and M. Bruch. 2004. Toxic cyanobacteria management in Australian waters. *LakeLine* 24(4):29-32

Carmichael, W and P. Gorham. 1977. Factors influencing the toxicity and animal susceptibility of *Anabaena flos-aquae* (Cyanophyta) blooms. *J. Phycol*. 13:97-101

Chorus, I., V. Niesel, J. Fastner, C. Wiedner, B Nixdorf and K-E. Lindenschidt. 2001 Environmental factors and microcystin levels in water bodies. P 159-177. In: I. Chorus (Ed.) Cyanotoxin: occurrence, causes, consequences. Springer, Berlin.

Graham J. L., John R. Jones, Susan B. Jones, John A. Downing, and Thomas E. Clevenger. 2004. Envronmental factors influencing microcystin distribution and concentration in Midwestern United States. *Water Res*. 38:4395-4404.

Lindon. M. J. and Heiskary, S. A. 2009. Blue-green algal toxin (Microcystin) levels in Minnesota lakes. *Lake and Reserv. Manage*.

World Health Organization. 2003. Guidelines for safe recreational water environments. Coastal and fresh waters. Volume 1. Geneva, Switzerland

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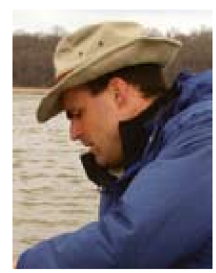

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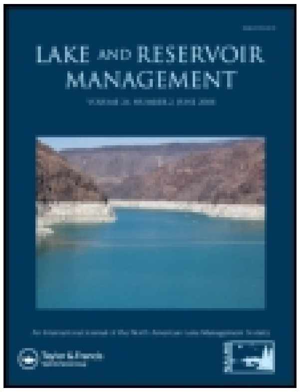

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Summary of microcystin concentrations in Minnesota lakes

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NOTE

Summary of microcystin concentrations in Minnesota lakes

Abstract

Minnesota has compiled a fairly extensive database on the cyanobacterial toxin microcystin (MC). These data have been acquired through targeted lake studies, incident-based investigations, and stratified random surveys as a part of the US EPA's 2007 and 2012 National Lakes Assessments. This paper updates previous efforts and provides a statistical summary of all Minnesota MC data collected by the Minnesota Pollution Control Agency from 2004 to 2012. These data provide a basis for (1) describing the influence sampling regimes have on reported MC values, (2) describing regional patterns in MC across 3 distinct Minnesota ecoregions, and (3) describing the relative risk of encountering elevated MC with respect to sampling regimes. This summary places MC concentrations in perspective for Minnesota and also provides a comparative database, which other entities that collect MC may use to place their results in perspective.

Key words: blue-green algae, cyanobacteria, harmful algal blooms, microcystin, Minnesota lakes, National Lakes Assessment

Since summer 2004, Minnesota has compiled a fairly extensive database on the cyanobacteria (commonly known as blue-green algae) toxin microcystin (MC). These data have been acquired through routine lake studies, incident-based investigations (Heiskary and Lindon 2009), and stratified random surveys as a part of the 2007 and 2012 US Environmental Protection Agency (USEPA) National Lakes Assessments (NLA). The studies (datasets) compiled through 2007 were described in Lindon and Heiskary (2009). These data were used to describe the range of MC in Minnesota lakes and the likelihood of encountering elevated levels of MC. Since that time, we have increased Minnesota's MC database via the 2012 NLA.

This brief paper updates previous efforts and statistically summarizes all Minnesota MC data collected by the Minnesota Pollution Control Agency (MPCA) from 2004 to 2012. We describe the intent of sampling efforts, statistically assess data from each effort, and describe differences among random and routine studies. The 2012 NLA MC data were used to assess regional patterns in MC, compare MC collected at mid-lake and a random near-shore site, and make statewide comparisons with the 2007 NLA data. This summary places MC concentrations in perspective for Minnesota and provides a basis for estimating relative risk of encountering elevated levels of MC in Minnesota lakes. It also serves as a comparative database, which other entities that collect MC may use to place their results in perspective.

Material and methods

Detailed descriptions of study design, sample collection, and laboratory methods were provided in Lindon and Heiskary (2009). The purpose of one targeted MC study in Lindon and Heiskary (2009) was to document seasonal changes and interrelationships in MC, water chemistry, and related measures based on monthly samples from May to September in 12 eutrophic–hypereutrophic lakes located in the same geographic area. In that study, MC and other environmental variables, including total phosphorus (TP), total Kjeldahl nitrogen, nitrate-N, alkalinity, conductivity, pH, chlorophyll *a* (Chl-*a*), algal composition, Secchi depth, and dissolved oxygen and temperature profiles, were collected at a pelagic site via a 2 m integrated sampler. In addition, near-shore MC and Chl-*a* samples were collected at a downwind site on most dates.

Near-shore or incident-based samples (e.g., in response to a reported dog death) were typically taken in the shallow waters near the shoreline where humans or animals may come in contact with severe cyanobacterial blooms. These samples were collected as grab samples just beneath the surface. Near-shore locations often varied dependent on wind direction and presence of algal blooms. In contrast, location of pelagic (index) sites was constant among sample events. Routine and incident-based studies are referred to as "targeted" studies for the purpose of this paper. In general, targeted lakes were relatively large (typically $\geq$50 ha) with public access and extensive public usage. Watershed landuse was often dominated by agricultural or developed landuses.

A stratified random process was used by USEPA to select lakes for the 2007 and 2012 NLA. These studies are designed to yield probabilistic estimates of condition at the national, regional, or state levels (USEPA 2012). In 2007, minimum lake size was 4 ha and in 2012 was 1 ha, resulting in a large number of small lakes in the NLA studies compared to the targeted studies. For example, in 2012, 75% of Minnesota's NLA lakes were <50 ha and 15% were <4 ha. These percentages are somewhat similar to the overall population of lakes in Minnesota, where 78% are <50 ha and 40% are <4 ha. Because public access is not a requirement in the study design, selected lakes were frequently deeply embedded in forested/wetland areas or prairie/cultivated areas and often had limited public usage.

In 2007 and 2012, MPCA sampled 50 lakes for statewide assessment of condition (as per USEPA statistical design; Messer et al. 1991). In 2012, MPCA added an additional

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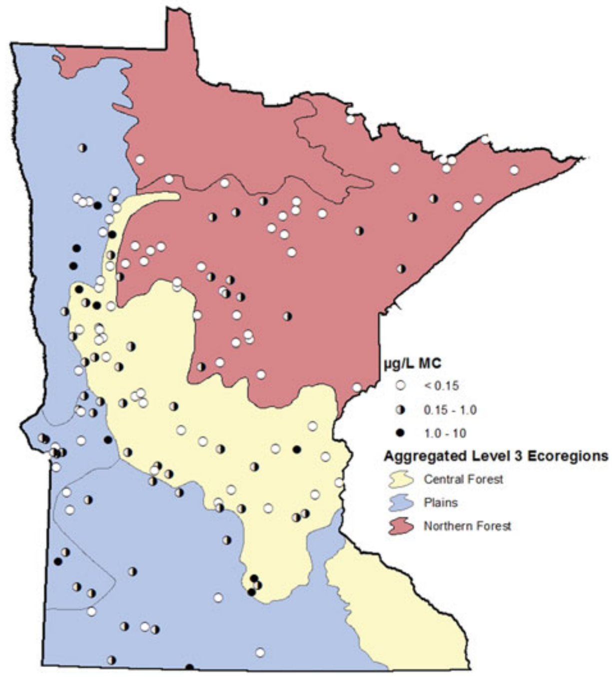

Figure 1. Map of 2012 NLA microcystin concentrations. Based on pelagic (index) samples. Note: all pelagic samples <10 μg/L.

Table 1. Statistical summary of all Minnesota (MPCA-collected) MC data by type or category of study. NLA data include pelagic and random near-shore samples combined. All concentrations in μg/L. UCL95 = upper control limit 95th percentile, Pctl = percentile.

	Overall MC dataset	Targeted Studies	2007 NLA Combined	2012 NLA Combined
# of Lakes	277	68	62	147
# of Samples	663	345	109	209
% Nondetects	26%	8%	39%	53%
% > 1 μg/L	43%	66%	17%	16%
Maximum	80,000	80,000	44	8.2
*Mean	317.2	608.4	1.4	0.3
*Std Error	139.9	268.0	0.4	0.1
*Std Dev	3601.2	4977.3	4.4	0.9
*UCL95	547.6	1050.4	2.1	0.4
*25th Pctl	<0.15	0.5	< 0.15	<0.15
*Median	0.6	3.0	0.2	<0.15
*75th Pctl	4	14	1.3	0.3

*Calculated using Kaplan-Meier Nondetect data analysis method (Helsel 2005) KM-Stats v1.4.

100 lakes from USEPA's randomized draw of lakes to allow ecoregion-based assessments for the 3 aggregated ecoregions that comprise Minnesota: Northern Forests, East Temperate Forests, and Great Plains. This randomized 150-lake frame (50 per ecoregion) provided statistically based coverage of lakes across Minnesota (Fig. 1). In addition to MC, a complete suite of water chemistry and biological samples were collected from each lake (see parameter lists in USEPA 2012).

NLA lakes were sampled on one occasion during the summer (Jun–Sep) index period. The 2007 national steering team discussed whether MC should be collected at the pelagic site or at a near-shore site. Due to insufficient funding at the national level to do both sites, the decision was made to collect MC at the pelagic site, consistent with all other water samples. This discussion prompted MPCA to collect an additional near-shore sample from one randomly selected (prior to arriving at the lake) near-shore site on its 50 lakes for a comparison among pelagic and near-shore MC. In 2012, NLA adopted a similar protocol and collected MC at a pelagic and a near-shore site.

All MC samples for MPCA were held on ice prior to processing by Minnesota Department of Health (MDH). MDH analyzed MC using a bench-top Abraxis ADDA ELISA method, which had a method detection limit (MDL) of 0.15 μg/L total MC, and the standardized analysis curve provided with the kit. MC samples underwent a triple freezing cell lysis procedure prior to analysis. The MC concentrations for all studies were based on microcystins, nodularians, and their congeners summarized as one MC concentration (total MC). The ADDA ELISA had an assay method maximum quantifiable range of 5 μg/L that required dilution of samples with reagent-grade deionized water when concentrations were above this range, which can result in reduced accuracy depending on the amount of dilution. MDH MC quality assurance analysis, using MCLR spike recovery quality assurance, indicated that 67% of samples had percent recovery within 90–110%, and 100% had recovery within 75–125%; and the coefficient of variation (CV) between sample and replicate was <15% for 56% of samples and <25% for 100% of samples.

World Health Organization (WHO) risk guideline categories established for recreational waters (WHO 2003) provide a basis for categorizing MC data and describe relative risk of health effects during recreational exposure to MC. The categories we used to assess our data were drawn from Chorus and Bartram (1999) and slightly modified to include: nondetects (<0.15 μg/L) no risk, <1–0.15 μg/L very low risk, 1–10 μg/L low risk, 10–20 μg/L moderate risk, 20–2000 μg/L high risk, and >2000 μg/L very high risk.

Nondetects were a common feature of these datasets (Table 1–3), hence our addition of a "no risk" category. Because of the large number of nondetects, statistical analysis was conducted using the Kaplan-Meier nondetect data analysis method (Helsel 2005).

Table 2. Statistical summary of Minnesota NLA 2007 and 2012 pelagic and random near-shore samples (limited to lakes where both samples were collected). All concentrations in μg/L. UCL95 = upper control limit 95th percentile, Pctl = percentile.

	2007 NLA Pelagic	2007 NLA Near-shore	2012 NLA Pelagic	2012 NLA Near-shore
# of Samples	51	51	50	50
% Nondetects	40%	45%	62%	54%
Minimum	<0.15	<0.15	<0.15	<0.15
% > 1 μg/L	27%	29%	12%	4%
Maximum	5.3	44	8.2	6.9
*Mean	0.98	1.92	0.37	0.44
*Std Error	0.21	0.89	0.18	0.17
*Std Dev	1.50	6.33	1.25	1.20
*UCL95	1.33	3.41	0.67	0.73
*25th Pctl	<0.15	< 0.15	<0.15	<0.15
*Median	0.2	0.2	<0.15	<0.15
*75th Pctl	1.4	1.5	0.19	0.34

*Calculated using Kaplan-Meier Nondetect data analysis method (Helsel 2005) KM-Stats v1.4.

Results and discussion

MPCA's MC data are statistically summarized collectively (referred to as overall MC dataset) and in 3 subsets: targeted studies, NLA pelagic and near-shore data from 2007, and NLA pelagic and near-shore data from 2012 (Table 1). The different goals and objectives of the monitoring efforts are clearly reflected in the monitoring results. For instance, mean MC for targeted lakes was 600 times higher than the random NLA study lakes (Table 1), and percent nondetects was 5–6 times lower than that observed in the 2007 and 2012 NLA studies. While each NLA lake was sampled only once, monitoring was deemed representative of conditions in the mid-summer index period for the overall population (USEPA 2012).

The large difference in mean MC concentrations between the targeted and NLA datasets (Fig. 2) was heavily influenced by a few extreme values (maximum MC was 80,000 μg/L in the targeted dataset vs. only 44 and 8.2 μg/L, respectively, for 2007 and 2012 NLA). In the case of these datasets, the "mean" is not a useful statistic for characterizing or comparing the datasets. The median value in the targeted dataset was ~15 times higher than the median 2007 and 2012 NLA MC concentration (Table 1). Similarly, the interquartile range (IQR; 25th–75th) was small for the NLA datasets (Table 1; Fig. 2), and both were heavily influenced by nondetect values compared to the targeted dataset, which had an IQR that spanned nearly 2 orders of magnitude.

The percentage of MC samples below the MDL varied substantially between the targeted and NLA datasets (Table 1). The targeted dataset had a rate of only 8% nondetects, which is a direct reflection of program intent and sampling methods. In contrast, sampling at a pelagic (index) site on the random NLA lakes yielded nondetect percentages that were more than 5-fold higher in 2007 and 2012.

Table 3. Minnesota 2012 NLA ecoregion-based MC summary. Pelagic samples only. All concentrations in μg/L. UCL95 = upper control limit 95th percentile.

	Northern Forests	Eastern Temperate Forests	Great Plains
# of Lakes	51	52	51
% Nondetects	71%	46%	40%
Minimum	<0.15	<0.15	<0.15
Maximum	0.60	2.20	8.20
*Mean	0.10	0.30	0.60
*Std Error	0.01	0.06	0.17
*Std Dev	0.10	0.40	1.30
*UCL95th	0.30	1.30	2.60
*Median	0.10	0.17	0.20

*Calculated using Kaplan-Meier Nondetect data analysis method (Helsel 2005) KM-Stats v1.4.

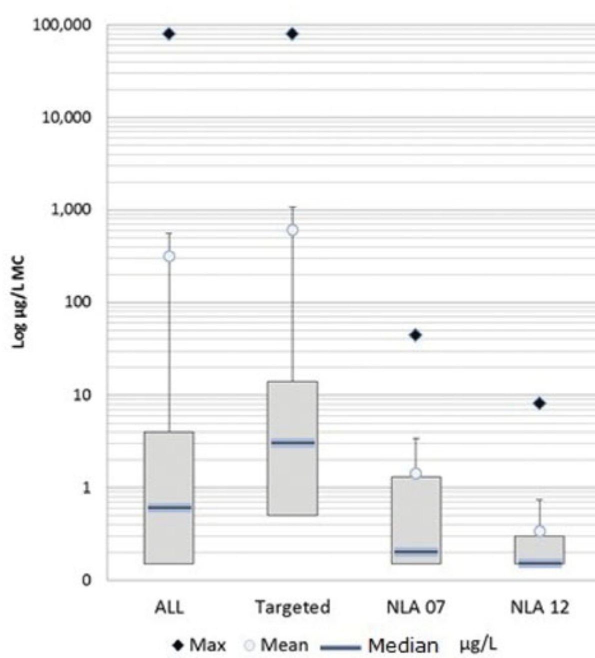

Figure 2. MC boxplots for entire Minnesota dataset, all targeted studies, and random NLA data from 2007 and 2012. Note: values below MDL of 0.15 μg/L cannot be shown on these plots but were used in the calculation of statistical summaries.

Based on MPCA's 2007 dataset, the near-shore mean, 95th percentile, and maxima were higher than the pelagic; however, there was no difference in the IQR for the 2 datasets and a minimal difference in percent nondetects (Table 2). In 2012, there was no substantial difference in mean or median values for the pelagic and near-shore sites, and 95% of all values were <1 μg/L (Table 2). A comparison of 2007 and 2012 pelagic values indicated substantial differences in mean, median, IQR, and 95th percentile values for the 2 years, with higher values in 2007. A comparison of near-shore values from the 2 years yields similar results. Based on the cumulative results from 2007 and 2012 (Table 1 and 2), there were no appreciable differences among pelagic and near-shore samples.

The increased spatial coverage of the 2012 NLA (50 lakes sampled in each of Minnesota's 3 ecoregions) allowed statistically valid characterization of MC across the state and among regions. Mean MC was lowest in the Northern Forest, followed by the Eastern Temperate Forest, and highest in the Great Plains (Table 3). Regional patterns in MC were similar to the regional patterns of lake trophic status across Minnesota (Heiskary and Wilson 2008), where lakes are least productive in the forested northeast, and eutrophic to hypereutrophic in the agriculturally dominated south and southwest (Great Plains), with a transition zone between the 2 that corresponds to the Eastern Temperate Forest ecoregion (Fig. 1). The 2012 NLA TP and Chl-*a* data support this finding with IQR as follows: Northern Forests: TP 10–28 μg/L and Chl-*a* 3–14 μg/L; Eastern Temperate Forests: TP 19–78 μg/L and Chl-*a* 4–41 μg/L; and Great Plains: TP 21–154 μg/L and Chl-*a* 14–127 μg/L. The most distinct among-region differences for MC were found in the Northern Forests and Great Plains where the maximum value for the Northern Forests was the same as the mean for the Great Plains.

Frequency distributions of the overall and NLA datasets relative to the previously described categories provide a basis for describing relative risk of encountering the various MC recreational exposure risk levels in Minnesota lakes (Fig. 3a-c). Based on the random NLA samples, we anticipate no detectable MC ~50% of the time when combined with MC <1 ug/L, and very low or no risk MC more than 80% of the time (Fig. 3b, c). Low risk values occur about 10% of the time, and moderate-to-high risks are anticipated <1%. When all MPCA data are considered, very low and no risk is anticipated 60% of the time, low risk is anticipated 20% of the time, and moderate risk about 10%. High and very high risk account for about 10% of the values (Fig. 3c).

The NLA randomized sampling approach provides a useful perspective on MC occurrence and magnitude at a national, regional, or state level. Minnesota's 2007 and 2012 NLA datasets indicate that randomly measured MC concentrations are very low in Minnesota lakes, and regional patterns in MC concentrations are similar to patterns in monitored trophic status among the state's diverse lake regions. These data further suggest minimal differences among MC as measured at pelagic sites compared to random near-shore sites, and no difference in terms of WHO recreational risk category. Thus, if there are monetary constraints for future NLA studies, collecting MC only at the pelagic site may be

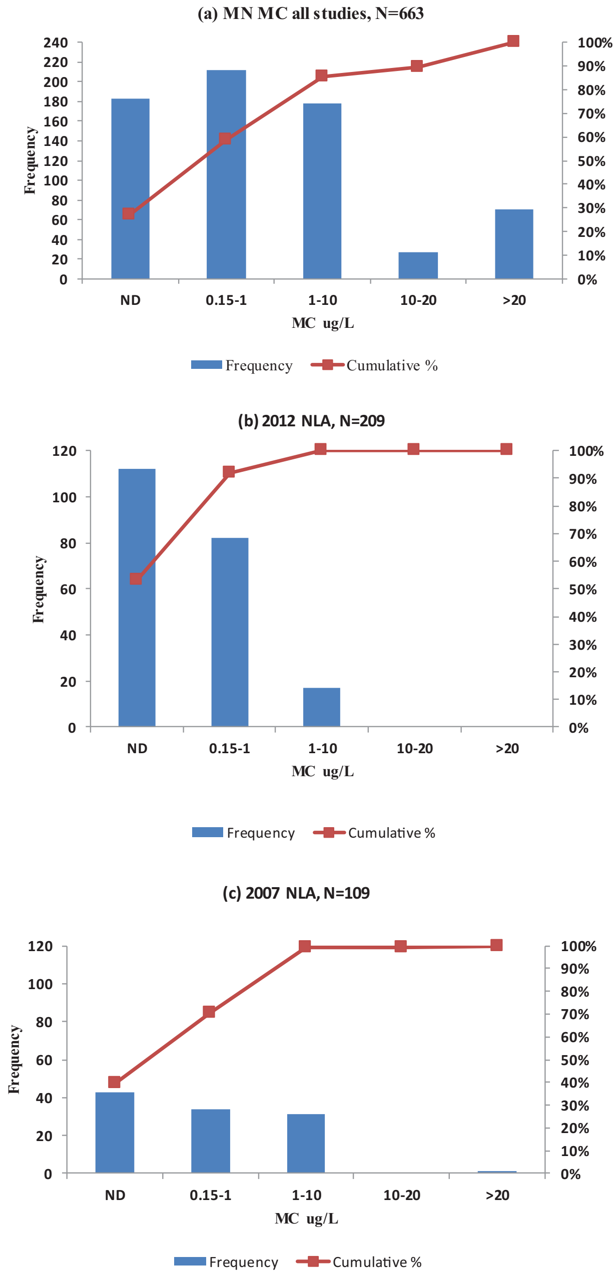

Figure 3. MC frequency distributions based on (a) overall data, (b) 2012 NLA, and (c) 2007 NLA. Groupings correspond to categories as described in text. ND implies <MDL.

adequate for national, regional, or state comparisons. Note that our observation of minimal differences among MC at pelagic compared to near-shore sites only applies if random selection of near-shore sites is used. If targeted sampling of near-shore sites is employed, differences among pelagic and near-shore results would likely be substantial.

If an investigator is interested in characterizing the MC range or relative risk of encountering elevated levels of MC in a given lake or set of lakes, a different sampling strategy must be employed. In this case, the lakes must be sampled over the course of the recreational season, and near-shore (downwind shoreline) samples should be collected in addition to pelagic samples. Minnesota's data from targeted studies support this recommendation (Table 1; Fig. 2). Lindon and Heiskary (2009) provide further insights based on these targeted studies.

High risk and very high risk MC concentrations are found in Minnesota lakes (as evidenced by the overall dataset); however, these levels are most often associated with severe and very severe nuisance cyanobacterial blooms (Lindon and Heiskary 2009), which are readily evident to the average lake user. Hence, the MPCA's proactive message to recreational lake users advising a preventative approach with messages such as "when in doubt, best keep out," is sound practice as a part of a broader effort to address the issue of harmful algal blooms. The broader effort has included public outreach (e.g., proactive television and radio and newspaper press releases) and collaboration with MDH, Minnesota Department of Natural Resources, Minnesota Veterinary Medicine Association, and local units of government, as well as our long-term focus on reduction of nutrient loading to lakes, with the specific intent to reduce the intensity and frequency of harmful algal blooms (MPCA 2014). In the future, we intend to review more recent toxicity information from the MDH and develop estimates of recreational and fish consumption exposure to MC, which will provide a basis for development of state-specific guidance that would reduce reliance on the WHO thresholds. The data described in this article will contribute to that effort.

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References

Chorus I, Bartram J, editors. 1999. Toxic cyanobacteria in water. World Health Organization. London (UK): E & FN Spon.

Heiskary SA, Lindon MJ. 2009. Microcystin in Minnesota lakes. LakeLine. 30(2):25–30.

Heiskary SA, Wilson CB. 2008. Minnesota lake eutrophication criteria development. Lake Reserv Manage. 24(3): 282–297.

Helsel DR. 2005. Nondetects and data analysis. Statistics for censored environmental data. Wiley. 250 p.

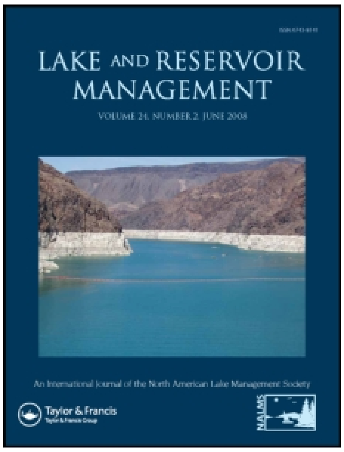
Lindon, MJ, Heiskary SA. 2009. Blue-green algal toxin (microcystin) levels in Minnesota lakes. Lake Reserv Manage. 25(3):240–252.

Messer JJ, Linthurst RA, Overton WS. 1991. An EPA program for monitoring ecological status and trends. Environ Monit Assess. 17:67–78.

[MPCA] Minnesota Pollution Control Agency. 2014. Blue-green algae and harmful blooms; [cited 14 Apr 2014]. Available from: http://www.pca.state.mn.us/index.php/water/water-types-and-programs/surface-water/lakes/blue-green-algae-and-harmful-algal-blooms.html

[USEPA] US Environmental Protection Agency. 2012. Survey of the nation's lakes: Field operations manual. 841-B-11-003. Washington (DC) USEPA; [cited 3 Mar 2014]. Available from: http://water.epa.gov/type/lakes/assessmonitor/lakessurvey/upload/NLA2012_FieldOperationsManual_120517_FINAL_CombinedQRG.pdf

[WHO] World Health Organization. 2003. Guidelines for safe recreational water environments. Coastal and fresh waters. Volume 1. Geneva (Switzerland).



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Blue-green algal toxin (microcystin) levels in Minnesota lakes

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Blue-green algal toxin (microcystin) levels in Minnesota lakes

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Abstract

Matt Lindon and Steven Heiskary 2009. Blue-green algal toxin (microcystin) levels in Minnesota lakes. Lake Reserv. Manage. 25:240–252.

Increased interest in blue-green algal toxins in recent years has led to increased monitoring to assess occurrence and levels of toxins in Minnesota lakes. Microcystin (MC), a hepatotoxin, is one of the primary toxins studied in Minnesota and elsewhere in North America. The Minnesota Pollution Control Agency has measured MC in numerous lakes across Minnesota as a part of three separate efforts: (1) A targeted survey in 2006 to assess MC levels in 12 eutrophic lakes in two south central Minnesota counties; (2) A stratified-random survey of 50 lakes in Minnesota as a part of the National Lake Assessment Project; and (3) Incident-based samples from various lakes during 2004–2007 with reports of severe nuisance algal blooms, potential for human health risk and/or documented dog deaths as a result of algal toxins. This investigation focuses primarily on the 2006 study and linkages between MC and other chemical, physical and biological measures. Of 133 MC samples, 94% were above the Method Detection Limit (MDL = 0.15 ug/L). Based on World Health Organization guidelines, 80% of all MC samples ranked in the "low risk" category (<10 μg/L), 8% as "moderate risk" (>10–20 μg/L), 11% as "high risk" (20–2000 μg/L), with an overall maximum of 8400 μg/L. Microcystin exhibited significant positive correlations (R_s) with pH and chlorophyll-a and significant negative correlations with alkalinity and Secchi depth. Data from the other two efforts place the 2006 results in perspective and provide a comprehensive representation of MC concentrations in Minnesota lakes and an improved basis for communicating risk to the public.

Key words: algae scums, blue-green algal toxins, cyanobacteria, microcystin, shoreline blooms

Blue-green algae, more appropriately referred to as cyanobacteria, are a common component of the algal community in lakes and rivers in Minnesota and elsewhere in the world. Certain forms of blue-greens have the ability to produce toxins and have been implicated in animal deaths and human-health related problems. These toxins, which include anatoxin, saxitoxin, microcystin (MC) and a more recently described toxin, cylindrospermopsin, vary in their toxicity. Of these, MC is the most commonly measured in many studies. It is an acute hepatotoxin (liver affecting toxin) and is possibly linked to liver tumor promotion (Chorus and Bartram 1999). Microcystin is produced by several genera of blue-green algae including: *Anabaena, Coelosphaerium, Lyngbya, Microcystis, Oscillatoria, Nostoc, Hapalosiphon* and *Anabaenopsis*.

Blue-green algae and the production of toxins (Carmichael and Gorham 1977) has long been a concern, and recent literature shows numerous efforts in countries such as, Australia (Brookes and Bruch 2004), Germany (Chorus et al. 2001), and the United States (Graham et al. 2004) intended to improve understanding of this issue, the factors that lead to toxicity and the ability to manage the blooms that cause the toxicity. Blue-green algae have several properties that contribute to their success in lake communities. Perhaps the most significant is the ability to control their buoyancy to optimize light and nutrient conditions, properties that allow the build-up of scums under certain conditions. Algae at the surface–water interface can take advantage of abundant light as well as atmospheric carbon and nitrogen. The build-up of algal scums is not only related to nutrient concentration and buoyancy but is also influenced by climatic factors such as wind, sunlight and other chemical and physical factors.

Blue-green algal toxicity is not a new issue in Minnesota. Olson (1949, 1960) and Buell (1938) documented several incidences of blue-green algal blooms in Minnesota that led to animal deaths, including cattle, horses and dogs dating back to the late 1800s. Studies conducted in response to these incidents associated toxicity with the blue-green

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genera *Anabaena*, *Aphanizomenon*, *Coelosphaerium*, *Lyngbya*, and *Microcystis.*

In recent years (2004–2007) several dog deaths, potential human health impacts and reports of very severe nuisance blooms again prompted renewed interest in blue-green algal toxicity. The Minnesota Pollution Control Agency (MPCA) in the course of these "incident" investigations typically collected MC and supporting water quality data. In 2005 the MPCA joined with the Department of Natural Resources (MDNR), Department of Health (MDH) and the Minnesota Veterinary Medicine Association (MVMA) to form the Minnesota Blue-green Algal Toxicity Workgroup for the express purpose of increasing awareness on blue-green algal toxicity within agencies, the veterinarian community, and the public. A poster, several news releases, fact sheets and an updated web site were all used to increase awareness. Workgroup discussions led to the realization that Minnesota had inadequate information on magnitude and frequency of occurrence of MC in Minnesota lakes.

In summer 2006 a study was conducted to characterize the magnitude and variability of MC in a set of 12 eutrophic lakes in south central Minnesota (Fig. 1). This study (Lindon and Heiskary 2007) sought to answer the following questions:

- What is the likelihood of encountering measurable MC at a mid-lake pelagic site in eutrophic lakes?
- What is the likelihood of the same when measuring MC at a near-shore site?
- What is the distribution of MC for both mid-lake and near-shore sites? Are these distributions significantly different?

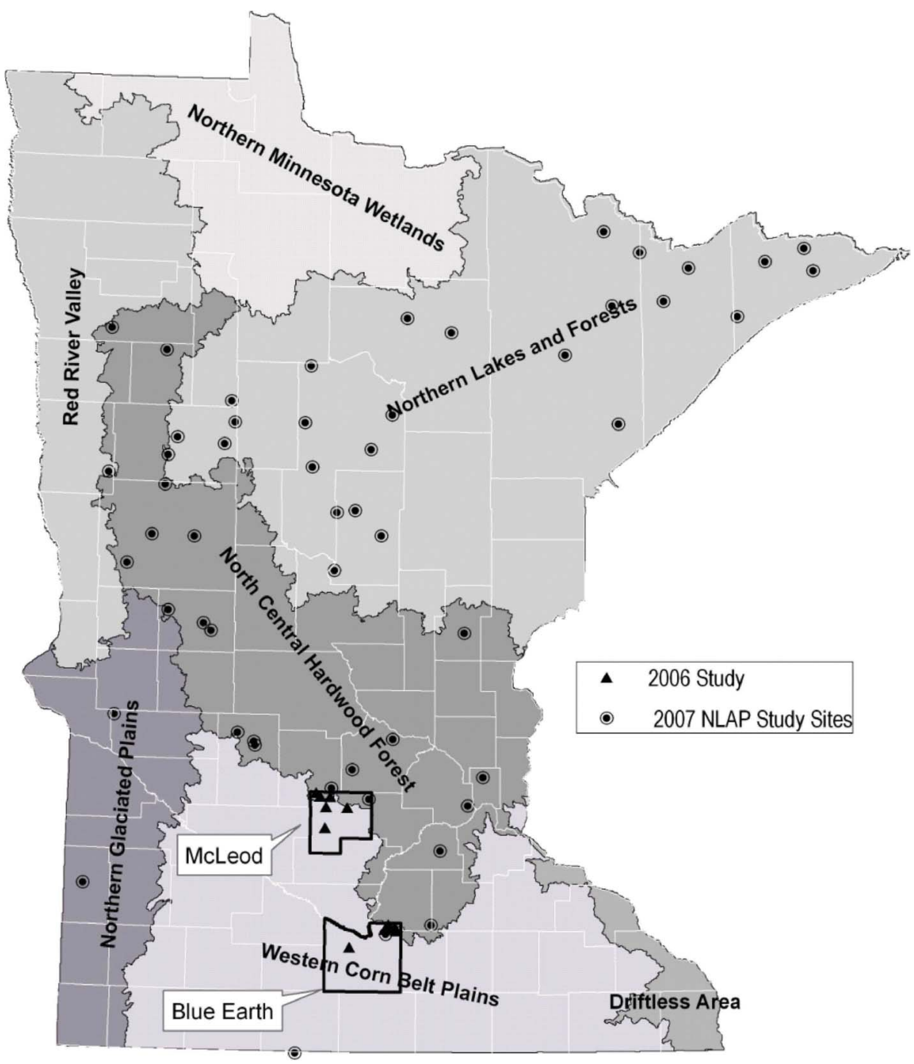


Figure 1.-Study lake locations and ecoregion map. 2006 study and 2007 National Lake Assessment Project (NLAP) sites noted.

Table 1.-2006 Study lake morphometric and watershed characteristics

ID	Lake	County	Area ha	Littoral %	Z max m	Z mean m	Watershed Ha
07-0047	George	Blue Earth	57	76	8.5	1.8	162
07-0053	Duck	Blue Earth	116	82	7.6	2.4	289
07-0054	Ballantyne	Blue Earth	143	86	17.6	1.8	1405
07-0060	Eagle	Blue Earth	370	100	2.7	0.9	1472
07-0096	Loon	Blue Earth	331	100	2.1	1.5	1144
07-0044	Madison	Blue Earth	1,113	64	59	10	10,828
43-0073	Hook	McLeod	132	100	3.2	1.5	1225
43-0084	Marion	McLeod	237	99	5.5	2.0	1722
43-0085	Otter	McLeod	111	100	3.6	0.9	115,378
43-0104	Stahl's	McLeod	57	100	11.2	4.0	1032
43-0115	Cedar	McLeod	779	100	2.4	1.5	3675
43-0034	Silver	McLeod	202	100	3.2	1.5	357

- How do values from this study compare to World Health Organization (WHO) recreational and drinking water guidelines?
- Is there seasonality to MC in these lakes?
- As bloom intensity (chlorophyll-a) increases, is there a greater likelihood of encountering high MC?
- What limnological and physical factors are associated with high MC?
- How can these findings be used to communicate risk to lake users?

Two additional MC data sets are used as a basis for comparison with the 2006 data: (1) MC samples were collected in 2007 as a part of the National Lakes Assessment Project (NLAP). In contrast to the 2006 study, the NLAP lakes consisted of 50 separate lakes selected randomly as a part of this nation-wide statistically-based sampling effort (Fig. 1). Of the 50 lakes only one, Eagle Lake (Blue Earth County), was also included in the 2006 study. Details on NLAP may be found at: http://www.pca.state.mn.us/water/nlap.html. (2) A second data set was derived from MPCA incident-based monitoring in response to citizen concerns regarding severe nuisance blooms, dog deaths and/or potential human-health problems for 2004–2007. There was no overlap of lakes in this data set with the other two studies.

Study site

The 2006 study focused on south central Minnesota near the North Central Hardwood Forest (NCHF) and Western Corn Belt Plains (WCBP) ecoregion transition (Fig. 1). Both ecoregions have numerous eutrophic to hypereutrophic lakes (Heiskary and Wilson 2008). No previous MC data were found for any of the study lakes. The 2007 NLAP lakes were distributed statewide (Fig. 1) consistent with the stratified random study design. In addition to these two studies, "incident-based" MC samples were collected as a part of investigations on select lakes in 2004–2007. The majority of these lakes were located in central or southern Minnesota; none were part of the 2006 or NLAP studies.

Based on percent littoral area, 9 of 12 2006 study lakes were considered shallow (>80% littoral; Heiskary and Wilson 2008). Watershed areas were quite variable (Table 1) ranging from 162 ha (George) to almost 115,400 ha (Otter). This results in watershed:lake area ratios ranging from about 3:1 (George) to over 1000:1 (Otter). Large watershed:lake area ratios often result in high total phosphorus (TP) and water loading from the watershed and, in extreme instances like Otter Lake, result in very low water residence time (high flushing rate).

Summer 2006 was characterized by drought conditions throughout much of central Minnesota. May through August temperatures were above normal while September was below the long-term norm in both areas. Precipitation was generally below normal for the May through August period and returned to normal to above normal in September. The northern portion of the study area (McLeod County) experienced five 1-in or greater precipitation events from May through September, while the southern portion was somewhat drier. Summer 2007 was characterized by warm and dry conditions, with May and June being particularly warm relative to the long-term records.

Materials and methods

Sample location, collection and laboratory analysis

In the 2006 study, pelagic (also referred to as mid-lake) sites were selected based on pre-existing sampling sites, where possible, and were typically located near the site of

Table 2.-Minnesota Department of Health laboratory methods and precision estimates

Parameter	Reporting Limit & Units	Method number	Precision	
			Mean difference[1]	Percent of observed[2]
Total Phosphorus	3.0 μg/L	EPA365.1	4.8 μg/L	2.7%
Total Kjeldahl N	0.1 mg/L	EPA351.2	0.05 mg/L	2.8%
Total Suspended Solids	1.0 mg/L	SM2540D	2.8 mg/L	9.6%
Total Sus. Volatile Solids	1.0 mg/L	SM2540E	—	—
Alkalinity	10.0 mg/L	SM 2320B	—	—
Chloride	1.0 mg/L	EPA 325.2		
Color	5.0 Pt-Co	EPA 110.2		
Chlorophyll-a		SM10200H	1.7 μg/L	7.4%
Pheophytin		SM10200H	—	—

[1] Average of individual means of 10 duplicates.
[2] Difference expressed as a % of observed concentration.

maximum depth. Near-shore sites were located near a downwind shoreline area that allowed accumulation of algae and often resulted in a distinct algal scum on the surface of the water. Pelagic sites were constant among sample events, whereas near-shore sites varied dependant on the wind direction, intensity and presence of an algal bloom.

Samples were collected monthly from May through September. Standard water quality parameters were collected at the pelagic site using a 2-m integrated sampler. Near-shore water quality and all MC samples were collected as surface grab samples. When scums were present, near-shore samples were collected in the midst of the scum. Water chemistry samples and field measurements were taken near the MC sample.

Several field observations were made at each sample event. Dissolved oxygen (DO), temperature, pH, and conductivity profiles (at 1-m intervals) were made at each pelagic site, and surface measures were typically taken at the near-shore site. Secchi transparency was measured at all pelagic sites. Other observations included a subjective assessment of the physical condition and recreational suitability of the lake (Heiskary and Walker 1988). Physical appearance ratings range from 1 = "crystal clear" to 5 = "severely high algae levels, scums and odor"; recreational suitability ratings range from 1 = "beautiful could not be any nicer" to 5 = "swimming and aesthetic enjoyment nearly impossible because of algae levels."

Chlorophyll-a (Chl-*a*) samples were filtered on the day of collection, folded and placed in Petri dishes and wrapped in foil. Samples were chilled on ice or frozen prior to shipment to the MDH for analysis. Samples for qualitative assessment of the algae were subsampled at the time of filtering and preserved in Lugols. Dr. Howard Markus, using the Minnesota Rapid Algal Analysis Procedure, later identified these samples to family or genera in most cases. This technique provided a semi-quantitative estimate of the relative biomass of the phytoplankton community, focused on the dominant forms in the sample, and allowed an estimate of the relative amount of MC-producing (MCP) blue-green genera in the sample.

The MDH lab in St. Paul analyzed all water quality samples, with the exception of phytoplankton, providing method numbers and associated quality assurance (QA) information (Table 2).

Lakes in the 2007 NLAP study were sampled on one occasion in either July or August 2007. Water quality samples (including MC) were collected from a mid-lake (index) site. Microcystin was also collected from a random near-shore site on each lake in conjunction with other near-shore measurements. The random sites were established in the office prior to going into the field so there was no subjectivity in their selection. Full details on NLAP parameters measured and sampling procedures may be found at: http://www.pca.state.mn.us/water/nlap.html.

MC analysis and data management

The MDH analyzed MC using a bench-top Abraxis ELISA method, with a method detection limit (MDL) of 0.15 μg/L total MC (for purposes of statistical analysis a nondetect substitution of 0.075 μg/L was used). Samples of MC underwent a triple freezing cell lysis procedure. The MC analysis conducted for this study is summarized as a quantification of MC congeners including nodularins. It has an assay method maximum quantifiable range of 5 μg/L that requires dilution of samples when concentrations are above this range, which can result in reduced accuracy, depending on the amount of dilution. Quality assurance of MC, based on samples from the summer of 2006, can be summarized as:

- Percent recovery: 67% within 90–110% and 100% within 75–125%

Table 3.-Summer-mean pelagic water quality 2006: McLeod County lakes. Last column represents the Western Cornbelt Plain (WCBP) ecoregion reference lake interquartile range (Heiskary and Wilson 2008)

Parameter units	Cedar	Stahl's	Hook	Otter East	Otter Main	Silver	Marion	WCBP Range
TP μg/L	85	35	121	296	351	323	82	65–150
Chl a μg/L	45	13	79	88	88	252	41	30–80
Chl-a max μg/L	55	23	150	194	153	365	52	60–140
Secchi meter	0.4	1.0	0.3	0.2	0.1	0.2	0.4	0.5–1.0
TKN mg/L	1.9	1.5	2.7	2.4	2.57	5.4	1.8	1.3–2.7
Alkalinity mg/L	172	168	116	238	246	130	142	125–165
Color Pt-Co	20	20	26	28	32	26	20	15–25
pH SU	8.8	8.5	9.5	8.6	8.5	9.5	9.2	8.2–9.0
Cl mg/L	15	14	28	26	27	60	32	13–22
Total. Sus. Solids mg/L	26	7	38	41	100	49	26	7–18
T. Sus. Volatile. mg/L	16	4	28	15	24	42	16	
T. Sus. Inorganic. mg/L	10	3	10	26	76	7	10	3–9
Spec. Cond. μS/cm	280	275	234	623	519	340	340	300–650
Pheophytin ug/L	9.9	2.7	7.5	20	19.7	60.2	8.3	

- Coefficient of variation (CV) between sample and replicate: 56% < 15% and 100% < 25%

World Health Organization risk guideline categories (WHO 2003) established for recreational waters and drinking water provide a basis for placing the MC data in perspective and describing relative risk. The categories used in this study (from WHO guidelines) were: <1 μg/L = very low risk; 1–10 μg/L = low risk; 10–20 μg/L = moderate risk; 20–2000 = μg/L high risk; and >2000 μg/L = very high risk. The very low risk category was added to include measurements that were very near the MDL for MC and below the 1 μg/L drinking water guideline for MC-LR, one of the most common and toxic MC compounds.

Most of the statistical analysis was conducted in Excel spreadsheets. Because of non-normal distributions of MC, the nonparametric Spearman rank correlation (Rs) was used to evaluate relationships between MC and various chemical, physical and biological variables. Based on these results and plotting of data, cross-tabulation was conducted to further characterize relationships. In this technique the independent variable (e.g., Chl-*a*) was paired with its respective MC concentration and independent values were ranked (low to high) and sorted into four approximately equal-sized groups.

Table 4.-Summer-mean pelagic water quality 2006: Blue Earth County lakes. Last column represents the NCHF ecoregion reference lake interquartile range (Heiskary and Wilson 2008)

Parameter units	Ballantyne	Duck	Eagle	George	Loon	Madison	NCHF Range
TP μg/L	40	70	142	105	157	81	23–50
Chl a μg/L	19.3	42	75	40	82	47	5–22
Chl-a max μg/L	24	66	104	76	102	67	7–37
Secchi meter	0.8	0.8	0.3	0.5	0.3	0.7	1.5–3.2
TKN mg/L	1.4	1.5	3.1	2.0	2.9	1.8	0.60–1.2
Alkalinity mg/L	142	154	118	91	136	144	75–150
Color Pt-Co	14	10	30	24	16	18	10–20
pH SU	8.6	8.7	9.2	9.2	8.9	8.7	8.6–8.8
Cl mg/L	19	21	19	16	24	21	4–10
Total. Sus. Solids mg/L	10	13	43	23	67	10	2–6
T. Sus. Volatile. mg/L	6	10	33	17	49	8	
T. Sus. Inorganic. mg/L	4	3	10	6	18	2	1–2
Spec Cond μS/cm	286	284	231	188	246	268	300–400
Pheophytin μg/L	2.7	8.3	8.0	5.2	6.0	8.8	

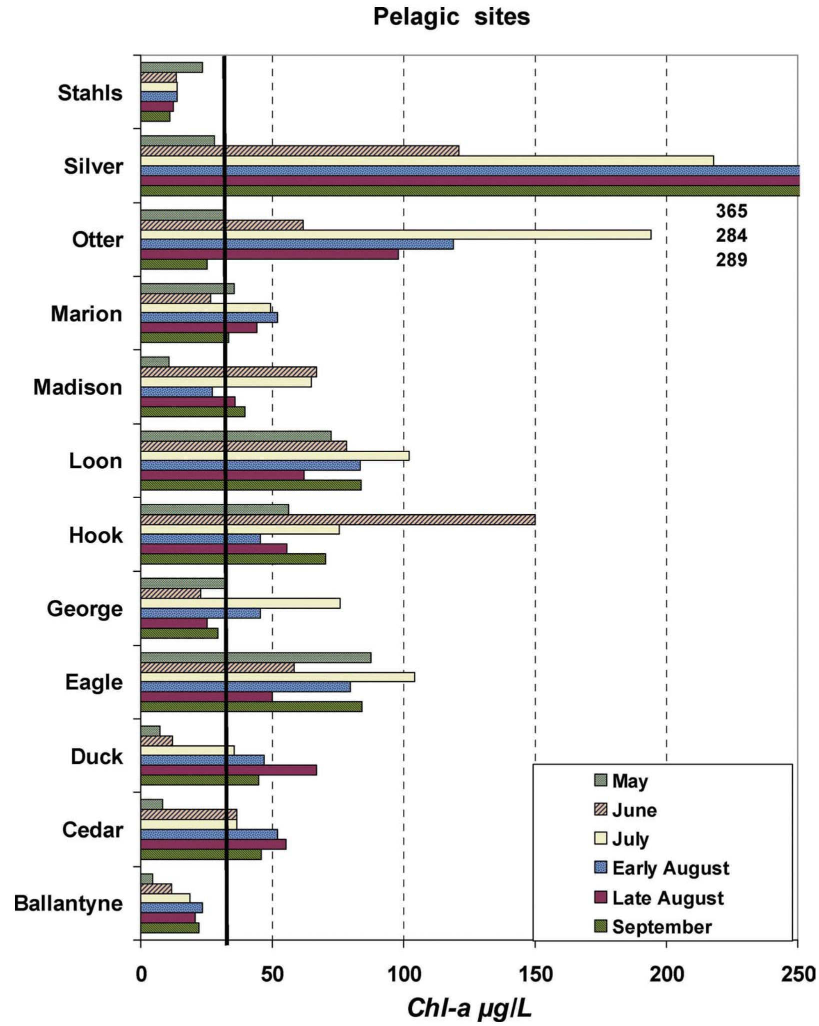


Figure 2a.-Pelagic chlorophyll-a concentration by lake and date for 2006 study. Severe nuisance bloom level (30 μg/L) noted by heavy black line for perspective. Concentrations beyond scale are noted immediately below bars and ordered by date.

Concentrations of MC were grouped by WHO risk categories, and the distribution of these categories was assessed within each group. Box plots for comparison of MC distributions among the data sets were created in SYSTAT V12.

Results

2006 seasonal patterns in chemical, physical and biological measures

Surface water temperatures ranged from about 14 to 16°C in May to peak temperatures of 26–30°C in late August. Surface temperatures peaked in the northern (McLeod County) lakes in July, while the southern (Blue Earth County) lakes peaked in early August. Rapid cooling in most lakes was evident in September, consistent with a rapid decline in air temperature. Water temperatures were conducive for blue-green algal growth (>22°C) from June through late August on most lakes.

All lakes were eutrophic to hypereutrophic based on TP, Chl-*a* and Secchi (Tables 3 and 4). TP ranged from $\leq$40 μg/L (Stahl's and Ballantyne) to >300 μg/L (Otter and Silver). Based on previously defined Chl-*a* levels (Heiskary and Walker 1988) "severe nuisance" (Chl-*a* > 30 μg/L) and "very severe nuisance" (Chl-*a* >60 μg/L) were common throughout the summer at the pelagic sites (Fig. 2a).

Microcystin exhibited no consistent seasonal pattern at either the pelagic or near-shore sites (Fig. 3a and 3b). Silver and Hook lakes exhibited the highest pelagic concentrations and had the only concentrations that fell in the moderate risk level (Fig. 3a). In contrast, 7 of 12 lakes were below

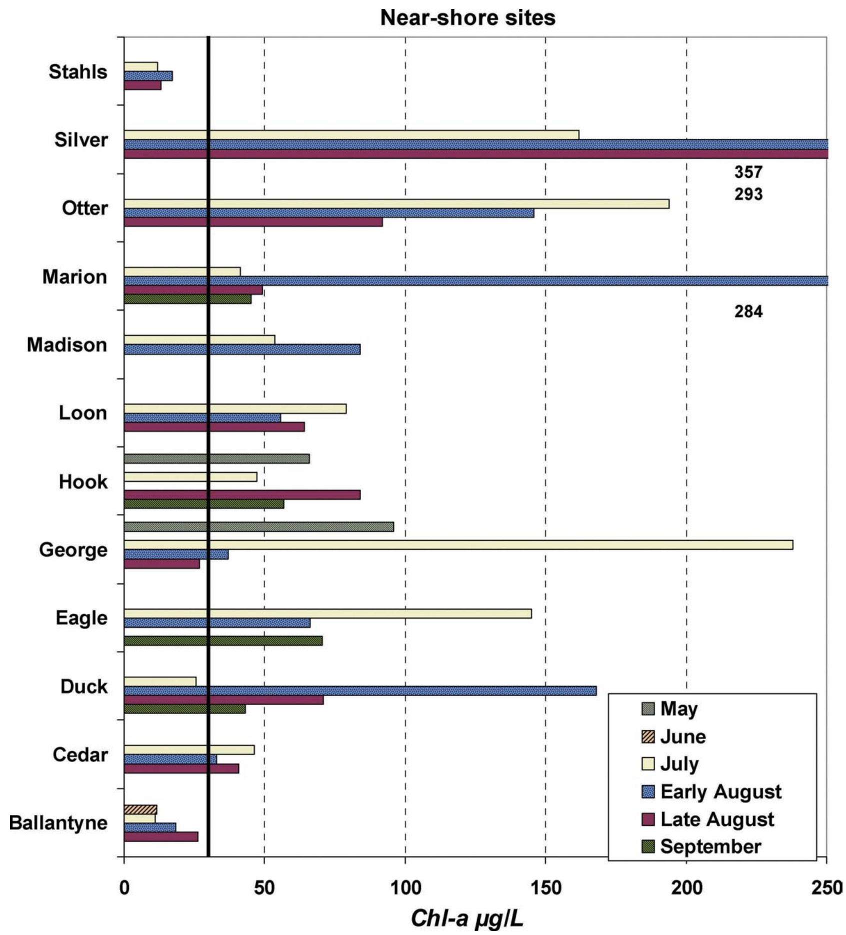


Figure 2b.-Near-shore chlorophyll-a concentrations by lake and date for 2006 study. Severe nuisance bloom level (30 μg/L) noted by heavy black line for perspective. Concentrations beyond scale are noted immediately below bars and ordered by date.

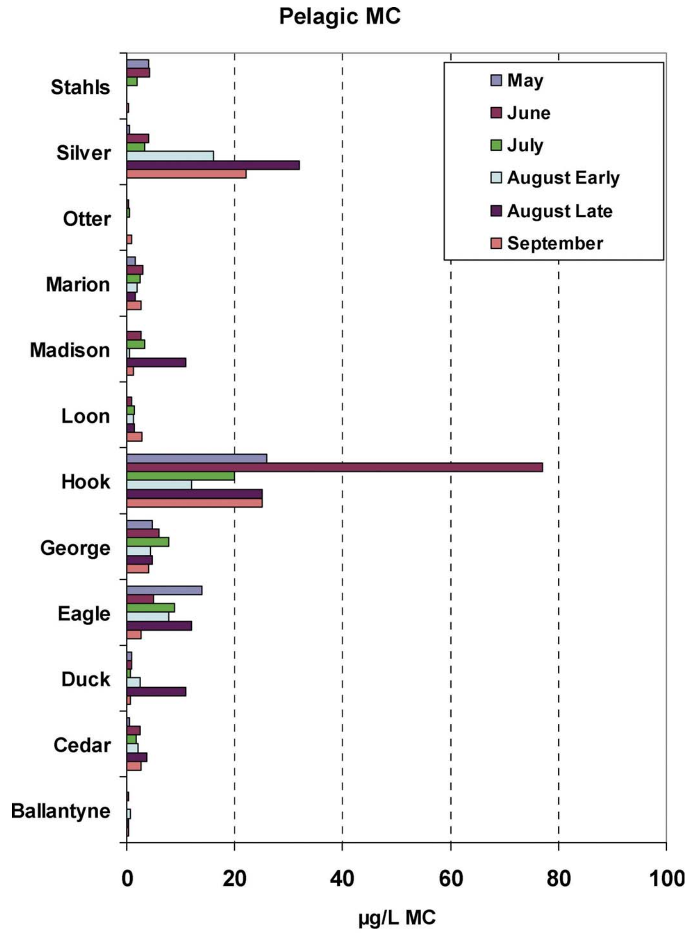
the low risk threshold (10 μg/L) for the entire summer at the pelagic site. High to very high risk concentrations were noted at near-shore sites on three lakes: Madison, Hook and George (Fig. 3b). Some of the highest concentrations were from samples collected in May and June, which was not anticipated.

Microcystin was highly variable within and among lakes (Fig. 3a and 3b). Six percent of MC results were below the MDL. More than 25% of the data were between 0.9 μg/L and the non-detect substitution of 0.075 μg/L (Fig. 4a). Concentrations of $\geq$1 μg/L were unevenly distributed up to 8400 μg/L. Because MC maxima are of most concern, they were not considered as outliers. Near-shore and pelagic sites exhibited different distributions and have statistically different means based on a log normalized t-test and 95% confidence intervals; however, near-shore and pelagic medians were not significantly different. About 85% of the pelagic samples were considered very low to low risk (Fig. 4a). Likewise, a high percentage of the near-shore samples were in these categories as well. Distributions for the moderate to high risk categories were not substantially different (Fig. 4a) among the pelagic and near-shore sites; however, the only very high risk measures were found at the near-shore sites (Fig. 4a).

Though it was common for the pelagic sites to have distinct green coloration and high Chl-*a*, surface scums were limited to the near-shore sites. Even at the near-shore sites, distinct surface scums were not common (Fig. 4b). Comparatively, near-shore sites with surface scums exhibited higher and more variable MC compared to sites without scums (Fig. 4b).

2006 MC and other environmental factors

Spearman's rank correlation (R_s) resulted in four moderate and three high correlations with MC (Fig. 5). Parameters exhibiting strong positive R_s with MC included: pH, microcystin producers (MCP) Chl-*a* (Chl-*a* $\times$ % MCP), % MCP, total suspended volatile solids (TSV), Chl-*a* and physical



(a)

Figure 3a.-Pelagic microcystin (MC) concentrations by lake and date for 2006 study.

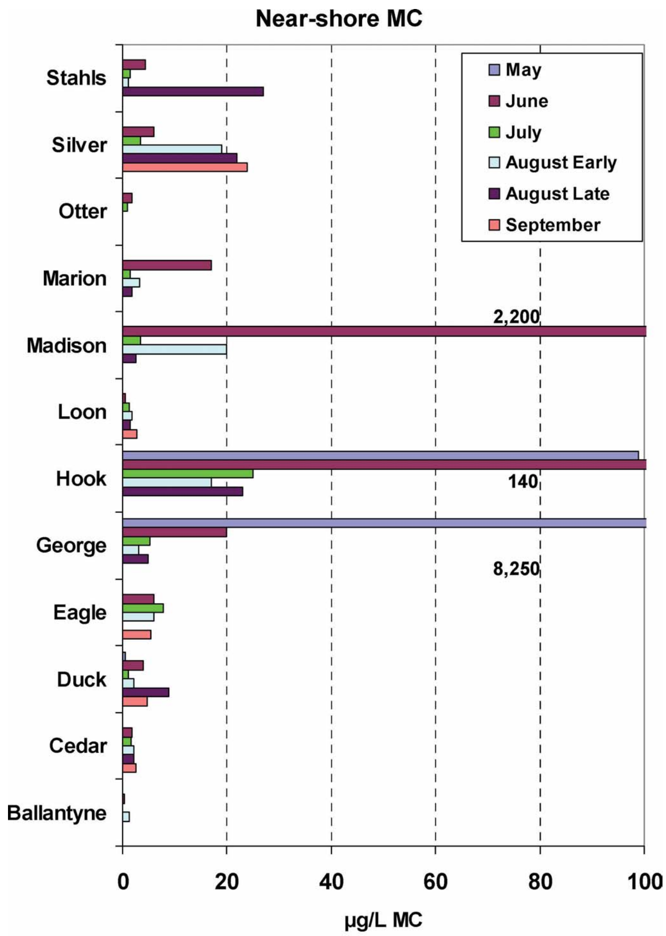
appearance ratings. Inverse relationships were found with alkalinity, Secchi depth and specific conductance. Parameters highly correlated with MC were typically related to algal biomass (Chl-*a*) or productivity as well.

Based on results (Fig. 5) and a lack of distinct linear relationships, we used cross-tabulation to explore the relationship among MC and three variables, Chl-*a*, Secchi and pH, selected because of their high correlation with MC and ease of measurement in lakes. This provided a means to assess the likelihood (risk) of encountering varying categories of MC over a range of Chl-*a*, Secchi or pH measurements (Fig. 6a–6c).

Chl-*a* concentrations and trends were highly variable among the study lakes (Fig. 2a and 2b), and no significant linear relationship was evident between MC and Chl-*a*. Combining MC and Chl-*a* classes (Fig. 6a), which approximate varying degrees of "bloom intensity," provides a basis for describing the "risk" of encountering specified levels of MC as a function of bloom intensity. Moderate risk MC was not encountered until blooms exceeded 30 μg/L (severe nuisance blooms; Fig. 6). As blooms exceeded 30–50 μg/L the frequency of moderate to high risk MC increased to $\sim$30%. All high risk MC concentrations were associated with Chl-*a* $>$30 μg/L.

A strong inverse relationship between Secchi depth and algal biomass (Chl-*a*) has long been noted; Secchi depth also exhibits an inverse relationship with MC (Fig. 5). Similar to the relationship between MC and bloom intensity, a threshold effect was observed; as Secchi declined below 0.5 m, risk of MC concentrations in the moderate to high risk categories increased (Fig. 6b).

The variable with the strongest relationship with MC was pH ($R_s = 0.73$; Fig. 5). More than 90% of the pH values $>$9.0 were associated with severe nuisance bloom levels (Chl-*a* $>$30 μg/L). With the exception of one sample, all lakes with moderate to high risk MC had a pH of $\geq$9.0 (Fig. 6c). Conversely, alkalinity exhibited a moderate inverse relationship with MC ($R_s = -0.61$). In the 2006 study all



(b)

Figure 3b.-Near-shore microcystin (MC) concentrations by lake and date for 2006 study. MC concentrations beyond scale noted on graph.

pH values ≥9.0 were associated with alkalinities ranging from 80 to 150 mg/L.

MC comparison among studies

A comparison of the 2006 study of 12 eutrophic lakes with the NLAP and incident-based samples provide further perspective on MC concentrations in Minnesota lakes (Fig. 7). Based on this comparison the distributions of these three data sets are significantly different, which is expected given the population of lakes sampled and focus of each study. The 2006 eutrophic lakes exhibit a larger range and more extreme MC concentrations compared with the NLAP data (Fig. 7). Both data sets reveal higher MC concentrations at near-shore sites compared to mid-lake index sites. The incident-based sampling yielded some extremely high event-based values, which again is expected given the intensity (magnitude) of the blue-green blooms sampled and the subjective nature of site selection.

Discussion

Cyanobacteria have the ability to produce several toxins that may be acutely and chronically toxic. Extensive study world-wide on this issue documents toxic events attributed to cyanobacteria, describe the toxicity and action of the various toxins, and describe development of action levels and thresholds that express the relative risk of these toxins (e.g., Chorus and Bartram 1999, Chorus et al. 2001). Other studies, such as Graham et al. (2004) describe the distribution of particular toxins (MC) and some environmental factors that may contribute to production of the toxin.

The 2006 study focused on MC in eutrophic to hypereutrophic lakes in south-central Minnesota. Several questions to advance knowledge on the extent, magnitude and frequency of MC in Minnesota lakes were posed prior to the study (see introduction) and provide a basis for the following summary comments.

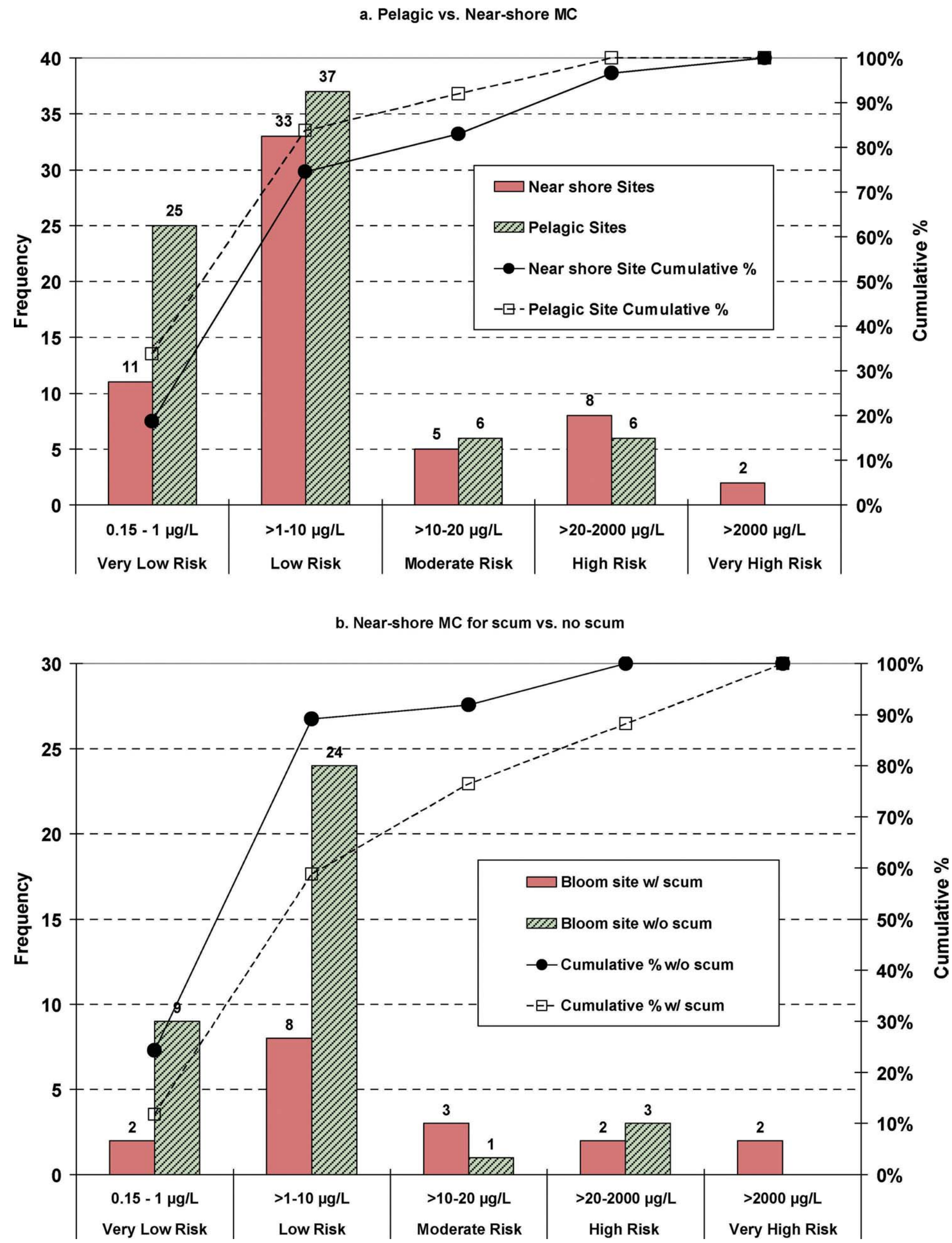


Figure 4.-Microcystin (MC) frequency distributions for 2006. Comparisons for (a) pelagic vs. near-shore MC and (b) near-shore (MC) for scum vs. no scum.

The likelihood of encountering measurable MC at a pelagic site in a eutrophic lake is quite high based on the 2006 study (Fig. 4a). When combined with the near-shore samples, 94% of the MC measurements were above the MDL. Near-shore samples (Fig. 7) exhibited a larger range, higher mean and much higher maximum value (8400 μg/L) compared to the pelagic samples (69 μg/L). The likelihood of moderate to very high risk MC was found to be greater at sites with a distinct surface scum. These results are consistent with observations by Graham et al. (2004) who note that MC in scums may be much greater than at pelagic locations.

WHO guidelines provided a basis for evaluating the relative risk of the MC concentrations in this study; 80% of all MC concentrations were in the WHO low risk category for recreational waters (82% pelagic and 72% near-shore). The remainder of the pelagic samples was in the moderate to

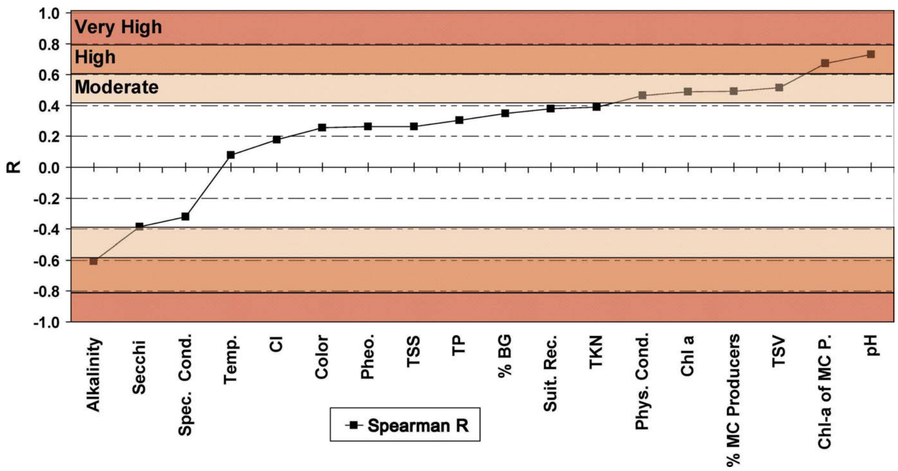


Figure 5.-Spearman Correlation Coefficients (R_s) for microcystin (MC) and various chemical, physical and biological variables. See Table 3 for complete parameter names and units.

high risk category (Fig. 3a). Only two near-shore samples were in the very high risk category (Fig 3b).

Seasonal patterns in MC, similar to typical patterns observed for Chl-*a* and nuisance algal blooms, were anticipated; however, based on the 2006 study, there was no distinct seasonality to MC concentrations, due in part to two lakes that exhibited very high MC in May and June at near-shore sites. The incident-based sampling and dog deaths (2004–2007) also indicate that elevated MC may occur at any time in the summer. In 2007 incident-based sampling was initiated in June in response to complaints of very severe algal blooms and a documented dog death.

Field and laboratory studies have demonstrated that relationship between cyanobacteria, MC and environmental factors is invariably complex (Graham et al. 2004). Some work indicates that variations in strains among toxin-producing species have more impact on MC production than environmental factors (Chorus and Bartram 1999), and isolating key environmental factors affecting MC is often difficult without detailed analysis. Results from the 2006 study reveal strong positive relationships with MC (in order of R_s) for pH, Chl-*a* of MCP, TSV, %MCP, Chl-*a* and physical appearance rating. A strong relationship with Chl-*a* and %MCP is consistent with the findings of previous researchers (e.g., Chorus and Bartram 1999, Chorus et al. 2001) who noted that toxic incidents involving MC or other blue-green algal toxins are most frequently associated with large surface bloom-forming genera.

Relatively distinct relationships were observed among MC and Chl-*a*, pH and Secchi. When Chl-*a* remained <30 μg/L, MC was in the very low to low risk categories (Fig. 6a). As Chl-*a* increased to >30–50 μg/L the risk of moderate to high MC increased to 30%. High correlation of MC with pH is consistent with correlations observed by Paerl and Ustach (1982) and is an expression of algal productivity (Chl-*a* $R_s = 0.58$), which is expected given that cyanobacteria prefer a high pH environment (Shapiro 1973). The strong inverse relationship with MC and alkalinity is likely a function of algal productivity. Wetzel (2001) noted that "rapid photosynthesis can rapidly reduce total dissolved inorganic carbon (DIC) and increase pH." Thus, alkalinity is not likely a direct driver of MC production; rather it may reflect the superior competitive advantage that blue-greens have at low DIC concentrations, which is most likely to occur in eutrophic, low alkalinity systems at high pH.

High risk MC was found only when Secchi depth was ≤ 0.5 m. Because surface blooms of MCP blue-greens, such as *Anabaena* and *Microcystis,* routinely result in very low transparency, this relationship is intuitive. This is in contrast to the non-MCP *Aphanizomenon* that forms large "rafts" that float at the surface and often allow higher transparency.

The 2006 study, as with most MC studies, does not allow precise prediction as to which blue-green algal blooms will produce MC in the moderate to very high risk range; however, the study suggests that Minnesota's current recommendation to the public to avoid contact with very severe nuisance blooms is sound. Very severe nuisance blooms (Chl-*a* >30 μg/L) are readily recognizable by the public. Moderate to high risk MC was associated with high pH (≥ 9.0) and low Secchi (<0.5 m), two parameters that are easy to measure. This suggests that a combination of visual examination of the water to assess bloom intensity (e.g., subjective assessments of physical appearance and recreational suitability) combined with measurement of all or some of the three variables (Chl-*a*, Secchi and pH) can allow resource managers to estimate the potential risk of encountering moderate to high risk MC. Further, three independent data sets

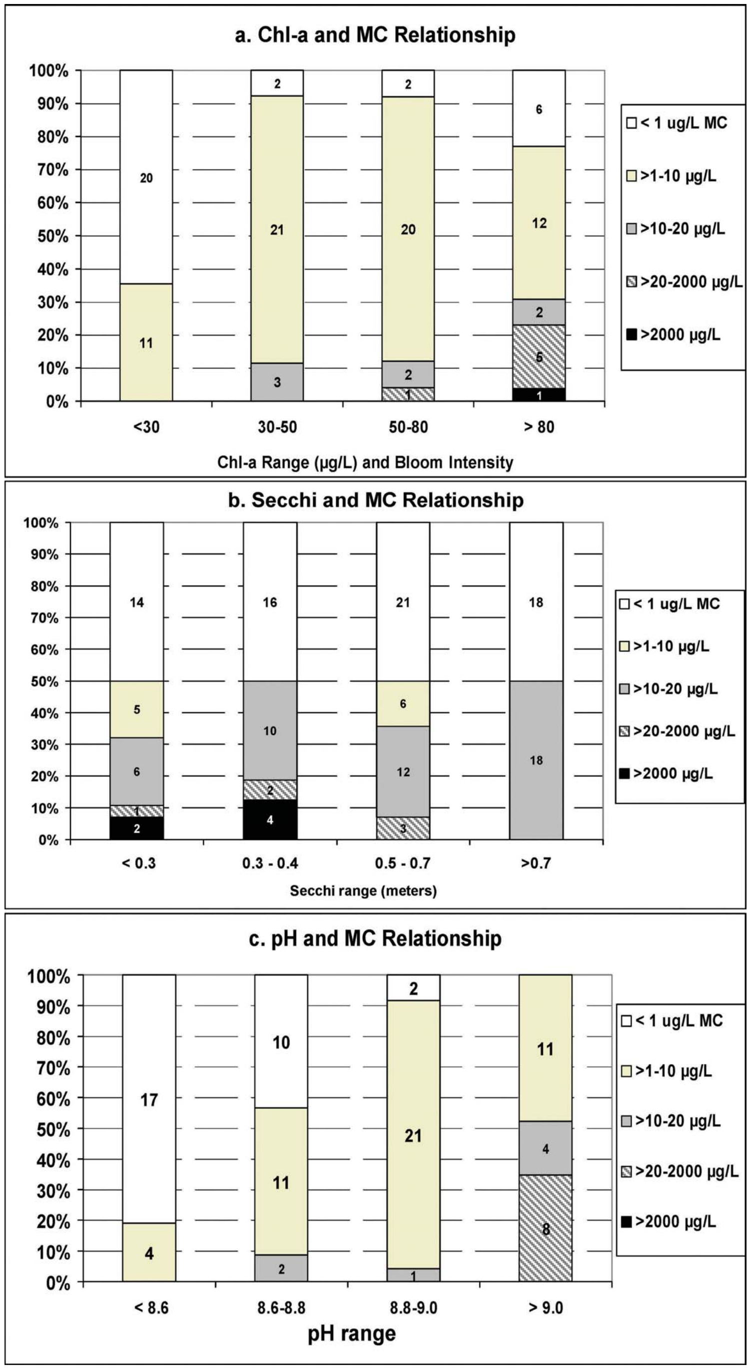


Figure 6.-Cross tabulation of microcystin (MC) relative to: (a) Chlorophyll-a (bloom intensity) (108 pairs), (b) Secchi (69 pairs) and (c) pH (91 pairs) based on 2006 data. Number of observations in each category noted.

indicate that MC is measurable across a wide range of Minnesota lakes and ecoregions and, collectively, these data (Fig. 7) provide a good basis for evaluating MC data from future monitoring efforts in Minnesota.

Minnesota does not have widely accepted thresholds (nor do most states) for assessing MC risk for aquatic recreational use, although some states (e.g., Nebraska) do issue health alerts based on MC measurements and have established thresholds for this purpose (Nebraska DEQ 2009). We used WHO thresholds as a basis to assess risk, but no attempt was made to assess their validity for assessing risk in Minnesota's waters. For Minnesota, and perhaps other states, it may be desirable to more closely review existing WHO

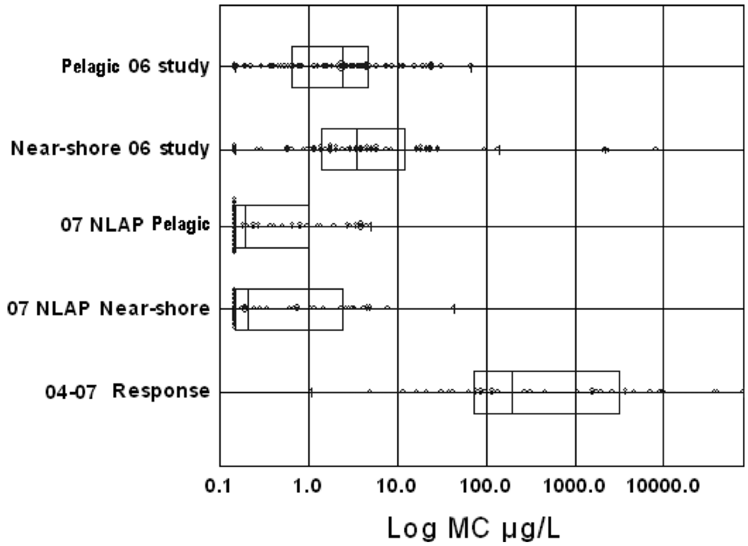


Figure 7.-Microcystin (MC) box and whisker plots by study and site location for 2006 pelagic and near-shore samples; 2007 NLAP pelagic and near-shore samples, and 2004–2007 incident-based samples.

thresholds to determine if mutually agreed upon thresholds could be developed for the purpose of assessing the risk to humans and animals that may come in contact with or consume water containing MC. From a risk communication standpoint, note that several other toxins (e.g., saxitoxin and anatoxin) may be produced by cyanobacteria as well as other algae, and it may be valuable in future studies to determine their relative concentrations and how they vary relative to MC, Chl-*a* and other factors.

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References

Brookes, J. and M. Bruch. 2004. Toxic cyanobacteria management in Australian waters. LakeLine 24(4):29-32.

Buell, H. 1938. A community of blue-green algae in a Minnesota pond. Ecology 19:224-232.

Carmichael, W. and P. Gorham. 1977. Factors influencing the toxicity and animal susceptibility of *Anabaena flos-aquae* (Cyanophyta) blooms. J. Phycol. 13:97-101.

Chorus, I. and J. Bartram (eds.). 1999. Toxic cyanobacteria in water. World Health Organization, E & FN Spon, London.

Chorus, I., V. Niesel, J. Fastner, C. Wiedner, B. Nixdorf and K-E. Lindenschidt. 2001 Environmental factors and microcystin levels in water bodies. P. 159-177. *In* I. Chorus (ed.). Cyanotoxin: occurrence, causes, consequences. Springer, Berlin.

Graham J.L., J.R. Jones, S.B. Jones, J.A. Downing and T.E. Clevenger. 2004. Environmental factors influencing microcystin distribution and concentration in Midwestern United States. Water Res. 38:4395-4404.

Heiskary, S.A. and W.W. Walker. 1988. Developing phosphorus criteria for Minnesota lakes. Lake Reserv. Manage. 4(1):1-10.

Heiskary, S.A. and C.B. Wilson. 2008. Minnesota's approach to lake nutrient criteria development. Lake Reserv. Manage. 24(3):282-297.

Lindon. M.J. and S.A. Heiskary. 2007. Microcystin levels in eutrophic south central Minnesota lakes. Part of a series on Minnesota Lake Water Quality Assessment. MPCA St. Paul, MN. 53 pp. http://www.pca.state.mn.us/publications/reports/wq-lar3-11.pdf. Accessed 21 Nov 2008.

Nebraska Department of Environmental Quality. 2009. 2009 toxic blue-green algae and bacteria sampling results. http://www.deq.state.ne.us/. Accessed 5 Jun 2009.

Olson, T.A. 1949. History of toxic plankton and associated phenomena. 1949. Sewage Works Engineering and Municipal Sanitation 20:71.

Olson, T.A. 1960. Water poisoning—a study of poisonous algae blooms in Minnesota. Am. J. Public Health 50:883-884.

Paerl H.W. and J.F. Ustach. 1982. Blue-green algal scums: an exploration for their occurrence during fresh water blooms. Limnol. Oceanogr. 27(2):212-217.

Shapiro, J. 1973. Blue-green algae: why they become dominant. Science 179:382-384.

Wetzel, R.G. 2001. Limnology. Lake and River Ecosystems. 3rd edition. Academic Press.

WHO. 2003. Guidelines for safe recreational water environments. Coastal and fresh waters, Volume 1. World Health Organization. Geneva, Switzerland.