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Blue-green Algal Toxin (Microcystin) Levels in Minnesota Lakes

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Abstract—Recent interest in blue-green algal toxins 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 MPCA has measured MC in numerous lakes across Minnesota as a part of three separate, but related, efforts: 1) A targeted survey conducted in 2006 to assess MC levels in 12 eutrophic lakes in two south-central Minnesota counties to determine the prevalence and seasonal trends in MC and to assess environmental variables associated with elevated MC; 2) A stratified-random survey of 50 lakes in Minnesota as a part of the National Lake Assessment Project (NLAP); 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 article will focus on the 2006 study and links between MC and other chemical, physical and biological measures. Data from the other two efforts are used to place the 2006 results in perspective and provide a good representation of MC concentrations that may be encountered in Minnesota's lakes. Better understanding of this toxin and its relationship and linkages to other water quality parameters will allow for better risk management and public awareness.

Introduction

Cyanobacteria, often referred to as blue-green algae, 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 problems. These toxins, which include anatoxin, saxitoxin, microcystin and a more recently described toxin, cylindrospermopsin, vary in their toxicity. Of these, microcystin (MC) is the most commonly measured in most studies. MC is an acute hepatotoxin (liver-affecting toxin) produced by several genera of blue-green algae including: Anabaena, Coelosphaerium, Lyngbya, Microcystis, Oscillatoria, Nostoc, Hapalosiphon and Anabnaenopsis. MC has also shown to be carcinogenic (Chorus and Bartram 1999).

While there has long been concern regarding blue-greens and the production of toxins (Carmichael and Gorham 1977), 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. 2005) 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. This property also allows for the buildup of scums under the right conditions. Algae at the surface water interface can take advantage of abundant light, as well as atmospheric carbon and nitrogen. The buildup 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, 1954, and 1960) documents several incidences of blue-green algal blooms in Minnesota that have led to animal deaths, including cattle, horses and dogs. Some of these accounts date back to the late 1800s with animal deaths attributed to contact with blue-green blooms on Lake Elysian, near Waterville. Documented incidences were also noted in the Fergus Falls area in 1900 and various other incidents from 1918 to 1934. Studies conducted at that time associated the toxicity with the blue-green genera: Anabaena, Aphanizomenon, Coelosphaerium, Lyngbya, and Microcystis. Toxic blue-green blooms were noted on Lake of the Isles Lagoon and Kenilworth Lagoon in 1918 (Buell 1938).

In the mid 1980s, isolated reports of animal deaths (typically dogs), presumably caused by blue-green algal toxins, prompted renewed interest in this subject and some work was conducted by the MPCA and collaborators to take a closer look at this issue. In recent years (2004 – 2007), several dog deaths, potential human health impacts and reports of very severe nuisance blooms have been reported. MC and supporting water quality data are typically collected in the course of these "incident" investigations.

In 2005 MPCA joined with the Minnesota Department of Natural Resources (MDNR), Minnesota Department of Health (MDH) and the Minnesota Veterinary Medicine Association to form the Minnesota Blue-green Algal Toxicity Workgroup to share information on blue-green algal toxicity, increase awareness within agencies and the veterinarian community and develop a public information campaign to raise awareness among the public. This resulted in development of a poster that was displayed in public places and veterinarian offices, several news releases and fact sheets, and an updated web site for further information and links to other states. These discussions also led the workgroup to the opinion that Minnesota had minimal information on magnitude and frequency of occurrence of MC in Minnesota's lakes.

In summer 2006 a study was conducted to characterize the magnitude and variability of MC concentrations in a set of eutrophic to hypereutrophic lakes. For this purpose, 12 lakes in south central Minnesota were selected with six each in the counties of Blue Earth and McLeod. The questions we sought to answer from this study include:

- What is the likelihood of encountering measurable MC at a pelagic (also referred to as mid-lake or index) site in a eutrophic to hypereutrophic lakes?
- What is the likelihood of the same when measuring MC at a near-shore site?
- What is the distribution of MC values for both mid-lake and near-shore sites? Are these distributions significantly different?
- How do values from this study compare to levels found elsewhere? How do they compare to World Health Organization guideline levels?
- Is there some seasonality to MC levels in these lakes?
- As bloom intensity (chlorophyll-a) increases, is there a greater likelihood of encountering high MC values?
- What limnological and physical factors appear to be associated with high MC concentrations?
- How can these findings be used to communicate risk to lake users?

Two additional MC data sets will be compared with the 2006 data. In 2007 MC samples were collected as a part of the National Lakes Assessment Project (NLAP). In contrast to the 2006 study, the NLAP lakes were randomly selected as a part of this nationwide, statistically based sampling effort. Details on NLAP may be found at http://www.pca.state.mn.us/water/nlap.html. A second data set is derived from MPCA incident-based monitoring in response to citizen concerns regarding severe nuisance blooms, dog deaths and/or potential human-health problems for the period 2004-2007.

Background

Study Area

The 2006 study focused on south central Minnesota near the North Central Hardwood Forest (CHF) and Western Corn Belt Plains (WCP) ecoregion transition (Figure 1)—two ecoregions with numerous eutrophic to hypereutrophic lakes.

Figure 1: 2006 study lake locations and ecoregion map

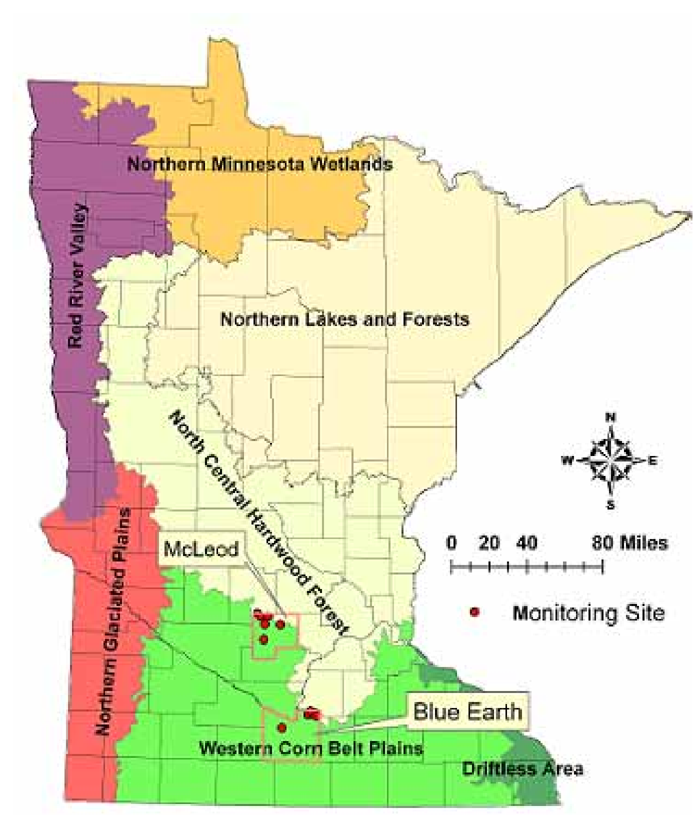
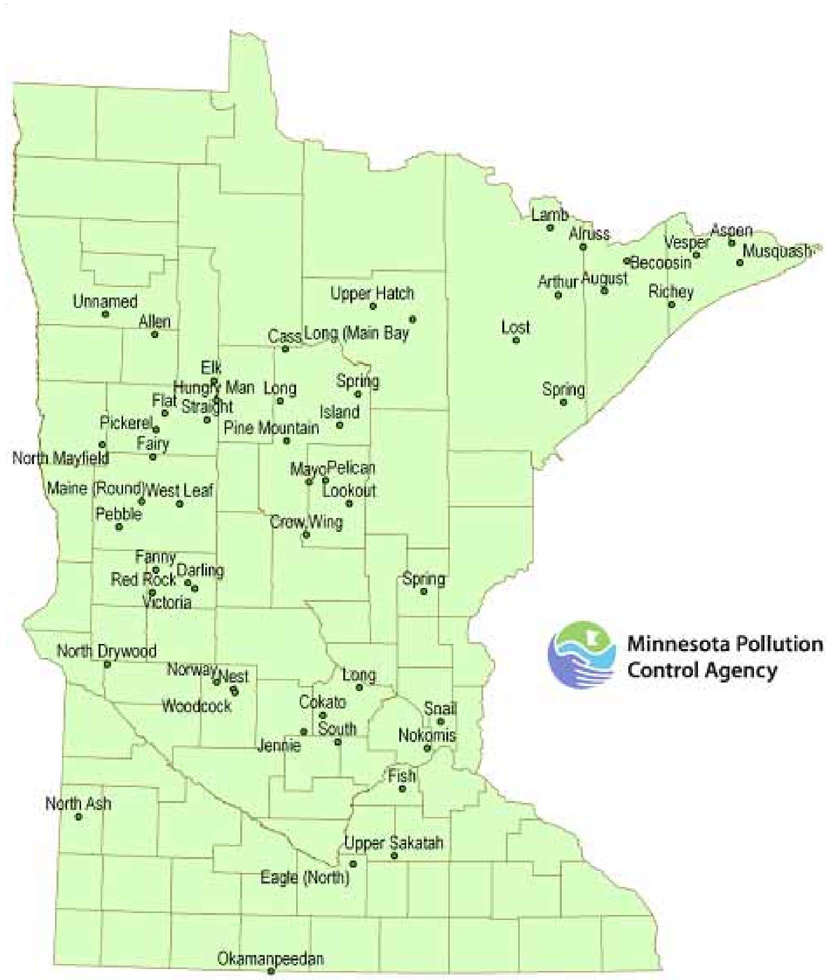


Figure 2: 2007 NLAP study lake locations



To the best of our knowledge, MC data was not collected previously for any of the study lakes. The 2007 NLAP lakes were distributed statewide (Figure 2) 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.

Climate

Summer 2006 was characterized by droughty conditions throughout much of central Minnesota. In general, 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 experienced five one-inch or greater events from May through September, while the southern portion was somewhat drier. Summer 2007 was characterized by warm and dry conditions as well 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 sites were selected based on established sampling sites whenever possible, and were typically located near the site of maximum depth. Near-shore sites were often 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. While pelagic sites were constant among sample events, the near-shore sites varied depending on the wind direction and 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 two-meter 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 one-meter intervals) were made at each pelagic site and surface measures were typically taken at the near-shore site. Secchi transparency was taken at all pelagic sites. Other observations included our standard subjective assessment of the physical condition and recreational suitability of the lake (Heiskary and Walker 1988) and basic observation on wind intensity and direction and percent cloud cover.

Chlorophyll-a (Chl-a) samples were filtered on the day of collection, filters 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 subset at the time of filtering and preserved in Lugols. These samples were later identified to family or genera in most cases by Dr. Howard Markus of the MPCA using the Minnesota Rapid Algal Analysis Procedure. This technique provides a semi-quantitative estimate of the relative biomass of the phytoplankton community and focuses on the dominant forms in the sample. This allowed for an estimate of the relative amount of MC-producing genera in the sample.

All water quality samples, with the exception of phytoplankton, were analyzed by the Minnesota Department of Health (MDH) lab in St. Paul. Method numbers, associated quality assurance information and all raw data are available in the detailed report (Lindon and Heiskary 2007).

Lakes in the 2007 NLAP study were sampled once in either July or August 2007. A majority of the water quality samples (including MC) were collected from a mid-lake (index) site. MC 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

Analysis was done by MDH using a bench-top Abraxis ELISA method, with a method detection limit 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. The MC analysis conducted for this study is considered as a quantification of MC congeners including nodularins and is intended to represent total MC. It has an assay method maximum quantifiable range of 5 µg/L that requires dilution of samples when concentrations are above this range. This can result in reduced accuracy depending on the amount of dilution. A summary of MC quality assurance, based on samples from the summer of 2006, is as follows:

- Percent recovery within 90-110% - 67%
- Percent recovery within 75-125% - 100%
- Coefficient of variation (CV) between sample and replicate <15% - 56%
- CV between sample and replicate <25% - 100%

World Health Organization (WHO) risk guidelines, established for recreational waters and drinking water, provide a basis for placing the MC data in perspective and describing relative risk. Though the guidelines are based on the congener LR as described in Guidelines for Safe Recreational Water Environments (WHO 2003), they are often used as a basis to evaluate total MC data. MC LR is considered the most frequently occurring form of MC (Chorus and Bartram 1999). The categories used are as follows:

- <1 µg/L very low risk
- 1- 10 µg/L low risk
- 10 - 20 µg/L moderate risk
- 20 – 2000 µg/L high risk
- > 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 those measurements that were very near the method detection limit for MC and below the 1 µg/L drinking water guideline for MC LR.

Results and Discussion

2006 seasonal patterns in chemical, physical and biological measures

Surface water temperatures ranged from about 14-16 C in May to peak temperatures on the order 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. A rapid cooling in most lakes was evident in September, consistent with a rapid decline in air temperature. Temperatures were conducive for blue-green algal growth (in excess of 22 C) from June through late August on most lakes.

All lakes were eutrophic to hypereutrophic based on TP, Chl-a and Secchi. TP ranged from less than 40 µg/L (Stahl's and Ballantyne) to over 300 µg/L (Otter and Silver). Based on previously-defined levels (Heiskary and Walker 1988) of Chl-a, "severe nuisance" (Chl-a > 30 µg/L) and "very severe nuisance" (Chl-a >60 µg/L) conditions were common throughout the summer at the pelagic sites on these lakes (Figure 3a). Chl-a was frequently higher at the near-shore sites (expected given the subjective nature of the near-shore sample collections) as compared to the pelagic sites (Figure 3b.)

MC was highly variable within and among lakes (Figures 4a & b). Over 25% of the data is between 0.9 µg/L and the non-detect substitution of 0.075 µg/L (Figures 5a & b). One µg/L and greater results were unevenly distributed up to 8,400 µg/L. Since MC maxima are of the most concern they were not considered as outliers. Six percent of MC results were below the detection limit. 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 percent of the pelagic samples were considered very low to low risk (Figure 5a). 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 among the pelagic and near-shore sites; however, the only very high risk measures were found at the near-shore sites (Figure 5a).

Toxic incidents involving MC or other blue-green algal toxins are most frequently associated with large surface-bloom-forming genera (Chorus and Bartram 1999; Chorus et al. 2001). 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 that common (Figure 5b). In a comparison of sites with and without surface scums, it was evident that the sites with surface scums exhibited higher and more variable MC concentrations compared to sites without scums (Figure 5b). Also, the likelihood of moderate to very high risk MC concentrations was found to be greater at sites with a distinct surface scum. These results are consistent with observations by Graham et al. (2004) when they note that MC in scums may be much greater than at pelagic locations.

MC exhibited no consistent seasonal pattern at either the pelagic or near-shore sites (Figures 4a & b). Silver and Hook lakes exhibited the highest pelagic concentrations and had the only concentrations that fell in the moderate risk level (Figure 4a). In contrast, seven of 12 lakes were below 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 (Figure 4b). Some of the highest concentrations were from samples collected in May and June, which was not expected.

2006 MC and other environmental factors

Field and laboratory studies have demonstrated that the relationship between cyanobacteria, MC concentration 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). Thus without getting to this level of analysis, isolating key environmental factors affecting MC is often difficult.

Associations among MC and several chemical, biological, and physical parameters were evaluated using the nonparametric Spearman's rank correlation coefficient (R_s). These comparisons resulted in four moderate and three high correlations with MC (Figure 6). Parameters exhibiting strong positive R_s with MC included: pH, Chl-a of MC producers, % Chl-a of MC producers, total suspended volatile solids (TSV), Chl-a, and subjective measures of physical condition. Negative relationships were found with alkalinity, Secchi depth and specific conductance.

Chl-a concentrations and trends were highly variable among the study lakes (Figure 3) and no significant linear relationship was evident between MC and Chl-a. Several lakes had extremely high Chl-a through the majority of the summer. Pelagic-site Chl-a concentrations from Silver and Hook lakes (Figure 4a) were among the highest in the study as was the case for MC (Figure 3a). In contrast, Chl-a for Loon and Otter lakes was also very high but MC was very low (Figures 3 and 4). MC had a moderate relationship with TSV (R_s=0.51; Figure 6); however, this relationship is likely a function of the high collinearity with Chl-a, since algae comprise much of the TSV in these lakes.

Figure 3a: 2006 pelagic (mid-lake) chlorophyll-a concentrations by lake and date

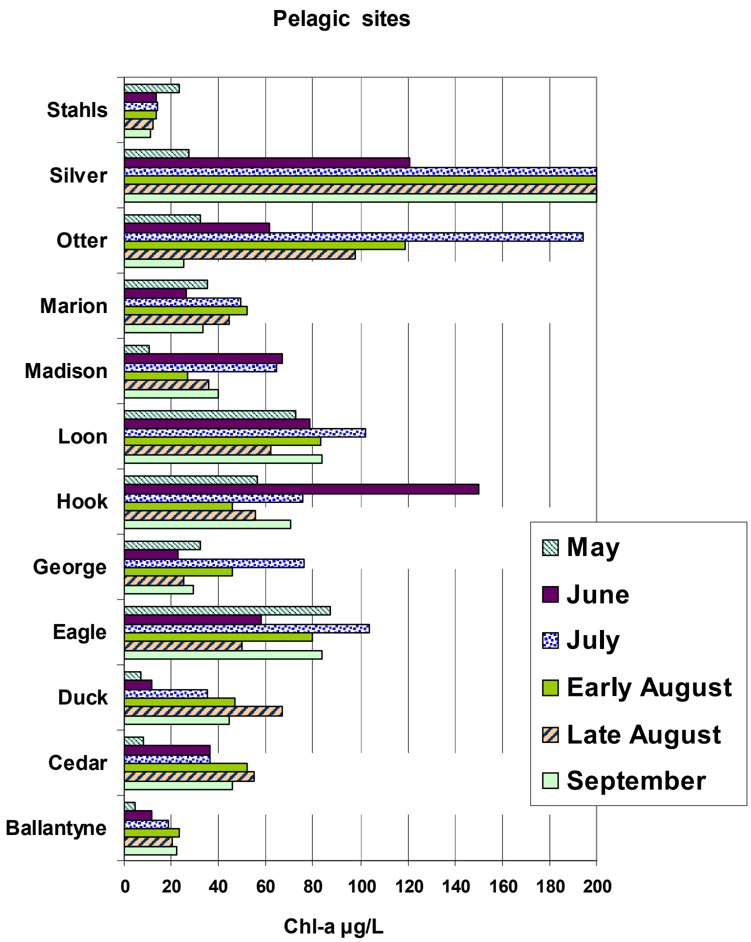
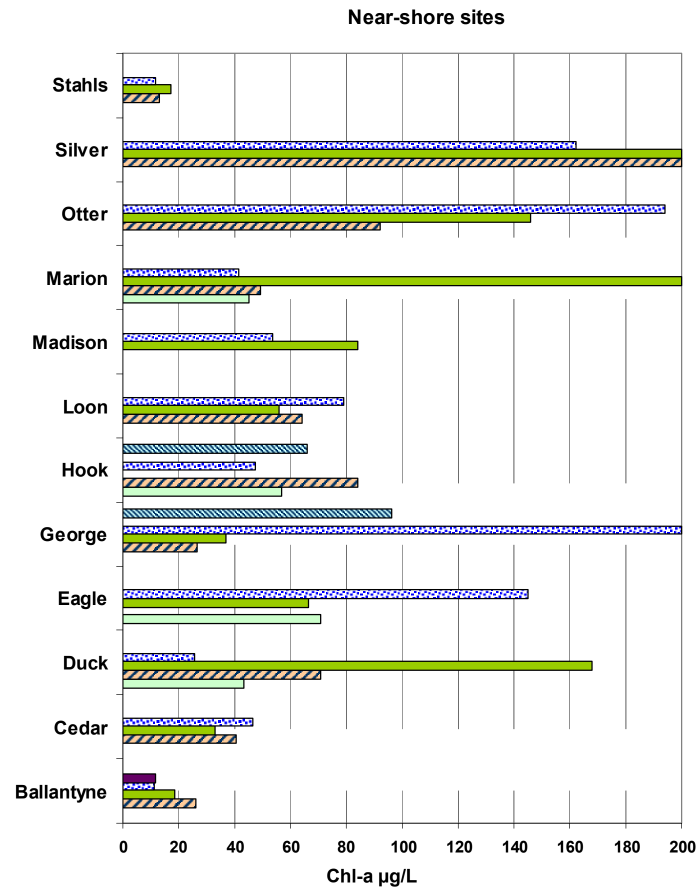


Figure 3b: 2006 near-shore chlorophyll-a concentrations by lake and date



Pelagic Chlorophyll-a values that exceed 200 µ/L

Lake	Value	Month
Silver	July	218
Silver	Early August	365
Silver	Late August	284
Silver	Mid-August	289

Near-shore Chlorophyll-a values that exceed 200 µg/L

Lake	Month	Value
Silver	Early August	357
Silver	Late August	293
Marion	Early August	284
George	July	238

Figure 4a: 2006 pelagic (mid-lake) microcystin (MC) concentrations by lake and date

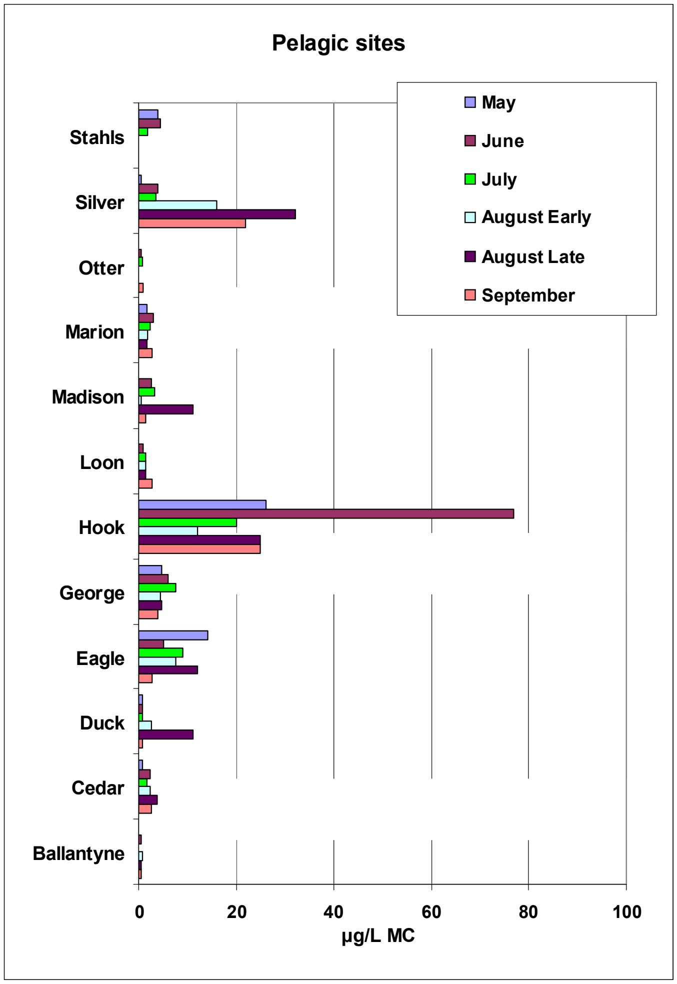


Figure 4b: 2006 near-shore microcystin (MC) concentrations by lake and date

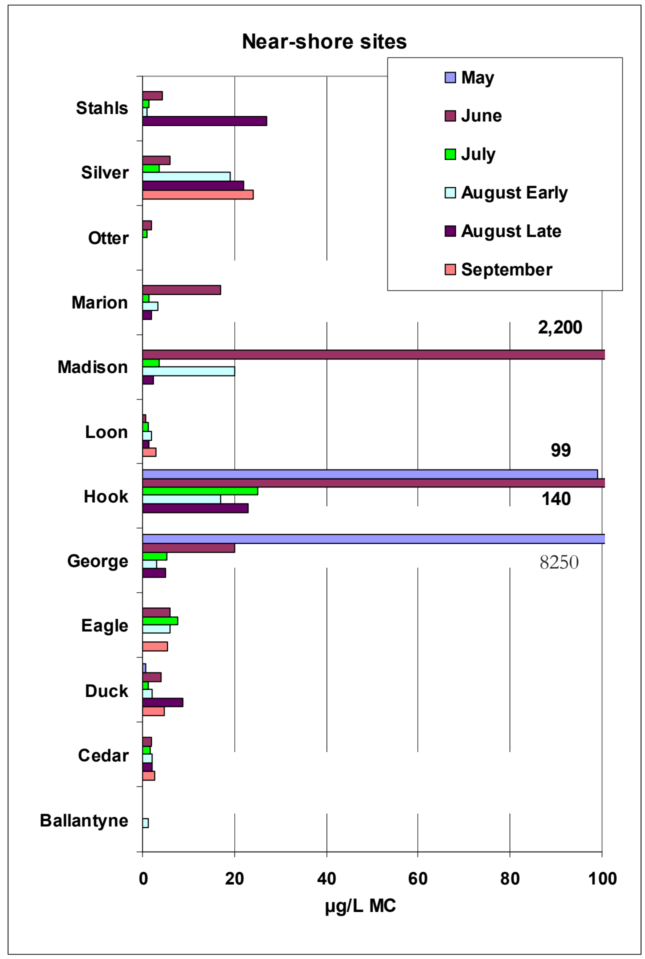


Figure 5a: 2006 MC frequency distribution comparison for mid-lake (pelagic) and near-shore sites

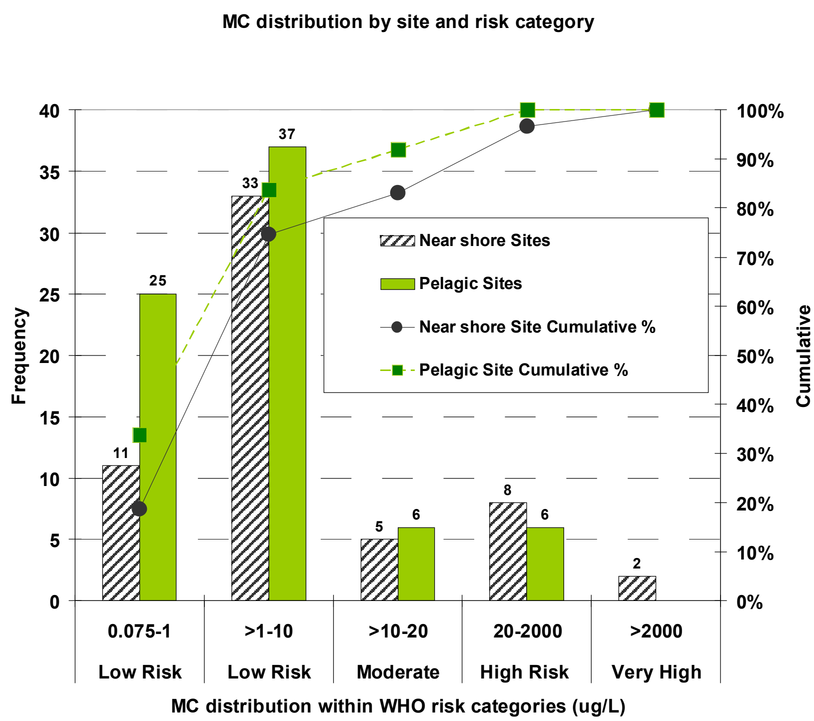
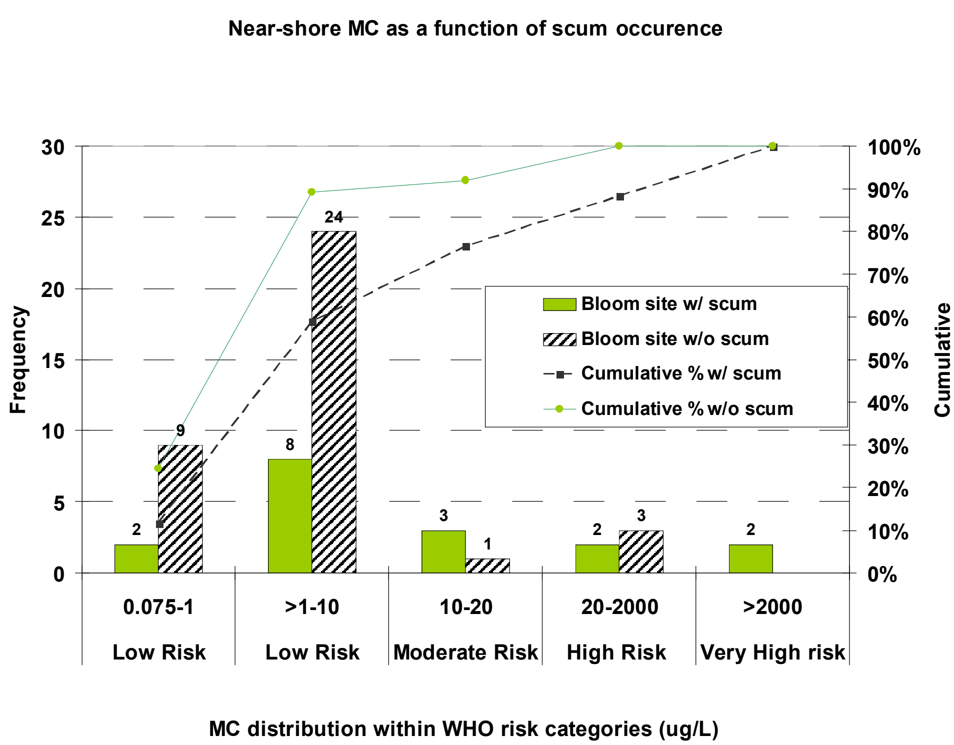


Figure 5b: 2006 near-shore MC frequency distribution comparisons for sites with scum vs. no scum



Combining MC and bloom intensity provides a basis for describing the "risk" of encountering specified levels of MC as a function of bloom intensity. Based on Figure 7a, moderate-risk MC concentrations were not encountered until blooms exceeded 30 µg/L ("severe nuisance"). As blooms exceeded 30 µg/L, the frequency of moderate- to high-risk MC increased to 12 percent and above 50 µg/L the likelihood of encountering moderate to high risk MC increased to 28 percent. All high risk MC concentrations were associated with Chl-a > 30 µg/L.

A strong inverse relationship between Secchi and algal biomass (Chl-a) has long been noted. Secchi depth also exhibits an inverse relationship with MC (Figure 6). Similar to the relationship between MC and bloom intensity, there is somewhat of a threshold effect; as Secchi declines below 0.5 m, there is an increased frequency (risk) of moderate- to high-risk MC (Figure 7b). Dense blooms of MC-producing blue-greens, such as Anabaena and Microcystis, routinely result in very low transparency.

The variable with the strongest relationship with MC was pH (R_s=0.73; Figure 6), which is consistent with correlations observed by Paerl and Ustach (1982). In the 2006 study, high correlation with pH is likely a reflection of algal productivity (Chl-a R_s=0.58). Wetzel (2001) notes "rapid photosynthesis can rapidly reduce total dissolved inorganic carbon (DIC) and increase pH." Further, cyanobacteria prefer a high pH environment (Shapiro 1973). Over 90% of the pH values >9.0 was associated with severe nuisance bloom levels (Chl-a >30 µg/L). All high-risk MC concentrations corresponded to pH levels >9.0 (Figure 7c). As a matter of perspective, the state water quality standard for pH is 9.0 and a typical pH range for WCP ecoregion lakes is 8.2 – 9.0.

Conversely, alkalinity exhibited a moderate negative relationship (R_s = 0.61). It is unlikely that alkalinity is a direct driver of MC production; rather it may reflect the superior competitive advantage that blue-greens have at low DIC concentrations, which are most likely to occur in eutrophic, low alkalinity systems at high pH. In the 2006 study all pH values of 9.0 or greater were associated with alkalinities of 150 mg/L or less.

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 8). Based on this comparison, it is evident that the distributions of these three data sets are

Figure 6: 2006 Spearman correlation coefficients (R) for various parameters relative to MC. Parameters sorted by R value. Negative (-) implies inverse relationship between parameter and MC.

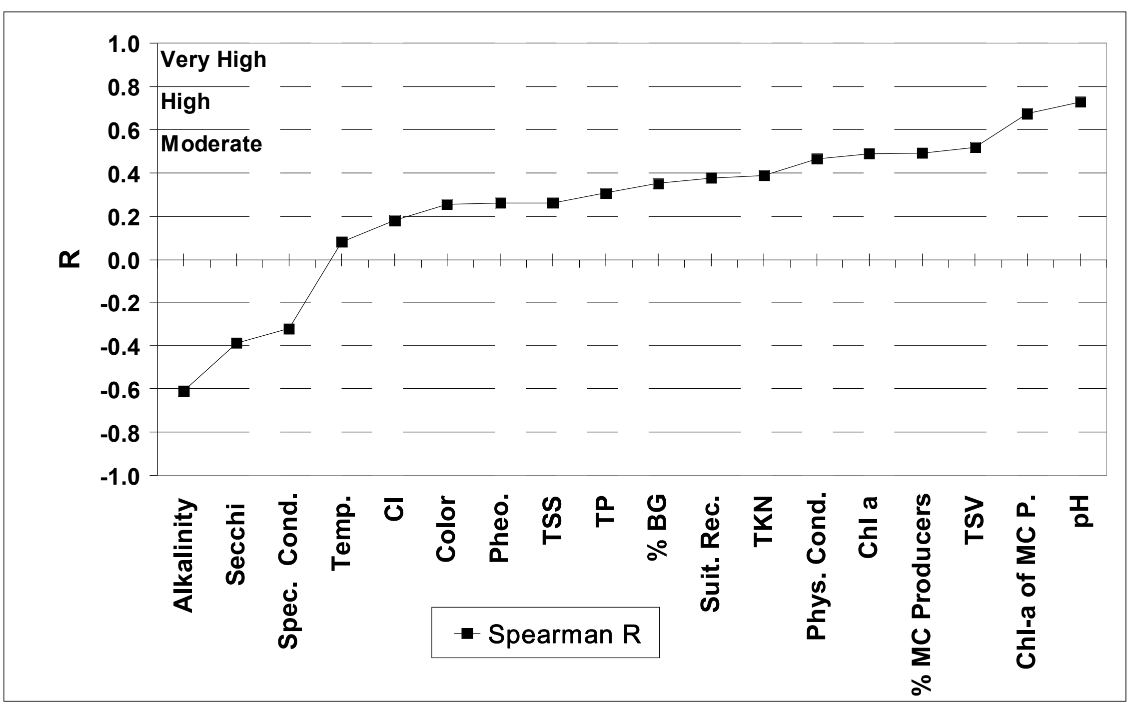
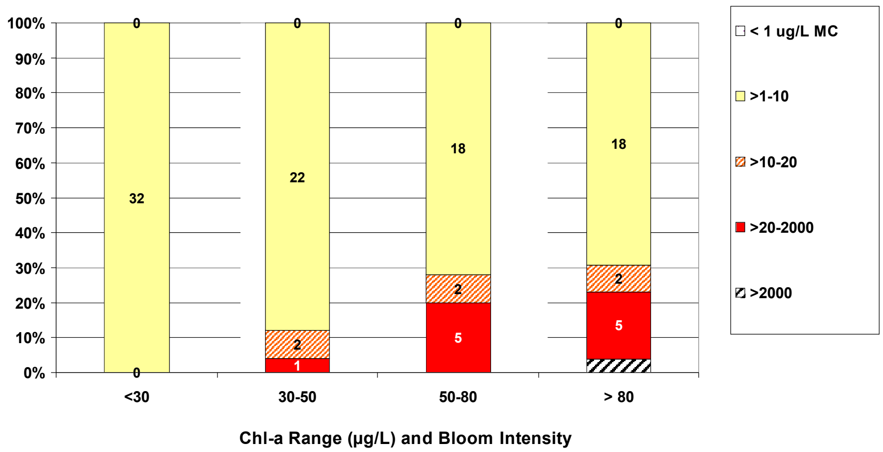


Figure. 7a,b, c: Microcystin (MC) as a function of a) bloom intensity (chl-a concentration); b) Secchi; and c) pH

a.



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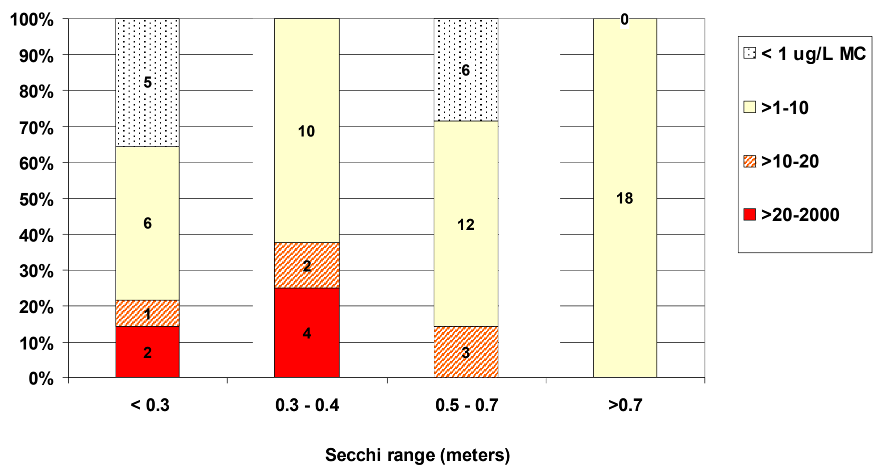

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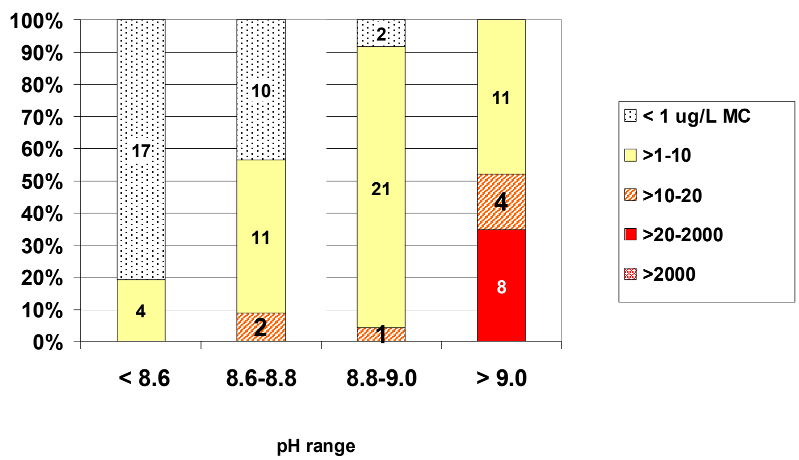
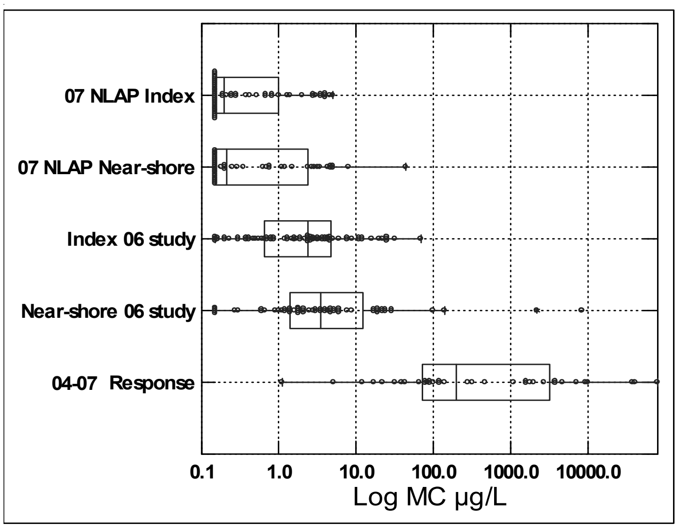


significantly different, which comes as no surprise given the population of lakes sampled and focus of each study. As expected, the 2006 eutrophic lakes exhibit a larger range and more extreme MC concentrations as compared with the NLAP data (Figure 8). Both of these data sets reveal higher MC concentrations at near-shore sites as compared to mid-lake index sites. The incident-based sampling yielded some extremely high event-based values, which again is not too surprising given the intensity (magnitude) of the blue-green blooms sampled.

Figure 8: Comparison of frequency distributions for: 2006 study (index and near-shore), 2007 NLAP study (index and near-shore), and complaint-based data.



Summary

Cyanobacteria have the ability to produce several different toxins, which may be acutely and chronically toxic. There has been extensive study worldwide on this issue. Numerous articles in the literature document 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. Other studies such as Graham et al. (2004) describe the distribution of particular toxins (e.g., MC) and some environmental factors that may contribute to production of the toxin.

The 2006 study focused on one of the toxins, MC, in eutrophic to hypereutrophic lakes in south-central Minnesota. Several questions intended to advance knowledge on the extent, magnitude and frequency of MC in Minnesota lakes were posed. The questions listed in the Introduction provide a basis for organizing the following summary comments:

- MC was above the MDL (>0.15 µg/L) in over 94% of the 133 samples collected from May – September at both near-shore and pelagic sites. Over 60% of the pelagic MC samples were 1 µg/L or less as compared to 25% of the near-shore samples. The near-shore samples exhibited a much larger range (Figure 4b) and much higher maximum value (8,400 µg/L) as compared to the pelagic samples (77 µg/L). Likewise near-shore mean and median MC was higher than the pelagic samples.

- WHO guidelines provided a basis for evaluating the relative risk of the MC levels measured in this study. 80% of all MC values 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 high risk category (Figure 4a). Only two near-shore samples were in the very high risk category (Figure 4b).

- It was anticipated there would be some seasonality to the MC concentrations, perhaps consistent with patterns often observed for Chl-a and nuisance algal blooms – whereby late summer is often characterized by elevated Chl-a and severe nuisance blue-green algal blooms. However, based on the 2006 study, there was no distinct seasonality to MC concentrations. This was due in part to a couple of 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.

- A relatively distinct relationship was observed among MC and algal bloom intensity. When Chl-a remained $\leq$ 30 µg/L, MC was in the very low to low risk categories (Figure 7a). As Chl-a increased to the 30-50 µg/L range, MC was in the moderate- to high-risk range in about 12% of the samples and as Chl-a increased to > 50 µg/L, the moderate to high MC risk increased to approximately 30%. High and very high risk MC were found only when Chl-a was > 30 µg/L.

- Several limnological and physical factors were tested for their association with MC. Because of extreme values and a non-normal distribution, nonparametric R_s was used for this purpose. Based on R_s strong positive relationships with MC were noted (in order of R_s) for pH, Chl-a of MC producers, TSV, %MC producers, Chl-a and physical condition rating. Strong negative relationships were found for alkalinity, Secchi and specific conductivity. This suggests that as the relative abundance of MC-producing genera increases, MC increases as well. The correlations with pH and alkalinity are to some degree an expression of algal productivity and buffering capacity of the lakes. With the exception of one sample, all lakes with high MC risk had a pH of 9.3 or greater. The negative correlation with Secchi is a function of the overall abundance of algae (Chl-a) and that several of the MC-producing genera have small cells that form dense colonies that limit light. In this study moderate- to high-risk MC was found only when Secchi was 0.5 m or less.

The 2006 study, as is the case with most studies on MC, does not allow for precise prediction as to which blue-green algal blooms will produce MC in the moderate to very high risk range. However, it does suggest that Minnesota's current recommendations to the public to avoid contact with severe nuisance blooms is sound advice. Severe and very severe nuisance blooms are readily recognizable to staff and public in general. Further, it was found that moderate to high MC was often associated with high pH ($\geq$ 9.3) and low Secchi (<0.5 m), two parameters that are easy to measure. Further, three independent data sets indicate that MC is measurable across a wide range of Minnesota lakes and collectively, they provide a good basis for evaluating MC results from future monitoring efforts

Risk communication

Minnesota does not have widely accepted thresholds (nor do most states) for assessing MC risk for aquatic recreational use. Hence WHO thresholds were used as a basis to assess risk; however, no attempt was made to assess their validity for assessing risk in Minnesota's waters. Further review of WHO and related MC thresholds should be conducted among resource management agencies. This review would ideally result in mutually agreed upon thresholds for assessing and communicating MC risk to humans and animals. Also from a risk communication standpoint, it is important to remember that several other toxins (e.g. saxitoxin and anatoxin) may be produced by Cyanobacteria as well as other algae. It may be of value to determine their relative concentrations and how they vary relative to MC, Chl-a and other factors in future studies.

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