Genetic and Environmental Factors Influencing *Microcystis* Bloom Toxicity

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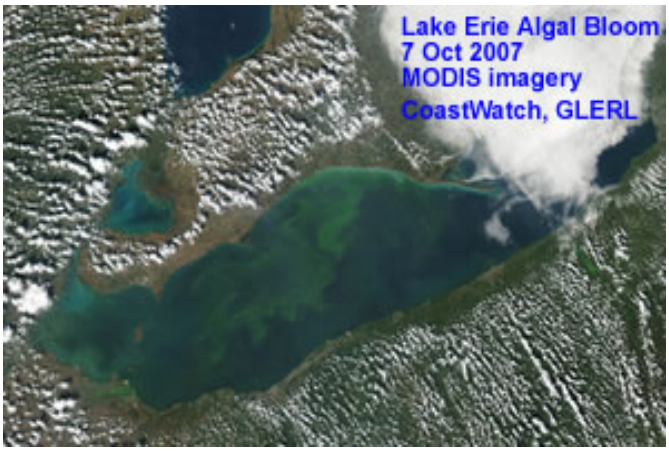
Overview

The ultimate goal of this project is to forecast the development and toxicity of the harmful algal bloom, *Microcystis*. Community structure of *Microcystis* and microcystin toxin production are controlled by both environmental factors (light, nutrients, grazers), and genetic composition. In order to forecast bloom toxicity, the numbers of toxic genotypes present in the *Microcystis* community and the environmental regulators of toxin production must be known. Currently, a quantitative PCR assay has been developed to look at both the genetic and environmental factors controlling microcystin production. This project will adapt the assay to quantify not only the number of potentially toxic strains present, but also how many are actively expressing the microcystin synthetase genes. The mcyB genotypes of these strains, which indicate microcystin production, will also be compared with the microcystin congeners produced to determine any significant relationship. Finally, the distribution of a potential new cyanobacterial harmful algal bloom (HAB) invader, *Cylindrospermopsis raciborskii*, will be determined using samples collected across Lake Erie during the summers of 2006 and 2007. The expected results of this project will be used to develop models for forecasting the growth and toxin production in *Microcystis* blooms in the Great Lakes and identify the potential for a new invasive cyanobacterial harmful algal bloom.



Proposed Work

Develop quantitative RT-PCR method to determine environmental conditions that promote microcystin production

In the past year, we developed a quantitative PCR (qPCR) assay based on the mcyB gene to determine how the number of toxic *Microcystis* cells changes in response to environmental forcing factors, such as Zebra mussel grazing. This assay confirms the presence of cells

capable of producing microcystin, but it does not determine if a given environmental stress turns on toxin production. For example, does *Microcystis* produce microcystin in the presence of grazers? We will build on the techniques already developed: extracting RNA, reverse transcription to cDNA and then quantifying by qPCR to measure mcyB gene expression. The assay will be used in experiments planned during the summer 2008 with Hank Vanderploeg and others (Vanderploeg-04) and will assess whether the exposure of *Microcystis* to Zebra mussel grazing induces toxin production by measuring mcyB expression. Additional environmental controls on microcystin production will be analyzed using samples collected during the summer of 2006 from previous nutrient and light manipulation experiments.

Identify the relationship between genotype and microcystin congener produced

Data from *Microcystis* isolated in the August 2004 and August 2005 sampling cruises on western Lake Erie and Saginaw Bay have suggested that there is a correlation between *Microcystis* genotype and the congener of microcystin produced. While the genes responsible for microcystin production are known (mcy genes), the genetic basis for the different microcystin congeners (-LR, -RR, etc) is not well understood. During the extensive *Microcystis* bloom in Muskegon Lake in the summer of 2007, samples were collected for comparative analysis of microcystin congeners (analyzed by Rick Rediske, Grand Valley State University) and genetic composition. We will compare the congener distribution with the genetic composition of the *Microcystis* at each station and time to determine if this connection is confirmed.

Identify presence of new invasive cyanobacterial harmful algal bloom, *Cylindrospermopsis raciborskii*

A proposed addition to the Aquatic Invasive Species list in the Great Lakes is the toxic *Cylindrospermopsis raciborskii* , a filamentous cyanobacteria that produces the hepatotoxin cylindrospermopsin. This species is common in tropical and subtropical lakes, but has recently been discovered in temperate locations, including Muskegon Lake (western Michigan, Hong et al. 2006) and was seen last summer in western Lake Erie (by Dyble, Wilhelm, Boyer, on August

2007 cruise). This species can live in low light and could be very successful in the turbid waters of Maumee Bay or deeper in the water column in a *Microcystis*-dominated system. Samples have been collected from Lake Erie for the summers of 2006 and 2007 and PCR primers have been developed to detect its presence from previous research. The distribution of this species in samples from the past 2 summers will be determined to assess *C. raciborskii* spread in Lake Erie. Positive samples will be sequenced to determine the genetic diversity and its degree of similarity to populations in other parts of the world. This work would provide an excellent basis for assessing the spread of this species into the Great Lakes and could predict future impacts of global warming on *C. raciborskii* distribution.

Accomplishments

- Developed a quantitative PCR assay for determining the number of toxin genes (mcyB) in a water sample; Rajreni Kaul, undergraduate student at MSU, contributed to the development
- Applied assay to samples collected biweekly (July - October) from three sites in western Lake Erie: just offshore Monroe (station A), inner Maumee Bay (station B) and Toledo Light (station M) Maumee Bay (B) had higher concentrations of toxic *Microcystis* in August; numbers dropped off quickly by September
    - Toledo Light station (M) peaked in September, low again by October
    - Lower, more consistent, concentrations at Monroe station (A) from August to October

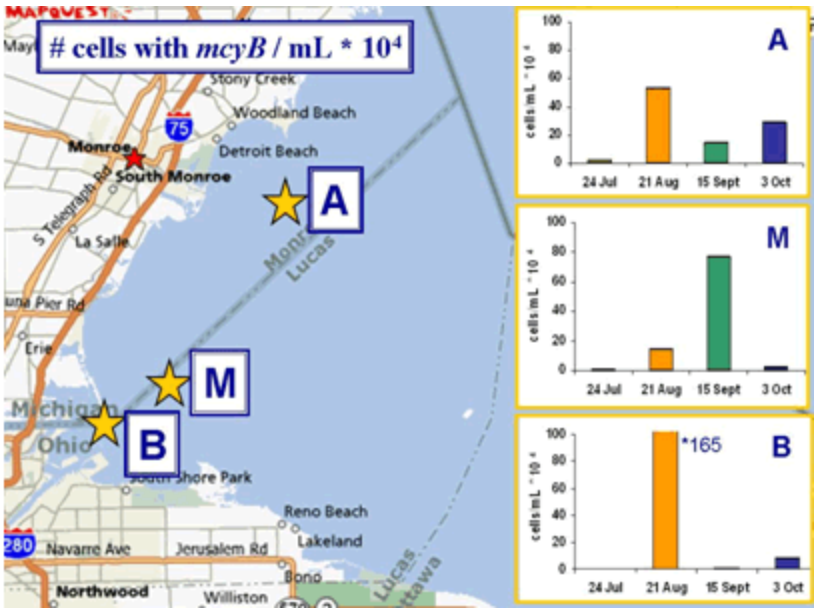


Figure 1: *Map of western Lake Erie with sampling stations marked (A, B, M) and the number of toxic Microcystis cells at each station at 4 times points in the summer of 2006 as assessed by a quantitative PCR assay for the mcyB gene (involved in microcystin synthesis).*

- Found 5 predominant genotypes with no consistent pattern in their distribution between sites and times
    - *Microcystis* communities change over course of a bloom
    - Genetic method needed to assess how bloom toxicity changes over summer

Scientific Rationale

The recent increase in cyanobacterial HABs in the Great Lakes has caused significant concern for human and ecosystem health due to the production of toxins by bloom species. In the Great Lakes, *Microcystis* dominates the bloom community and produces the hepatotoxin microcystin (Brittain et al. 2000, Carmichael 1994, 1997, Vanderploeg et al. 2001). Studies have documented the presence of microcystins in the Great Lakes, at times exceeding the recommended limit of 1 µg L-1 of microcystin established by the World Health Organization for drinking water supplies (Brittain et al. 2000, Vanderploeg et al. 2001). The increase in *Microcystis* blooms in recent years has caused concern due to the dependence on these waters as a resource and the health risks attributed to microcystins. The ability to measure the distribution and concentration of microcystin in the Great Lakes, including the various microcystin congeners, is therefore essential to protecting human and ecosystem health in this region.

Despite very limited studies, relatively high concentrations of microcystin have been found in Saginaw Bay, western Lake Erie, and in the western region of Lake Ontario (Brittain et al. 2000; Vanderploeg et al. 2001; Murphy et al. 2003). Microcystin concentrations of 3.5 µg L-1 were measured in Saginaw Bay in July 1995, and estimated to be as high as 24 µg L-1 in western Lake Erie in September 1995 (Vanderploeg et al. 2001). In a shallow harbor in western Lake Ontario, microcystin concentrations were as high as 400 µg L-1 in the summer of 2001 (Murphy et al. 2003). In 2003, microcystin concentrations throughout Saginaw Bay and western Lake Erie were commonly above 1 µg L-1 and as high as 58 µg L-1 in wind-accumulated scums (Dyble et al. submitted). Thus, concentrations of microcystin in the Great Lakes may pose a threat to human and ecosystem health.

Production of microcystins by *Microcystis* and other related cyanobacterial species is under complex genetic and ecological control. Production is controlled by the mcy genes, a bidirectionally transcribed complex of 10 open reading frames that control synthesis of polyketide synthases and peptide synthetases involved in microcystin synthesis (Dittman et al. 1997, Kaebernick et al. 2002). Not all *Microcystis* strains produce microcystin and non-toxic strains do not appear to carry mcy genes. In toxic strains, genetic differences within mcy result in the production of different amounts and types of microcystin. Toxic strains continuously produce microcystins (Kaebernick et al. 2000) and cell quotas vary with cell growth (Orr and Jones 1998) and between strains (Carmichael 1997). Changes in environmental factors that regulate growth, such as nutrients and light, can result in a 2-10 fold increase in toxicity. These environmentally induced changes in toxicity can be relatively minor compared to the 10-1000 fold increase in bloom toxicity associated with genetic shifts in community composition toward the predominance of more toxic strains (Zurawell et al. 2005). Therefore, predicting bloom toxicity requires an understanding of the genetic variation within the bloom and cannot be based on cell counts alone.

Quantitative PCR (qPCR) is becoming one of the best ways to compare environmental effects on the genetic level. Combining a measure of the number of mcyB genes (correlating to the number of toxic *Microcystis* cells present) and the expression of these genes, RT-qPCR shows the impacts of changing environmental factors on a cellular level. This method can provide information on how the number of toxic genotypes changes over the course of a bloom and will be used for determining the distribution of toxic vs. non-toxic strains. These qPCR assays will

be valuable in forecasting toxic blooms by determining *Microcystis* blooms with genotypes harmful to human and ecosystem health.

Governmental/Societal Relevance

Harmful algal blooms are of great importance to NOAA, GLERL, the scientific community and the public because of their potentially detrimental effects on ecosystem and human health. Legislature and the scientific community have supported HAB studies due to microcystin concentrations above the WHO recommended limit of 1 µg L-1 in western Lake Erie and Saginaw Bay. Traditional measures of detecting *Microcystis* by microscopic cell counts require more time than available for rapid management decisions. In addition, cell counts are not an accurate indicator of toxicity. *Microcystis* blooms can be comprised of toxic and nontoxic strains and a largely non-toxic bloom will not likely have a significant human health risk. The proposed research will develop methods for detecting toxic strains of *Microcystis* and determining whether blooms in western Lake Erie and Saginaw Bay may be detrimental to human and ecosystem health in this region.

Relevance to Ecosystem Forecasting

This project has direct links to forecasting the effects of toxic *Microcystis* blooms in the lower Great Lakes. In order to develop forecasts for the presence of toxic blooms, it is essential to know if blooms are comprised of toxic strains and what environmental and ecological factors are responsible for the selection of those genotypes. The results of this research can provide input for a biophysical model that will couple *Microcystis* growth and toxin production to basin hydrodynamics to provide forecasts that will be useful for monitoring and managing toxic blooms.

Products

Presentations

IAGLR, *Detecting and quantifying toxic Microcystis populations using genetic analyses*, 31 May 07, Penn State U, PA.

OHH Annual Meeting, *Current successes, challenges, and going forward at the Center of Excellence for Great Lakes and Human Health*, 25 March 2007, WHOI.

ASLO Ocean Sciences meeting, Orlando, FL, *Assessing the role of grazing on genetic composition of Lake Erie cyanobacterial HAB populations*, 3 Mar 08

KBS seminar series, Gull Lake, MI, *But it is toxic? Assessing the distribution of toxic Microcystis and environmental factors controlling microcystin production using genetic analyses*, 21 Mar 08

IAGLR, Peterborough, ON *Cyanobacterial HABs in the Great Lakes: Environmental stressors, genetic diversity and impacts on human health*, 21 May 08

Publications

Dyble, J., Fahnenstiel, G.L., Litaker, R.W., Millie, D.F., and P.A. Tester. Accepted. Microcystin concentrations and genetic diversity of *Microcystis* in the lower Great Lakes. *Environmental Toxicology.*

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