Volume 7 2006

Great Lakes Research Review

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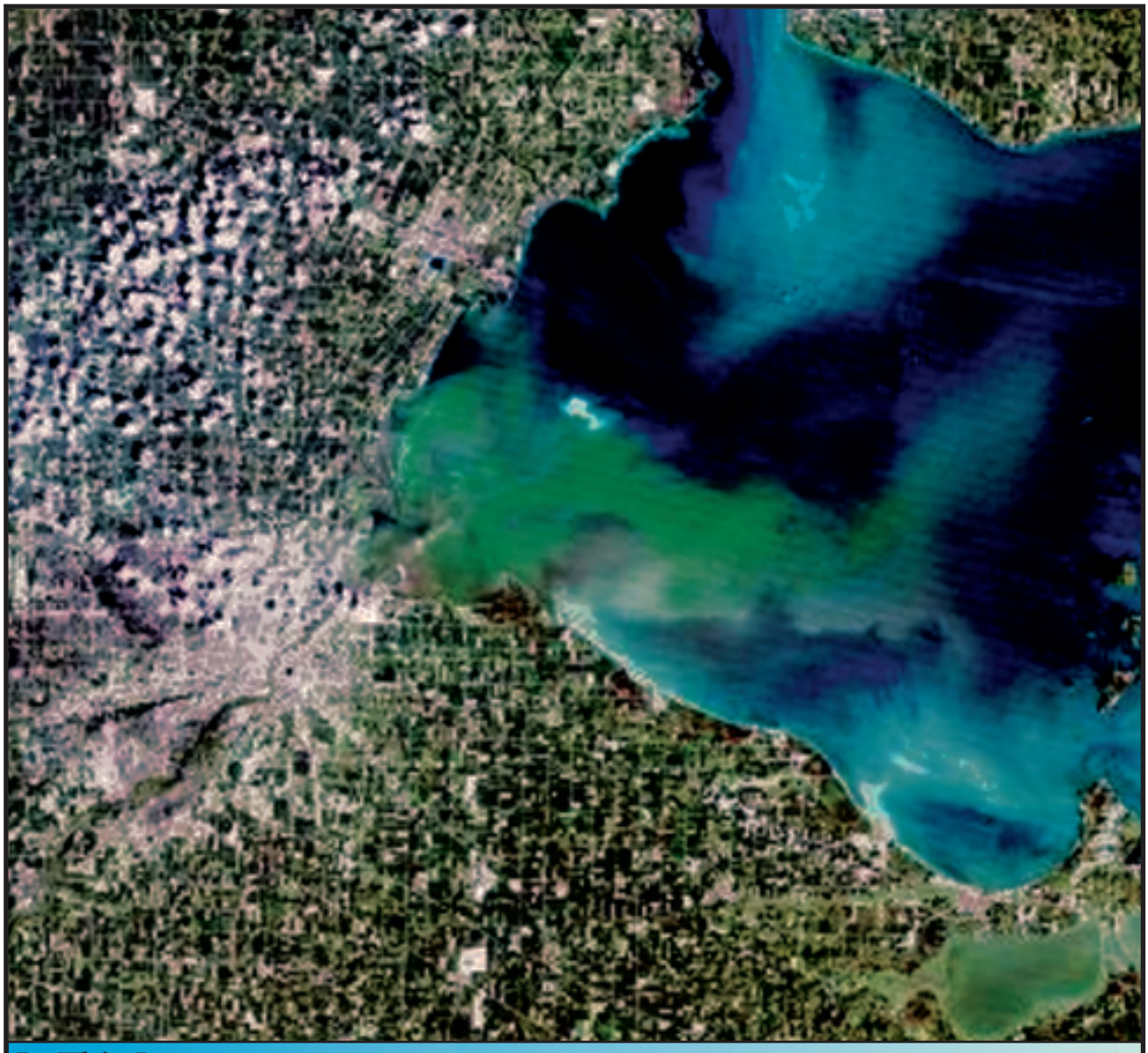
Monitoring Harmful Algal Blooms



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Great Lakes Research Review

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ABOUT THIS PUBLICATION:

Several years ago, staff from the Great Lakes Program, the Great Lakes Research Consortium, and New York Sea Grant realized an information gap existed between peer reviewed journal articles and newsletter type information related to Great Lakes research. The Great Lakes Research Review was created to fill that gap by providing a substantive overview of research being conducted throughout the basin. It is designed to inform researchers, policymakers, educators, managers and stakeholders about Great Lakes research efforts, particularly but not exclusively being conducted by scientists affilliated with the Consortium and its member institutions.

Each issue has a special theme. Past issues have focused on the fate and transport of toxic substances, the effects of toxics, fisheries issues, and exotic species. The most recent volumes have focused on the Lake Ontario, St. Lawrence River and Lake Erie ecosystems, and the research of the New York Great Lakes Protection Fund. The present issue describes some of the research by the MERHAB Project. We gratefully acknowledge all of the contributing authors who willingly shared their research efforts for this publication, especially Greg Boyer for his assistance-with editing and organizing authors. We also wish to thank the SUNY ESF Office of News and Publications for assistance.

THE UPCOMING ISSUE

For more information, contact Michael Connerton at mjconner@mailbox.syr.edu

ON THE COVER: Image of *Microcystis* bloom in Western Lake Erie, August 2003

Volume 7 2006

Great Lakes Research Review

Monitoring Harmful Algal Blooms in the Great Lakes

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Introduction

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"And all the waters that were in the river turned to blood. And the fish that were in the rivers died; and the river stank, and the Egyptians could not drink the water of the river, …"

Exodus 7:20-21

Harmful algal blooms are not new. They have been around centuries and their impacts on humans were recorded in biblical times. These blooms can negatively impact our drinking waters, pets, livestock, fish and the enjoyment of our aquatic resources.

Most research on harmful algal blooms has focused on marine environments where the negative impacts of "red tides" is both well studied and well understood. In 1993, the US government recognized that marine biotoxins represented a significant and expanding threat to human health and fisheries resources and established an interagency national plan to address both the research and monitoring needs of our nation (WHOI, 1993). This national plan spawned several national research agendas, most notably Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) and Monitoring and Event Response for Harmful Algal Blooms (MERHAB). While much of ECOHAB and MERHAB's efforts have focused on our coastal resources, great lakes issues are also recognized as an important component of these research agendas.

ECOHAB and MERHAB fund both small individual investigator projects focusing on a single problem and large regional efforts that span across traditional disciplines and boundaries. In 2002, NOAA's Coastal Ocean Program through MERHAB funded the first regional effort to look at toxic cyanobacteria in the lower Great Lakes ecosystem. This project, termed MERHAB-LGL, focuses on Lakes Erie, Ontario and Champlain. The MERHAB-LGL project has more than 30 specific objectives, all focused on the development of cost effective monitoring strategies to protect our drinking and recreational waters from cyanobacterial blooms. No technique is off limits and the project combines the efforts of analytical chemists, classical taxonomists, molecular biologists, experts in satellite imagery, hydrodynamic modelers and outreach experts as it works towards a unified approach to protecting the public. Sampling platforms range from the docks of local homeowners to large ships operating miles from shore for extended periods of time. This is interdisciplinary science at its best.

The following articles provide a general introduction to these efforts, as well as summarize some of our current work using citizen monitors, satellites, the microscope and the PCR thermocycler to protect our waters from toxic blooms. While we may not be able to stop our rivers from turning to blood, we will know when and if it is safe to drink and swim in the water.

Toxic Cyanobacteria in the Great Lakes: More than just the Western Basin of Lake Erie

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Cyanobacteria, or blue-green algae, are ubiquitous in nature and found in nearly all environments. Many species have selective advantages such as the ability to use atmospheric nitrogen for growth, or the production of gas vacuoles to control their exposure to light that allows them to dominate other phytoplankton during the later months of the season. These cyanobacterial blooms can lead to taste and odor problems in drinking waters and the formation of surface scums. Cyanobacteria can also produce extremely potent toxins and if this occurs, the blooms can be hazardous to animals and humans alike. One need only look at their historical names: slow death factor, fast death factor and very fast death factor, to appreciate their effects.

Saxitoxin (STX), one of several toxins produced by cyanobacteria, has a lethal dose (LD-50) of approximately 8 µg per kg body weight and is about 1000 times more toxic than a typical nerve agent such as sarin. A recent bloom of *Anabaena circinalis* from Australia contained up to 3,400 $\mu g\ gdw^{-1}$ of STX or related toxins, and was responsible for the death of 1600 cattle and sheep[7]. The microcystin peptides produced by *Microcystis* and numerous other cyanobacterial species are potent hepatotoxins. The occurrence of these toxins in drinking water has resulted in human fatalities and an increased incidence of liver cancer. The World Health Organization has established an advisory threshold of 1 microgram per liter ($\mu g\ L^{-1}$) for drinking waters and prepared an excellent review on the animal and human health aspects of cyanobacterial toxins[6].

Cyanobacteria in the Great Lakes

In 1995, Lake Erie experienced a large bloom of toxic cyanobacteria in the western basin. This bloom was caused by the species *Microcystis aeruginosa*, which produced the hepatotoxin microcystin-LR and lesser amounts of two minor toxins, demethyl (Asp3) microcystin-LR and microcystin-AR [3]. Since that initial description, toxic *Microcystis* has continually reoccurred in the western basin of Lake Erie with toxin levels often exceeding the WHO advisory level of 1 $\mu g\ L^{-1}$. These blooms have been studied as part of a number of programs (See "For More Information" in this article.)

Figure 1. LandSat image of *Microcystis* bloom in Western Lake Erie, August 2003 (Rinta-Kanto et al., 2005).

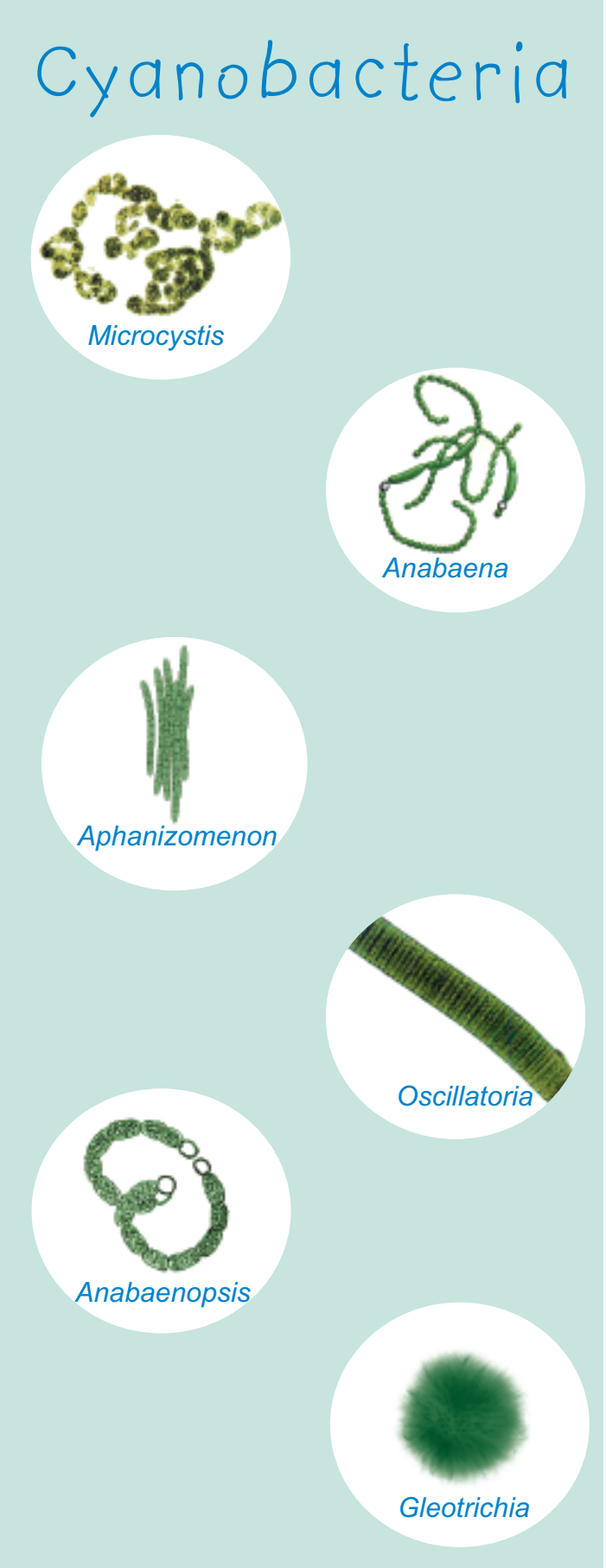
The *Microcystis* blooms in the western basin of Lake Erie often span large areas and are readily visible from outer space (Figure 1). The occurrence of these cyanobacterial blooms represents an important health issue as the western basin of Lake Erie supplies drinking water for several major metropolitan areas (e.g. Toledo, Cleveland), and serves as an important recreation area for millions of visitors to the Great Lakes area. However this issue is greater than just *Microcystis*, the microcystins hepatotoxins and the western basin of Lake Erie. Here we review some of

the current findings on cyanobacterial toxins from the MELEE and MERHAB programs. Other articles in this series deal in more depth with the issue of these toxins in Lakes Champlain and the embayments of Lake Ontario.

Introduction to Cyanobacterial Toxins

Chemically, cyanobacterial toxins fall into several diverse categories. Many phycologists learned the acronym "Anni, Fanni and Mike", referring to the production of anatoxin-a, saxitoxin, and microcystin by *Anabaena flos-aquae, Aphanizomenon flos-aquae, and Microcystis aeruginosa*. Unfortunately, the situation is much more complicated. In 1988, there were 10 reported microcystins. As of 2005, there were more than 70 different chemically identified microcystins (Figure 2). Microcystins have been reported in many kinds of cyanobacteria including *Microcystis, Anabaena, Oscillatoria (Planktothrix), Nostoc, Hapalosiphon, Anabaenopsis* and new sources are appearing yearly. In addition to the microcystins, there are many closely related peptides such as the nodularins anabaenopeptins, aeruginopeptin, and other bioactive peptides. Many of the microcystin-type peptides are hepatotoxic- toxic to animals' livers; though their lethal dose (LD-50) is highly dependent on the specific amino acids present [10]. For other compounds such as the anabaenapeptins or microginins, their biological activity toward humans is largely unknown. While the general conception is that microcystin-LR is the "major" microcystin produced by cyanobacteria (see Figure 2 for a description of the nomenclature), that generalization is often unjustified when one looks at the microcystin chemical structure more closely. For example, while microcystin-LR was the major congener isolated from the 1996 Lake Erie *Microcystis* bloom [3], more recent samples have contained a much richer diversity in their toxins present (Boyer, unpublished). Microcystin derivatives vary over 100-fold in their biological activity [10] and human health decisions based on the toxicity of microcystin-LR are likely to be in error when the other congeners are considered.

Separate from the hepatotoxic peptides are the neurotoxic alkaloids. These consist of anatoxin-a, anatoxin-a(S), saxitoxin and related analogs (Figure 3). The most important of these compounds from an environmental health aspect is probably anatoxin-a. Anatoxin-a was originally isolated from *Anabaena flos-*



aquae, but is also reported in *A. planktonica*, *Oscillatoria* species, and *Cylindrospermum* [13]. Traditionally thought to primarily occur in North America in the highly eutrophic prairie pothole lakes, in recent years anatoxin-a has been associated with the deaths of several dogs that came in contact with low-biomass blooms in oligo- or mesotrophic Lake Champlain [1]. Significant levels of anatoxin-a have also been reported in the Great Lakes [16], particularly in the western basin of Lake Erie where values have exceeded 0.5 $\mu g\ L^{-1}$. While these values do not seem high in comparison to those observed for microcystins (i.e. 20 $\mu g\ L^{-1}$), they are about 5-fold higher than the values measured for Lake Champlain that were associated with the dog fatalities [15].

Understanding which organisms produce anatoxin-a, their bloom dynamics and the stability of this toxin *in situ* is essential for designing monitoring and response criteria to protect human health. The structurally unrelated compound, anatoxin-a (S), is a naturally occurring organo-phosphate (Figure 3) produced by *A. flos-aquae* strain NRC 525-17 and a number of other *Anabaena* strains. It acts as an inhibitor of acetylcholinesterase, a chemical necessary for proper nerve function in animals.

Toxic cyanobacteria can also contain the Paralytic Shellfish Poisoning (PSP) toxins saxitoxin and neosaxitoxin (Figure 3). These toxins are identical to those produced by some "red-tide-forming" marine dinoflagellates, and which accumulate in shellfish that feed on those algae. The PSP toxins are actually a large family of 18-24 different analogs that are highly variable in their biological activity. Not all of the PSP analogs currently identified in marine dinoflagellates and shellfish have been reported in freshwater cyanobacteria but several of the most toxic such as saxitoxin, neosaxitoxin, and GTX1-4 have all been

A Quick Chemistry Lesson

Microcystin

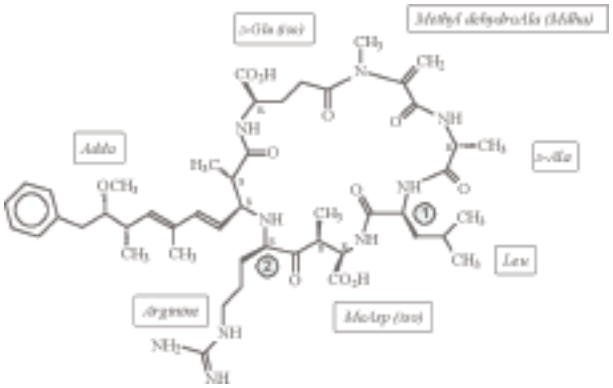


Figure 2. The generic chemical structure of a microcystin. Amino acid variations in the core ring occur primarily at the positions 1 and 2. For example, Microcystin-LR has the amino acids leucine (L) and arginine (R) at positions 1 and 2 respectively. Microcystin-RR has arginine at both positions. Nodularins are similar, with the five amino acids Adda-gGlu-Mdhb-bMeAsp-Arg making up the core ring system (Harada, 1996).

Neurotoxic alkaloids

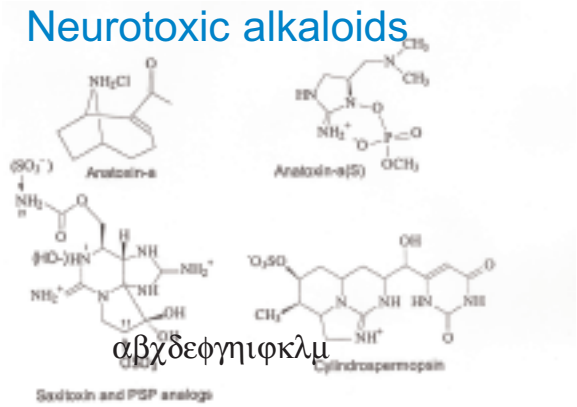


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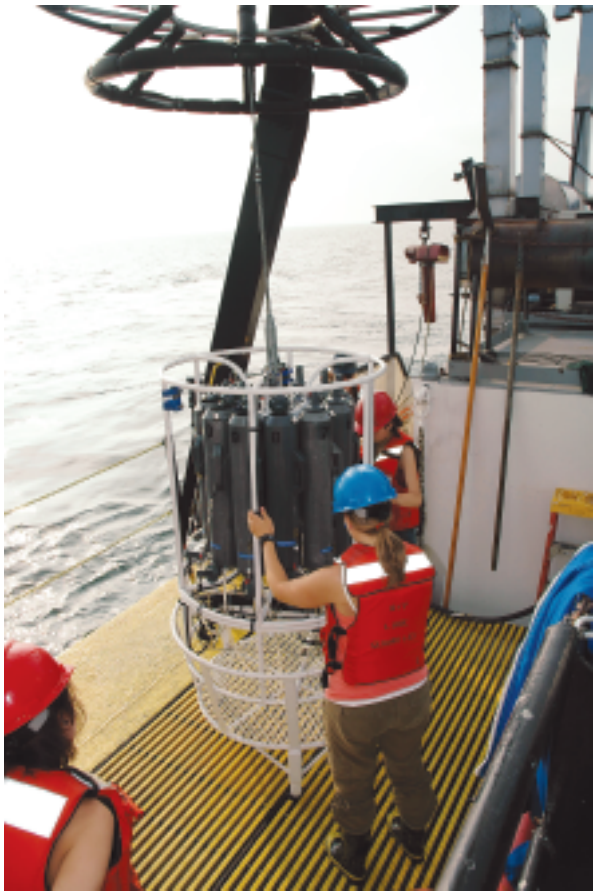
Figure 3. The chemical structure of the alkaloid cyanobacteria toxins. Anatoxin-a (ATX-a), anatoxin-a(S) and the PSP family of toxins, including saxitoxin (STX), neosaxitoxin (N-1- OH-STX), and the sulfated gonyautoxins (GTX1-4) are all neurotoxic. Less toxic PSP analogs include C1 and C2. Cylindrospermopsin (CYL) has hepatotoxic activity similar to the microcystins.

reported in freshwater cyanobacteria including *Aphanizomenon flos-aquae, Anabaena circinalis, Planktothrix sp., Lyngbya wollei,* and a Brazilian isolate of *Cylindrospermopsis raciborskii*. *Cylindrospermopsis raciborskii* from Hungary or Australia instead produces the hepatotoxic alkaloid cylindrospermopsin (Figure 3). An outbreak of this organism in the drinking water supply on Palm Island, Queensland Australia, led to a severe outbreak of hepatoenteritis among the inhabitants of the island [6]. The toxin cylindrospermopsin has also been reported from *Umezakia natans* in Japan, and *Aphanizomenon ovalisporum* in Israel [13]. Toxic cylindrospermopsin-forming species are generally associated with semi-tropical or arid environments such as Florida and Arizona but there are increasing reports of *C. raciborskii* occurring in the temperate zones of Europe and the USA including New York and Michigan[9]. Both *Cylindrospermopsis* and *Raphidiopsis* species have been identified in the phytoplankton flora of Lake Erie and the toxin cylindrospermopsin was detected in August samples collected from Lake Erie.



MERHAB researchers collect samples from Lake Erie onboard the USEPA *Lake Guardian*

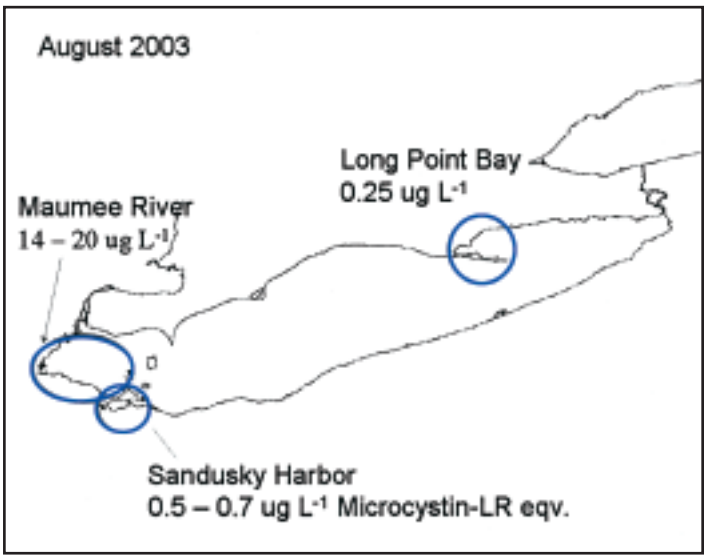


Figure 4: Distribution of microcystin toxins in Lake Erie in August 2003. Three distinct regions of the lake showed measurable levels of microcystins.

The Distribution of Cyanobacterial Toxins in Lake Erie

Since the initial report of microcystins in the western basin of Lake Erie, a number of research cruises have looked at the distribution of microcystins and other toxins in Lake Erie and nearby Great lakes. Initial reports of microcystins in the western basin of Lake Erie were soon followed by Lake-wide surveys that identified three areas of concern, all with different characteristics (Figure 4). The western basin of Lake Erie is characterized by high biomass blooms of *Microcystis aeruginosa* that produced microcystins at concentrations exceeding 20 μg L^{-1}. The origin of these blooms is unknown, but there have been suggestions that they are coming from the region of the Maumee River [2]. They may also be stimulated by the presence of zebra mussels or by recent changes in the light and phosphate regimes in this basin [14, 12].

A second area of concern is the region in and around Sandusky Harbor. This region is characterized by high nutrient loads and a vibrant population of cyanobacteria. *Microcystis* is only one of many species present and the blooms in Sandusky Bay are often dominated by *Aphanizomenon* and *Anabaena* species. Microcystins are again present in this region but molecular techniques indicate that the species responsible for toxin production is not *Microcystis* but rather *Planktothrix* species [11], (Rinta-Kanto, personal communication).

In the eastern basin, toxic blooms have been reported off the Buffalo shoreline but the only documented blooms to our knowledge are in and around Long Point Bay. There again the toxins seem to be associated with *Microcystis* species but there has been very little sampling for cyanobacterial toxins in the inner basin due to its shallow waters. The extent of these blooms is currently unknown and remains to be investigated.

Other Lakes and Other Toxins

Microcystin toxins have also been reported in the Saginaw Bay of Lake Huron [14] and in both Hamilton Harbor and the Bay of Quinte of Lake Ontario [5, 8], (S. Watson and G.L. Boyer, unpublished). In 2003, a large bloom of toxic *Microcystis* occurred in the eastern basin of Lake Ontario off of Oswego NY. This bloom produced microcystin concentrations that exceeded the WHO advisory limit of 1 μg L^{-1} and was located very near the Onondaga County water intakes (Figure 5). Blooms containing other toxins such as anatoxin-a and cylindrospermopsin have been increasingly reported in the lower great lakes watershed. Anatoxin-a has been found in basins in Lake Ontario, Lake Champlain and Lake Erie [16]. While the concentration of the other toxins are often well below the 1 μg L^{-1} advisory threshold established for microcystins, they illustrate that the problem of toxic cyanobacteria in the lower Great Lakes goes far beyond simply *Microcystis* in the western basin of Lake Erie. How the occurrence of this multitude of cyanobacterial toxins in potential drinking water supplies and recreational waters will impact management decisions, as well as their transport through the food chain remains to be determined.

Summary

The presence of *Microcystis aeruginosa* and the hepatotoxic microcystin was initially reported in the mid-1990s in the western basin of Lake Erie. Since that time, the presence of toxigenic or potentially toxic organisms, as well as the chemical identification of a number of cyanobacterial toxins, has increased to include all three basins of Lake Erie as well as Lake Ontario, Lake Huron and Lake Champlain. The presence of hepatotoxic microcystins has become common in these systems and there are confirmed reports of both the alkaloid hepatotoxin cylindrospermopsin and the neurotoxin anatoxin-a in these systems. These toxins have resulted in a number of animal fatalities, though their impacts on humans and movement through the food chain remains to be determined.

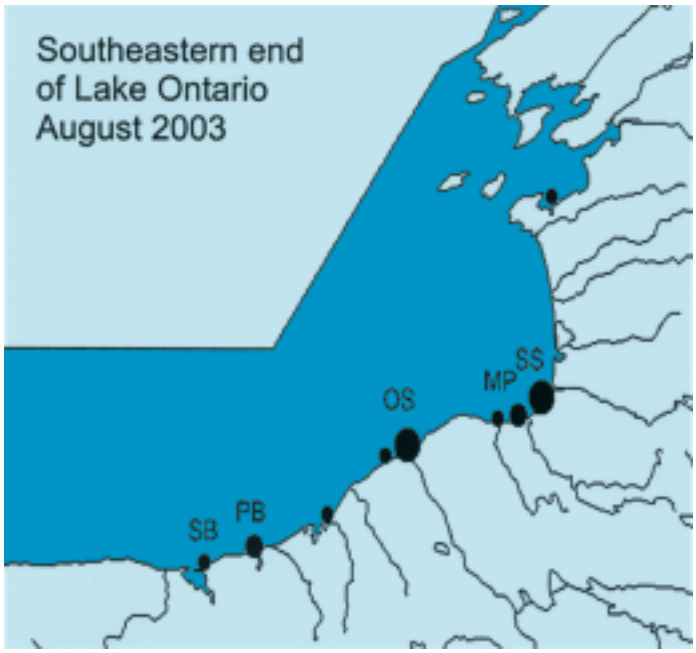


Figure 5. The occurrence of microcystin toxins in the eastern basin of Lake Ontario during August 2003. Microcystin values for the Oswego Station (OS; 0.93 mg L^{-1}) and Selkirk Shores (SS: 1.1 mg L^{-1}) were both near the WHO advisory limit of 1 mg L^{-1} for drinking water. Lower levels (0.2-0.4 mg L^{-1}) were observed at Mexico Point (MP), Sodus Bay (SB) and Port Bay (PB).

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For more information

Visit the websites of programs that have studied algal blooms in the Great Lakes:

The Microbial Ecology of Lake Erie (MELEE) Working Group
web.bio.utk. edu/wilhelm/melee.htm

NOAA's MERHAB-Lower Great Lakes Project www. esf.edu/merhab

NOAA's Center of Excellence for Great Lakes and Human Health at the Great Lakes Environmental Research Laboratory
www.glerl.noaa.gov/res/Centers/HumanHealth/

International Field Year on Lake Erie (IFYLE) 2005
www.glerl.noaa.gov/ifyle/

A Partnership Approach to Monitoring Cyanobacteria in Lake Champlain

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Summary

The increasing incidences of toxic cyanobacteria blooms worldwide have created a need for practical and efficient monitoring to protect public health. We developed a monitoring and alert framework based on World Health Organization (WHO) recommendations and applied it on Lake Champlain during the summers of 2002-2004. The protocol began with the collection of phytoplankton samples to maximize the chances of finding potential toxin-producing cyanobacteria. Samples were collected lakewide in partnership with ongoing monitoring efforts, but because open water sample sites did not capture conditions along the shoreline, we added nearshore and shoreline stations in problem areas using citizen monitors. Samples were examined qualitatively until potential toxin-producing taxa were found. Primary toxin analysis was for microcystins using ELISA (enzyme-linked immunosorbent assay) methods. Cell densities, locations of colonies, and toxin concentrations were reported weekly to public health officials. We found that screening for potential toxin-producing cyanobacteria and then measuring toxin concentrations when cell densities reached critical levels worked well to identify problem locations. The majority of the 84 samples with microcystin levels greater than 1 µg/L, the WHO level of concern, were collected in shoreline locations. With pre-season training and regular communication and support, citizen monitors can greatly enhance a monitoring effort and provide invaluable data at a very reasonable cost.

Sampling a bloom on Lake Champlain

The Need for Monitoring Partnerships

Cyanotoxins pose a risk both to those who use natural waters as sources for drinking water and those who use them for recreation. Therefore, there is an urgent need for data about the distribution of cyanobacteria and cyanotoxins in natural waters. Throughout the world efforts are underway to develop monitoring programs and alert protocols to help collect these data and inform public health officials about toxic blooms [2, 3, 5, 10]. The World Health Organization (WHO) has proposed an Alert Level Framework to be used as a guide for developing monitoring programs [2]. We have revised and adapted this framework to monitor for cyanobacteria and cyanotoxins in Lake Champlain. Cyanobacteria have always been a component of the Lake Champlain phytoplankton community [4, 7]; however, toxic blooms are a relatively recent concern. Cyanotoxins were first documented in Lake Champlain in 1999, when two dogs died as a result of consuming water containing large amounts of toxic cyanobacteria and the neurotoxin anatoxin-a [6]. Since 2000, studies have documented the regular presence of potentially toxic cyanobacteria and the occasional presence of both microcystin and anatoxin-a in the waters of Lake Champlain [1, 6, 9].

Because Lake Champlain is a large lake (120 miles long and 10 miles across at its widest point), considerable challenges arose in establishing a network of sampling stations to monitor cyanobacteria abundances in all areas. To increase our spatial coverage, we established partnerships that took advantage of existing sampling teams already working on the lake. Beginning in 2003, we also recruited and enlisted the help of citizens to assist in monitoring shoreline areas where exposure risks for people and pets are greatest. Citizen monitoring efforts have worked well in other kinds of monitoring programs, so we were confident that we could develop and implement an appropriately simple protocol for cyanobacteria sampling.

Sample Collection and Analysis

The monitoring and alert system we developed for Lake Champlain in 2002 is outlined in Table 1. In most instances, we followed the basic recommendations of the WHO. However, beginning in 2003, we eliminated chlorophyll-a triggers and adjusted cyanobacteria cell densities to more precisely capture hazardous conditions in Lake Champlain [9]. Following the WHO guidance, initial phytoplankton samples were only evaluated qualitatively until potential toxin-producing cyanobacteria were identified at a sampling

Table 1. Outline of monitoring and alert system employed in Lake Champlain.

Alert Level	*Sampling Frequency*	*Collect Samples for:*	*Trigger to Next Level*	*Public Action*
Initial	2/mo	Algal identification	Identification of toxin-producing cyanobacteria	None
Quantitative	2/mo	Algal enumeration; Chlorophyll *a*	>2000 cyanobacteria cells/ml in net samples or lay monitor samples	None
Vigilance	1/wk	Algal enumeration; Chlorophyll *a*	>2000 cyanobacteria cells/ml in net samples or lay monitor samples	Notify public health officials that cyanobacteria are abundant and blooms could form.
Alert Level 1	1/wk	Algal enumeration; Chlorophyll *a*; Toxin analysis	>1µg/L microcystin/L in whole water samples	Notify public health officials of potential risks to humans and animals.
Alert Level 2	1/wk	Algal enumeration; Chlorophyll *a*; Toxin analysis		Notify public health officials that significant risk to humans and animals exists. Public health advisories should be issued by appropriate agencies.

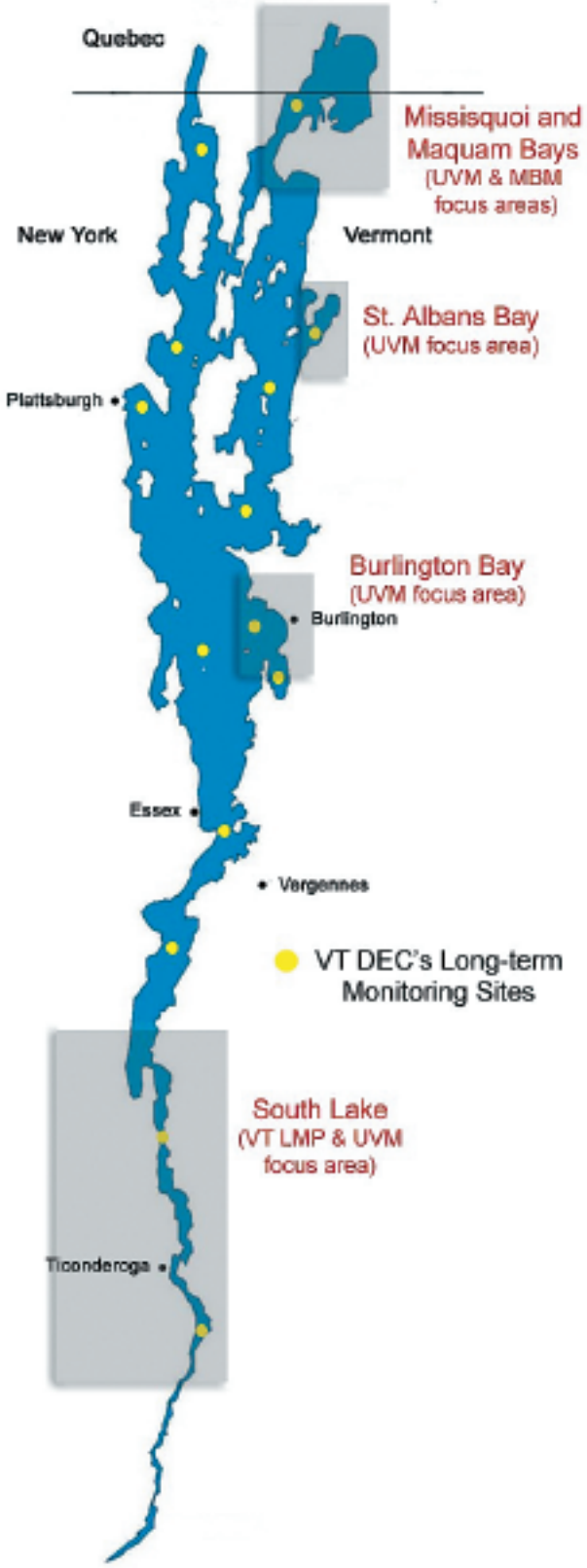


Figure 1. Map of Lake Champlain with approximate locations of intensive sampling area indicated by a box.

station. Once cyanobacteria densities reached Alert Level triggers, toxin analyses were initiated and public health officials around the lake were notified. Weekly updates were provided thereafter so that public officials could take action, such as closing public beaches, as they deemed appropriate.

In addition to the samples collected by our group at the University of Vermont (UVM), we worked in partnership with three other groups to monitor Lake Champlain: the Vermont Department of Environmental Conservation (VTDEC), which regularly visited 14 long-term stations for water quality monitoring; volunteers from the Vermont Lay Monitoring Program (VTLMP), who sampled in the south lake area; and volunteers that we recruited to sample shoreline locations in Missisquoi Bay and nearby Maquam Bay (MBM), in the north lake, on the Vermont side of the international border (Figure 1). The number of sampling sites and frequency of sample collection from 2002-2004 is summarized in Table 2.

The MBM volunteer citizen monitors were solicited with the help of the Lake Champlain Committee, a local citizen action group interested in lake health. To ensure that we collected consistent data, we held a training session for all of our volunteers. At this session, we gave each volunteer an instruction sheet, sample bottles, pre-cleaned filters in foil packets, and hand-operated vacuum pumps. We asked our volunteers to collect weekly grab samples at a specified shoreline location in about 1-2 feet of water, targeting areas of algal accumulation or scum. We showed them how to collect a one liter water sample and then prepare three subsamples for analysis. One subsample was preserved in Lugol's solution for microscopic analysis. A second subsample was filtered for chlorophyll analysis, and a third subsample was filtered for cyanotoxin analysis. Filters were frozen in their foil packets until pick-up for analysis – usually within 24 hours.

For both the qualitative and quantitative microscopic analyses, 1 mL of a well-mixed Lugol's preserved sample was placed into a Sedgwick-Rafter cell, allowed to settle for 5 minutes and evaluated using an inverted microscope at 100x. Natural units (colonies and colony fragments) instead of individual cells were counted for the most common colonial phytoplankton using a procedure developed at the Rubenstein Ecosystem Science Laboratory to reduce analysis time and increase efficiency [9].

Table 2. Locations and frequency of sampling by groups involved in the cyanobacteria monitoring program on Lake Champlain.

Collected by:	*Locations*	*Approximate Frequency*
UVM	4-7 sites in Burlington Bay 1-5 sites in Missisquoi Bay 1-5 sites in St. Albans Bay 1 site in southern lake	1/mo April-May, 2/mo Jun-Sep. 1/wk (after bloom formation) 1/wk (after bloom formation) 2/mo
VT DEC	14 sites lakewide	2/mo
VT LMP	~6 sites mostly in southern lake	If blooms were suspected
MBM Citizen monitors	8 (2003, 2004 only)	1/wk (after bloom formation)

Microcystin concentration was determined using an ELISA QuantiPlate™ kit commercially available from Envirologix (Portland, Maine). Samples in which potential anatoxin producers made up a substantial portion of the phytoplankton community were analyzed for anatoxin in addition to microcystin by Dr. Greg Boyer at the State University of New York, College of Environmental Science and Forestry (SUNY-ESF) using HPLC.

Distributions and Concentrations of Cyanotoxins in Lake Champlain

Throughout the growing seasons of 2002, 2003 and 2004, over 1000 phytoplankton samples were analyzed following our tiered protocol. The bulk of these samples, 800 in total, were evaluated quantitatively because potential toxin-producing cyanobacteria were present at the sample locations. Potential toxin-producing cyanobacteria (both microcystin- and anatoxin-producers) were commonly observed throughout Lake Champlain; however, they were most abundant in the northern bays of the lake. St. Albans Bay, Maquam Bay and Missisquoi Bay, all in the northeast section of the lake, attained Alert Level 1 densities (Table 1) in every year of the study with the most long-lasting and consistent blooms occurring in Missisquoi Bay. These blooms were dominated by large *Microcystis* spp. colonies. Several other isolated areas of Lake Champlain reached Alert Level 1 for short periods, including sites in the south lake, the Alburg Passage and the main lake.

The cyanotoxin detected most often in Lake Champlain was microcystin. Elevated anatoxin a concentrations were observed in 2000 and 2001, and dog deaths from anatoxin exposure have occurred in the past [6, 8]. Concentrations more recently have been very low despite the regular presence of potential anatoxin producers, such as *Anabaena* spp. Out of 126 samples tested for anatoxin in 2002-2004, only seven were above the detection limit for anatoxin. At least 84 samples were found to have microcystin levels of more than 1 $\mu g\ L^{-1}$. The highest concentrations of microcystin were found in Missisquoi Bay, with one shoreline scum registering approximately 6.5 mg/L [8].

There was frequently great variability in the abundance of algae and the concentration of cyanotoxin in samples collected on any sample date. For example, shoreline samples and open water samples collected less than 10 m apart in Missisquoi Bay sometimes differed by several orders of magnitude in both algal density and microcystin concentration (Table 3).

Table 3. Comparison of samples taken at the Rte.78 access site on Missisquoi Bay from shore and approximately 10m away from shore by boat.

		Average Potentially Toxic Cells/ml	*Average microcystin µg/L*
19-Aug-03	shoreline	210,000	6.2
	boat-based	1,954	0.2
26-Aug-03	shoreline	186,333	23.9
	boat-based	1,382	0.5
26-Jul-04	shoreline	85,267	78.3
	boat-based	2,552	5.0
1-Sept-04	shoreline	24,320,000	6490.0
	boat-based	4,154	0.7

Generally, areas where scums were forming, along the shoreline or in protected bays, had the highest microcystin concentrations. Ten of the highest 25 microcystin concentrations were measured in shoreline samples collected by our citizen monitors. In fact, we would not have documented some of the most severe risks to people and pets without these citizen monitors.

Effectiveness of the Partnership Approach

Partnering with established monitoring programs (VTDEC and VTLMP) and with our own group of dedicated citizen sample collectors (MBM) allowed us to monitor Lake Champlain relatively comprehensively in a time- and cost-efficient manner. Visually screening samples for potential toxin-producing cyanobacteria and only measuring toxin concentrations when cell densities reached critical levels worked well to identify problem locations. It is certainly possible that isolated instances of cyanobacterial blooms went undetected by the monitoring program, particularly in the small isolated bays where we did not conduct shoreline sampling; however, we are confident that we were able to find all major and prolonged bloom events in Lake Champlain.

The integration of shoreline sampling into our monitoring protocol became especially important because of the tendency of cyanobacteria to accumulate in these protected areas. Many of the highest toxin concentrations were found in the algal accumulations and scums along the shoreline. Routine monitoring of lakes is often done from relatively deep mid-lake stations by boat, and does not always represent conditions found along the shoreline. This is of concern in cyanobacteria monitoring programs because most lake users access the lake from the shore. Shoreline sampling must be included as a component of a successful cyanobacteria monitoring program in order to thoroughly assess the risks to which recreational users are exposed. Citizen monitors who live along the shoreline can watch the water for shoreline problem areas and very effectively sample them. We have expanded this aspect of our monitoring protocol and are currently investigating whether shoreline sampling may prove more effective than regular open water sampling in many areas of the lake.

Mary Watzin trains volunteers at Plattsburgh Beach on Lake Champlain

We developed a monitoring system that was effective at detecting the initiation of blooms and was not expensive or difficult to implement. Our citizen monitors collected samples in a rigorous and repeatable manner, and provided observations that gave us a good overall sense of conditions in problem areas.

> Shoreline property owners have a stake in the water conditions and are willing and responsible partners in a seasonal monitoring effort.

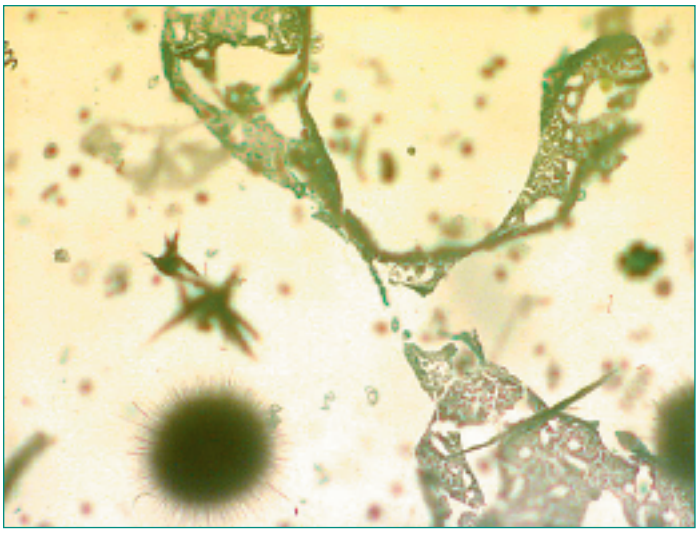
Samples collected by the Vermont DEC allowed us to maintain a lake-wide perspective about cyanobacteria distribution. As we discovered problem areas, we expanded our citizen sampling effort to include those areas. In 2005, for example, we built on our experience in 2003 and 2004 and added citizen monitoring stations along the New York shoreline and in other problem areas in the Inland Sea and the Alburg Passage.

With our adaptations, we find the WHO Alert Level Framework to be an efficient and effective monitoring protocol for all natural waters, not just those that are drinking water sources. The Framework's straightforward design provides a clear pathway for decision-making by public health officials and its relatively low cost makes it an appealing monitoring option. We especially recommend that other programs consider the use of citizen monitors in shoreline areas. Shoreline property owners have a stake in the water conditions and are willing and responsible partners in

a seasonal monitoring effort. We have found that when appropriate training is provided before the summer algal bloom season begins, and with regular communication and support, citizen monitors can greatly enhance a monitoring effort and provide invaluable data at a very reasonable cost.

Acknowledgements

We thank Todd Clason, Michael Levine and Dick Furbush (UVM), Pete Stangel (VT DEC) and our many volunteer monitors for their help in various aspects of the project. The Missisquoi Bay National Wildlife Refuge office assisted by serving as a central sample collection point for our northern sites and the Lake Champlain Committee assisted in finding monitors for Missisquoi Bay. Dr. Greg Boyer's lab at SUNY-ESF provided analysis for anatoxin. Funding for the project was provided by grants from NOAA through the MERHAB Program (Grant No. NA 160P2788) and the US EPA through the Lake Champlain Basin Program.



Microscopic image of a very large *Microcystis* colony with *Gloeotrichia* and *Aphanizimenon* in St Albans Bay, 2002

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Mapping Variations of Algal Blooms in the Lower Great Lakes

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Summary

As part of the MERHAB (Monitoring and Event Response for Harmful Algal Blooms) Lower Great Lakes project, we are exploring the use of remote sensing for mapping the spatial and temporal distribution of algal blooms in Lake Erie and Lake Ontario. We have processed SeaWiFS and MODIS data acquired in the summers of 2003, 2004, and 2005 over the investigated lakes. The remotely-sensed data were used to derive chlorophyll-a concentration. Comparisons were made between Case I and Case II water satellite-based estimates of chlorophyll-a concentrations and *in situ* cruise measurements for the 2003-2005 field seasons (July-August 2003, 2004, May-Sept 2005). We used algorithms which were developed for case I waters (where chlorophyll content and other dissolved and suspended materials are assumed to co-vary), and applied these algorithms to the more optically complex waters of Lake Erie and Lake Ontario [3, 6]. This approach has been successfully applied to Lake Michigan waters [5]. We demonstrate similar applications and successes in Lakes Erie and Ontario, where comparisons of satellite-based chlorophyll-a concentrations with *in-situ* chlorophyll-a measurements indicate that these algorithms provide reasonably good estimates of chlorophyll-a. We also demonstrate an even better correlation with chlorophyll-a concentrations derived from Case II water algorithm. Using a series of SeaWiFS and MODIS scenes that were acquired around the same approximate time and which show minimal cloud cover, we could map the annual re-occurrence (same location and time) of chlorophyll distribution patterns as well as unique algal bloom events in Lakes Erie and Ontario in the summers of 2003-2005.

We used the unique spectral characteristics of the phycocyanin pigment to discriminate between phycocyanin containing blooms and other blooms. These results have been validated using phycocyanin flourescence data showing relative phycocyanin abundance. Field spectrometer measurements made in the fall of 2005 are being used to further refine the optical components related to phycocyanin pigments, and to generate a model for phycocyanin quantification.

We are exploring the use of remote sensing for mapping the spatial and temporal distribution of algal blooms in Lake Erie and Lake Ontario.

Image Processing

We examined SeaWifs and MODIS (raw and chlorophyll-a concentrations) data for the summers of 2003 and 2004 over Lakes Erie and Ontario. Approximately 120 SeaWiFS scenes (2003, 2004) and 20 MODIS scenes (2004) were processed using SEADAS software and applying the OC4V4 (SeaWiFS), and the OC3 (MODIS) algorithms [6] for the calculation of water leaving radiance and chlorophyll-a concentrations. In addition, the Carder case II semi-analytic algorithm [4] was used to calculate values of chlorophyll-a from the same images. The default atmospheric models were utilized. Images were brought to a common projection using commercial image processing software (ENVI) to allow for comparison to be made between scenes and between remote sensing and *in-situ* observations.

Satellite vs. *In Situ* Measurements of Chlorophyll-a

In-situ chlorophyll-a measurements were compared with chlorophyll-a concentrations extracted from SeaWiFS (Figure 1); the remote sensing and *in situ* measurements were acquired around the same approximate date (within 48 hours). Figure 1 shows the data (spectral and *in-situ*) that was used to calibrate and to derive the case I water algorithms for open oceans; the inner diagonal solid lines mark ± 35% agreement relative to the 1:1 central solid line and the dashed lines are the 1:5 and 5:1 lines encompassing the data set. For comparison, we plotted our *in-situ* chlorophyll-a data and the Chrolophyll-a data derived from SeaWiFS for the same locations and using Case I and Case II algorithms. Inspection of Figure 1 suggests that all of our Case I samples plot within the envelope defined by the 1:5 and 5:1 lines

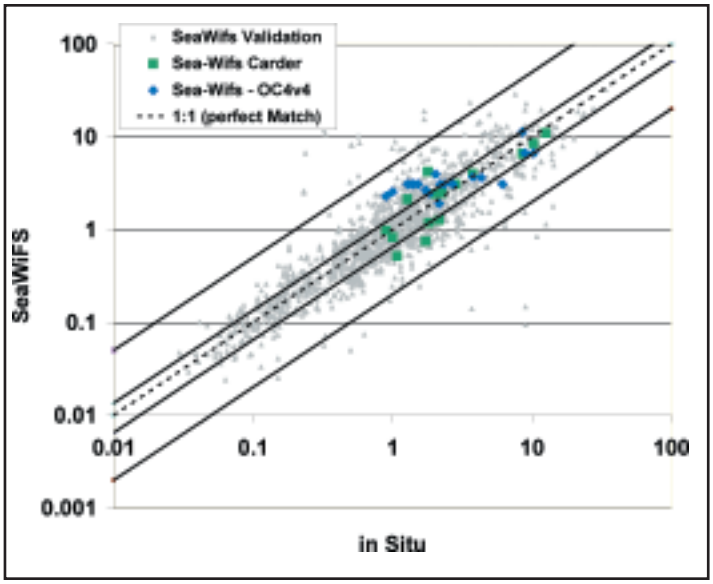
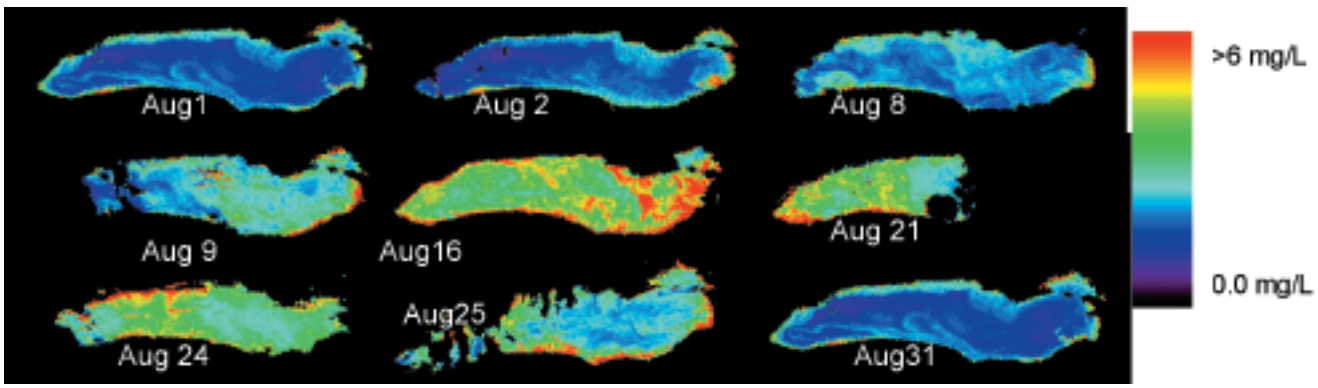


Figure 1 - Sea-WiFS Case I (OC4) and Case II (Carder) derived concentrations vs. *in situ* results overlain over Sea-WiFS validation results.

and for case II samples, the majority of them plot within the envelope defined by the ± 35% lines. These results suggest that the application of case I water algorithms provide reasonable approximations for the chlorophyll-a content in Lakes Erie and Ontario and that Case II algorithms provide more precise estimates. Differences between the Sea-WiFS-based and *in-situ* chlorophyll-a concentrations extracted using Case II algorithms could be attributed to: (1) differences in "sample size" (1 km^2 for satellite vs point samples for ground measurements), (2) variations in chlorophyll concentrations with depth that are reflected in the depth-integrated satellite measurements, but not in the *in situ* measurements, and (3) differences in the time of acquisition. Satellite measurements are not always acquired at the same time during which the ground sampling campaign is being conducted.

Figure 2. Mapping the progression of an algal bloom in the Oswego Harbor using a time series generated from SeaWifs and MODIS images acquired in August of 2004.



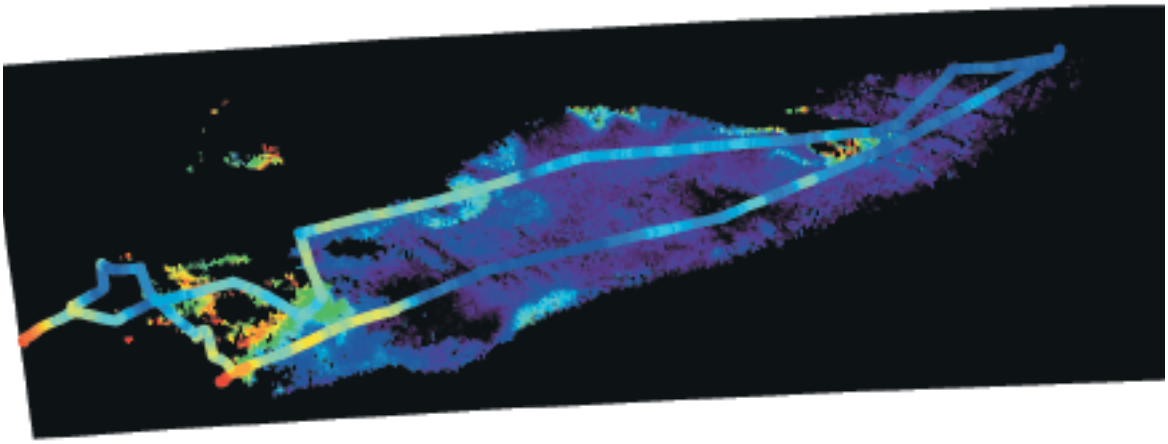


Figure 3. Relative abundance of phycocyanin from in-situ flourescence measurements (July 13-16, 2004) overlain on top of relative abundance of phycocyanin derived from Sea-Wifs imagery (July 13 and 15 2004).

Identification and Mapping of Algal Blooms

Examination of chlorophyll-a concentration images (extracted from SeaWiFS and MODIS) acquired in summers of 2003 and 2004 over Lakes Erie and Ontario revealed several algal blooms. Blooms were noted as early as early June in Lake Ontario, and through mid-October in Lake Erie. We integrated inferences from SeaWiFS and MODIS data as needed [1]. For example, mapping of a bloom which was noted in the Oswego Harbor at the beginning of Aug 2004 was enabled using a time series of chlorophyll-a images extracted from a combined set of SeaWiFS and MODIS data sets. The assembled time series provided a temporal coverage with minimal cloud cover (Figure 2).

Locating Phycocyanin Containing Blooms:

One of the primary goals of the MERHAB project is to be able to discriminate between potentially harmful and non-harmful algal blooms in the lower Great Lakes. *Microcystis* species and other toxin-producing cyanobacteria have been observed in Lake Erie [2, 7]. As *Microcystis* is a cyanobacteria containing the phycocyanin pigment, it should be possible to rule out the possible toxicity of many blooms by the absence of phycocyanin. We are developing algorithms to allow the identification of the location of potentially toxic cyanobacterial blooms from SeaWiFS satellite data, based on spectral characteristics of phycocyanin pigment.

To accomplish this goal, SeaWiFS Images were processed to remote Sensing Reflectance using SEADAS 4.8. A mixing model was adopted based on the following endmember components: Chlorophyll-a, phycocyanin and water [8], chlorophyll and water (extracted from SeaWiFS images in areas of >8 µg/L Chl-a and low phycocyanin as determined from fluorescence data), and water with chlorophyll-a levels below 1 mg/L.

This phycocyanin abundance extracted from SeaWiFS was compared with relative abundances of phycocyanin based on fluorescence data acquired during July 2004 (Fig. 3). Our success in spectrally distinguishing phycocyanin is consistent with the earlier findings in western Lake Erie [8]. Despite the broad

One of the primary goals of the MERHAB project is to be able to discriminate between potentially harmful and non-harmful algal blooms.

We are developing remote sensing algorithms to identify phycocyanin, a pigment known to occur in *Microcystis*.

wavelength regions covered by the TM Landsat bands, and the absence of TM band(s) in the wavelength region affected by the PC, Vincent et al. [8] successfully developed algorithms to detect PC from Landsat TM data for mapping cyanobacterial blooms in Lake Erie. We are currently refining our mixing model to include better end members for high turbidity and high dissolved organic matter areas in an attempt to remove some false positive identification, and to increase the areas where the model resolves.

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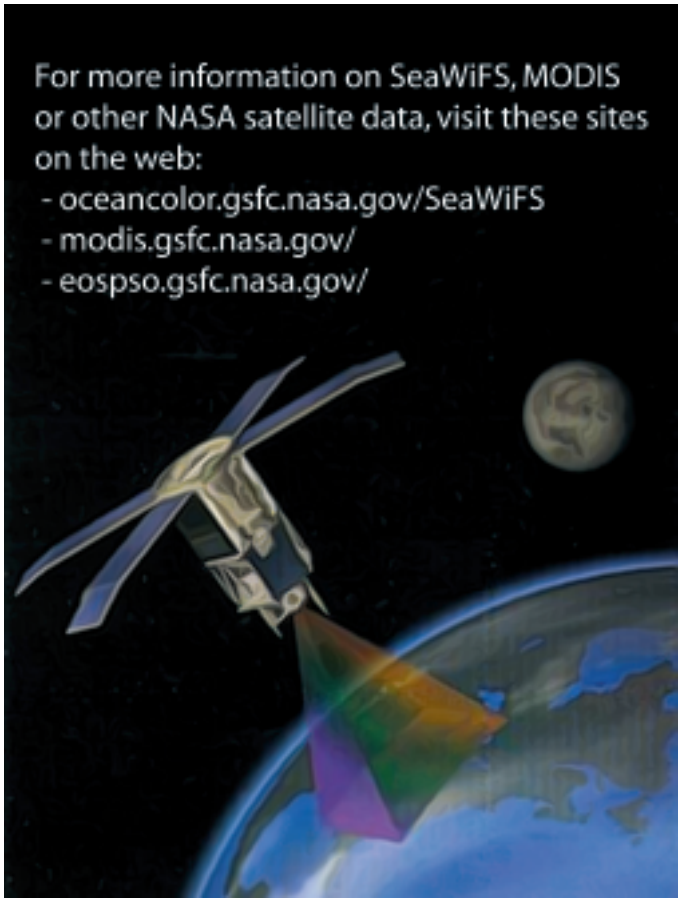

Lake Champlain Phytoplankton and Algal Toxins: Past and Present

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Lake Champlain, one of the largest lakes in North America has a total surface area of approximately 1100 km^2 and volume of 2.58 x 10^{10} m^3. Algal toxins have recently emerged as an issue with the death of several dogs in 2000 and 2001 along the shoreline of the main lake in New York and Vermont, most likely the result of the blue-green algae neurotoxin anatoxin-a from localized blooms [1,8]. Despite this emerging issue in Lake Champlain, very little is known about past and present phytoplankton communities in the lake [2, 4, 5, 7]. Previous studies suggest that the most common blue-green algae in Lake Champlain over the past 80+ years was *Anabaena* usually accounting for >75% of total blue-green abundance in most lake sites [5, 6]. This contrasts with recent lakewide assessments of phytoplankton and algal toxins in Lake Champlain [1, 4] that show other blue-green taxa, primarily *Microcystis*, as a dominant taxon lakewide.

Figure 1 illustrates lakewide algal community composition from sampling conducted during the MERHAB project in July 2004. Although abundances varied greatly, cyanobacteria were dominant, accounting for >50% of total in 23 of 37 sites. In the Northeast Arm of Lake Champlain all sites (13 total) contained primarily blue-green algae (Malletts Bay, St. Albans Bay and into Mississquoi Bay- Alburg, Chapman Bay, Highgate Cliffs, Highgate Springs, Route 78). In July 2004 when blue-greens were prevalent, *Microcystis* was always the most abundant taxon. Mississquoi Bay sites also contained a large proportion of *Aphanizomenon*. Of remaining sites where blue-greens are dominant, most are either in the southern lake (Northwest Bay, Cole Bay, Snake Den Harbor, Long Point) or northern lake (Horseshoe Passage, Lamotte Passage, Monty Bay, Treadwell Bay). Other algal taxa, primarily the diatoms *Asterionella*, *Aulacoseira* (formerly *Melosira*,[10]), and *Fragilaria* and *Synedra*, were more common in the main lake region (the area from Cumberland Bay to Jones Point on Figure 1).

SUNY Plattsburgh student Trevor Carpenter collecting samples on Lake Champlain

Recent lakewide assessments of algal community composition show a shift in the Lake Champlain community from historical studies. Present day Lake Champlain contains a blue-green community dominated by *Microcystis*. This represents a shift from historical patterns where *Microcystis* was reported as common only in south lake sites and *Anabaena* was the most common blue-green found in the Northeast arm, main lake and northern lake [5].

Why Have We Observed This Long Term Shift in Algal Communities?

MERHAB sampling in Lake Champlain is contributing much needed data to address some of the possible causes. Identifying causes of long-term patterns in lake algae will likely need further study and may never produce a complete explanation (keeping the research community busy for years to come).

What do other studies suggest about why *Microcystis* might be dominant? One study by Vanderploeg et al. [9] suggests that invasion by zebra mussels into a lake can facilitate a "shift" in the phytoplankton community, favoring increased *Microcystis* densities. This occurs because zebra mussels can ingest all types of algae but are not well adapted to ingest *Microcystis* which forms massive colonies. Selective filtration by zebra mussels is a possible agent responsible for lakewide increases in *Microcystis* and may also be a partial explanation for the long-term decline in *Anabaena*, because this genus does not form such large colonies. However, zebra mussels are still not abundant in the north lake, so other factors may also be contributing to the new pattern.

Explanations in ecology are not always that simple. For example, Carling et al. [2] documented shifts in Lake Champlain's zooplankton community in the mid-1990s, particularly a decline in Rotifers lakewide. Carling et al. [2] also suggest this could be a zebra mussel-mediated interaction. Major shifts in zooplankton have obvious implications for patterns in their food resource, the phytoplankton. It is entirely plausible that the lakewide shift in zooplankton is also one of the causative agents for observed shifts in algal composition from the historical "condition". Other possible causes might include changes in nutrient conditions, competition interactions with other algae and others.

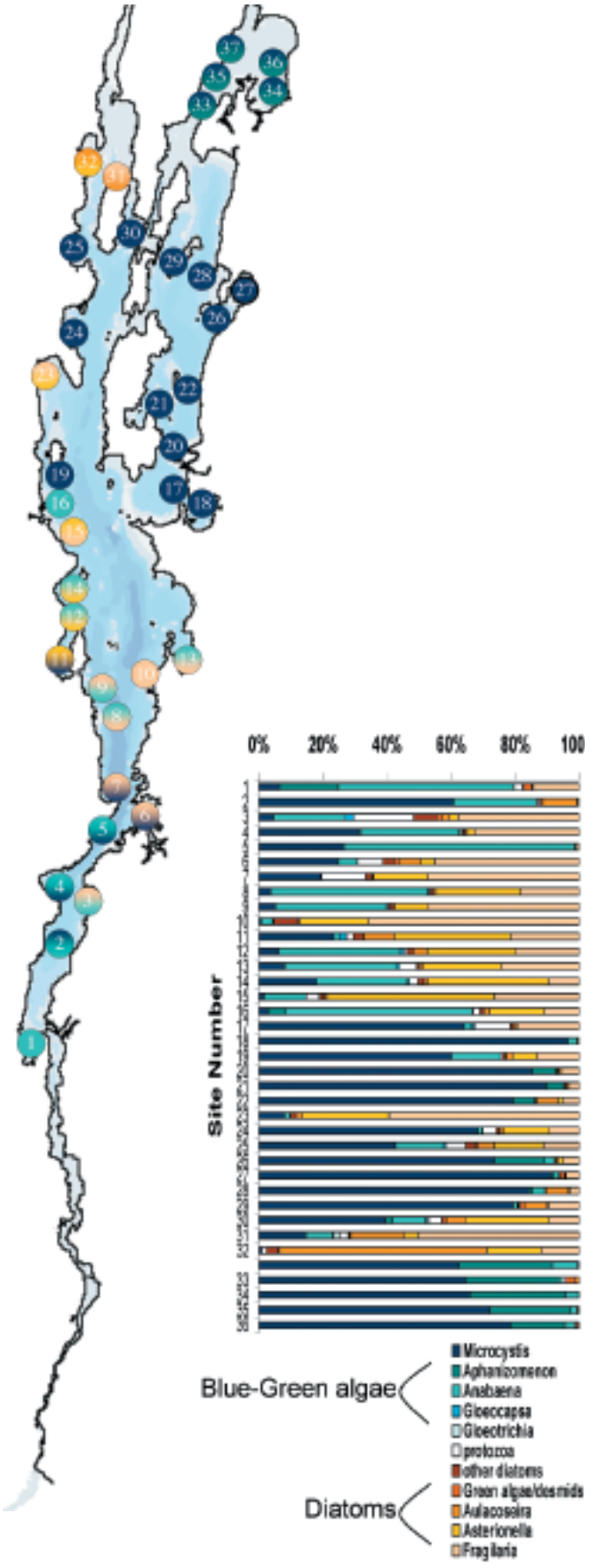

Figure 1. Algal community composition during July 26-28, 2004 lakewide sampling.

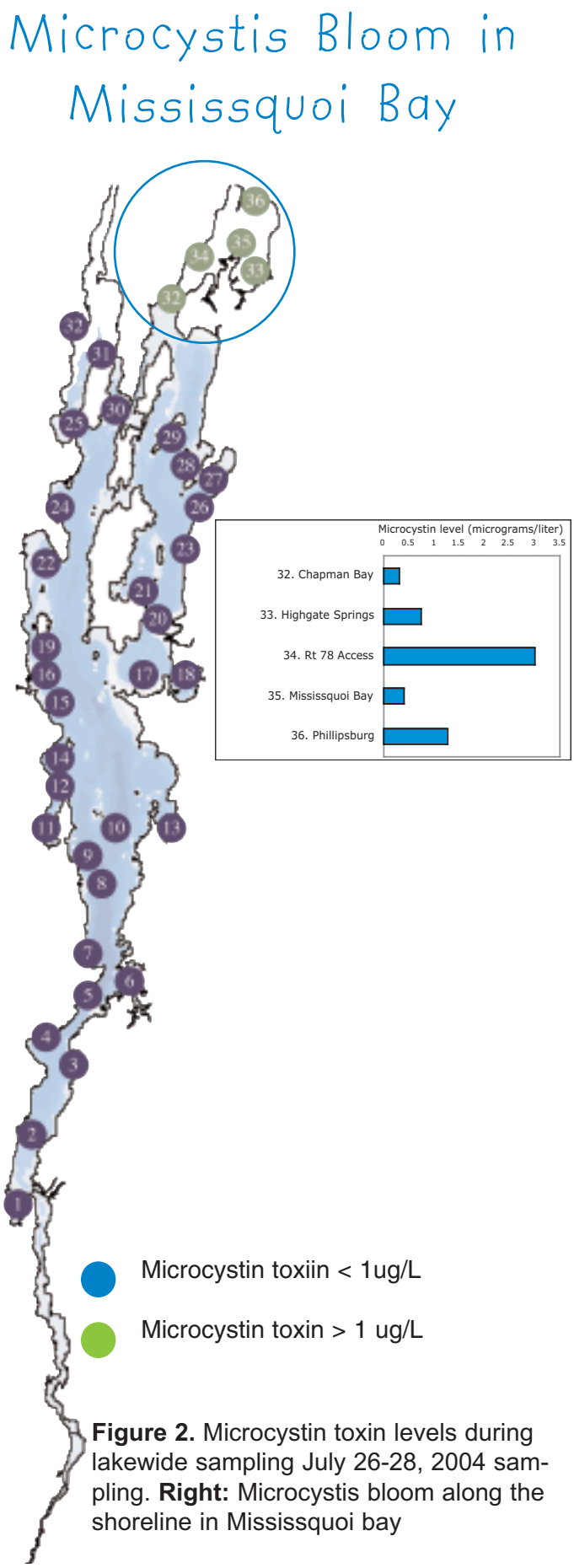

Figure 2. Microcystin toxin levels during lakewide sampling July 26-28, 2004 sampling. **Right:** Microcystis bloom along the shoreline in Mississquoi bay

Does Algal Toxin Level Relate to What We Find in the Algal Community at a Given Site?

Figure 2 shows the lakewide distribution of Microcystin toxin (determined from PPIA analysis [3]) during the same July 2004 sampling used to generate Figure 1. Patterns in Figure 2 are similar to data from previous years with algal toxin production highest in the Mississquoi Bay region of the Northeast Arm [1, 4]. Details of Mississquoi Bay seasonal sampling and public education are documented in Watzin et al. (this volume). Mississquoi Bay contains a dominant blue-green community, with the highest densities of algal toxin producing species (principally *Microcystis* and *Aphanizomenon*) when compared to other sites [4]. Of interest is that *Aphanizomenon* was not as prevalent in any other lake site except for those in Mississquoi Bay (Figure1) where it accounts for >25% of total algal abundance. Does this relate to the occurrence of toxins at these sites? Is there a density threshold for individual species that triggers toxin production? If so what is that density and how does it vary across species? Does the ratio of blue-green species (e.g., *Microcystis*/*Aphanizomenon*) matter? We are currently addressing these questions, among others, in Lake Champlain.

Acknowledgements

This study was supported by research grants from the New York Sea Grant program and the National Oceanic and Atmospheric Agency MERHAB program. Special thanks to Meghan Greene, Allison Labriola, Megan Pellam and Carriane Pershyn for help with the field sampling and laboratory work in 2004.

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The Application of Molecular Tools to Freshwater Harmful Algal Blooms: Identifying and Quantifying Toxic Cyanobacteria

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Freshwater harmful algal blooms are a global problem akin to the spread of disease: just when you think you have one outbreak under control, a reoccurring or novel organism can start proliferating. In recent years researchers in the Great Lakes have struggled with questions concerning the reoccurring seasonal occurrences of toxin-producing cyanobacteria in both Lakes Erie and Ontario. In Lake Erie these events have reoccurred on a nearly annual basis since 1995 [1]. Recent blooms have occurred throughout the western basin of Lake Erie as well as in Sandusky Bay and during cyanobacterial blooms the concentrations of microcystin have reached levels nearly 20 times those established by the World Health Organization (WHO) for safe drinking water (1 $\mu g\ L^{-1}$). Researchers have been studying blooms of toxic cyanobacteria for over a decade in Lakes Ontario and Huron.

An understanding of the reoccurrence of harmful algal blooms in environmental settings requires three questions to be answered: 1) what are the species forming the blooms, 2) what environmental factors trigger the formation of the blooms and 3) are the microorganisms consistently the same population that are emanating from a single reservoir, or a series of different populations that arise from different locations at different times? In the case of freshwater harmful algal blooms, answering these questions is of utmost interest to system managers, as the response to a single, reoccurring population would be predictably different than the management approach taken to address a multitude of different organisms. Better understanding of the environmental factors such as amounts of nutrients in the water and weather conditions, including temperature, wind speed, and solar irradiation that might trigger the formation of the bloom events could help in the development of forecasting systems for harmful algal blooms.

Toxic cyanobacteria are found in a variety of genera. During recent mass occurrences in the Great Lakes, mixed populations of cyanobacteria including *Microcystis*, *Anabaena*, *Planktothrix*, and *Aphanizomenon* were found associated with cyanotoxins. In such a population of bloom-forming cyanobacteria, only a small fraction of the cells is usually responsible for producing the toxin (Table 1). The non-toxin producing population consists of cells that are not able to produce toxin at all (non-toxic cells) and cells that are capable of producing toxin, but are not doing so at the moment (referred to as potentially toxic cells). Separating between these types is no easy task.

One other area where researchers are making significant inroads is the determination of specific species that can produce toxin. Given the cited diversity of toxin producers (above), it has become important from a management perspective to understand which organisms are producing toxins, as control of different cyanobacterial populations often requires different management strategies. Genetic relatedness can provide some clues to the origin of the bloom-forming community, and determine if the bloom originates from a single or multiple sources. The specific DNA sequences in the toxin biosynthetic operons also vary from genus to genus (Figure 1). Sequencing these genetic elements gives the molecular biologist an important tool to determine which genus or genera in mixed populations of cyanobacterial are the toxin-producing culprit. Knowing the responsible organism(s) is critical for the development of management strategies as differentcyanobacteria genera can respond very differently to changes in the nutrient addition and control techniques.

In studying the ecology of these freshwater cyanobacterial blooms, researchers have relied on standard tools (*i.e.*, microscopy, pigment analysis and cyanotoxin analysis) to determine which organisms are

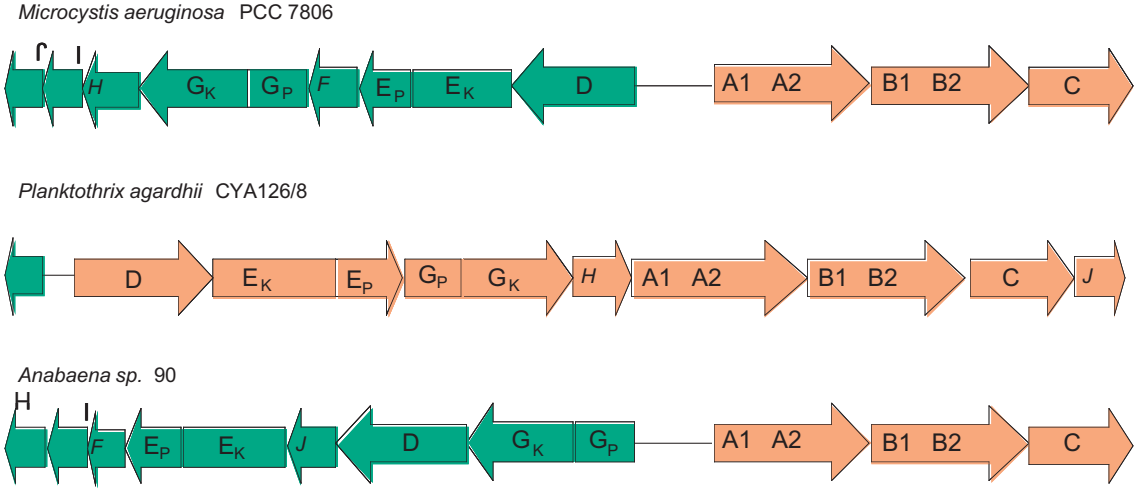
Table 1: The relative abundance of the different target genomes in samples collected from Lake Erie in 2003. Data adapted from Rinto-Kanto et al, 2005.

present and whether they are producing cyanotoxin. The classical approaches can provide some insight into the diversity of cyanobacterial populations and their toxigenic potential. However, the visual identification of cyanobacterial cells under a microscope is confounded by the fact the cells with different toxin producing capabilities have similar morphologies (*e.g.,* Figure 2). To this end, it is desirable to examine populations at the genetic level – determining the presence of genes associated with toxin production and using them to indicate the ability of a cell to produce a specific toxin. In genetically distinct cyanobacterial genera, generations of selection and genetic transfer have lead to multiple variations in the genetic systems associated with the production of these toxins. To the molecular ecologist, the knowledge of genes involved in toxin synthesis in each genus provides an opportunity to develop gene-based tools that can be used to identify general pathways for cyanotoxin production. The genes involved in toxin synthesis can be used for detection and identification of cyanobacteria employing these pathways. With the quantitative ability of these novel techniques, it has become possible for the first time to specifically quantify the cells bearing the genes required for toxin production. For example, Ouellette and Wilhelm [2] used DNA based detection methods to distingush between morphologically similar toxic and nontoxic *Microcystis* in Lake Erie algal blooms (Figure 2). and have been able to demonstrate that potentially toxic cells exist throughout the Lake Erie system [4].

Tracking Down the Source of Blooms

Besides detecting the presence or absence of potentially toxic cyanobacteria, analysis of specific DNA sequences from cyanobacterial populations allows investigators to determine how closely related the organisms in these populations are. Relatedness of organisms may provide some clues of the origins of the bloom forming community, whether they originate from one particular source or whether the cyanobacteria originate from multiple sources. Incorporating DNA-based techniques into studies of cyanobacterial blooms will therefore help investi-

Figure 1. Comparison of the organization of the microcystin toxin operon in three different species of toxic cyanobacteria. Genes with similar functions and similar sequences in this operon (the mcy cluster) are labeled with similar letters. Organization is compiled from Christiansen and Fastner (2003), Rouhiainen et al (2004) and Tillett et al (2000).



gators to gain a much deeper insight into the cyanobacterial communities: identification of the bloom forming cyanobacteria, understanding of the toxin production and elucidation of the relatedness of the cyanobacteria. Moreover, the novel molecular methods are highly sensitive, allowing detection of even just a few cells in water samples. The ability to detect even a small population of potentially toxic cells is extremely useful for establishing early warning systems for cyanobacterial blooms. Sample processing through DNA-based methods can be done rapidly, which enables processing of large sample volumes in a short time. One trained person can screen a dozen samples in one day. These are significant developments when compared to detection of blooms through a combination of microscopy and toxin analysis. Several research laboratories around the world have started applying these methods in the study of cyanobacteria and the same development is also taking place in studies of the Great Lakes.

In the future, integration of these molecular techniques into ecosystem scale observing systems (like the planned Great Lakes Observing system, (http://glos.us/) will provide scientists, system managers and health officials with more rapid diagnostics of potential bloom events. These sentinel tools require that present day science continue to elucidate both the organisms responsible for cyanotoxin production in the environment and the genetic elements associated with these capabilities.

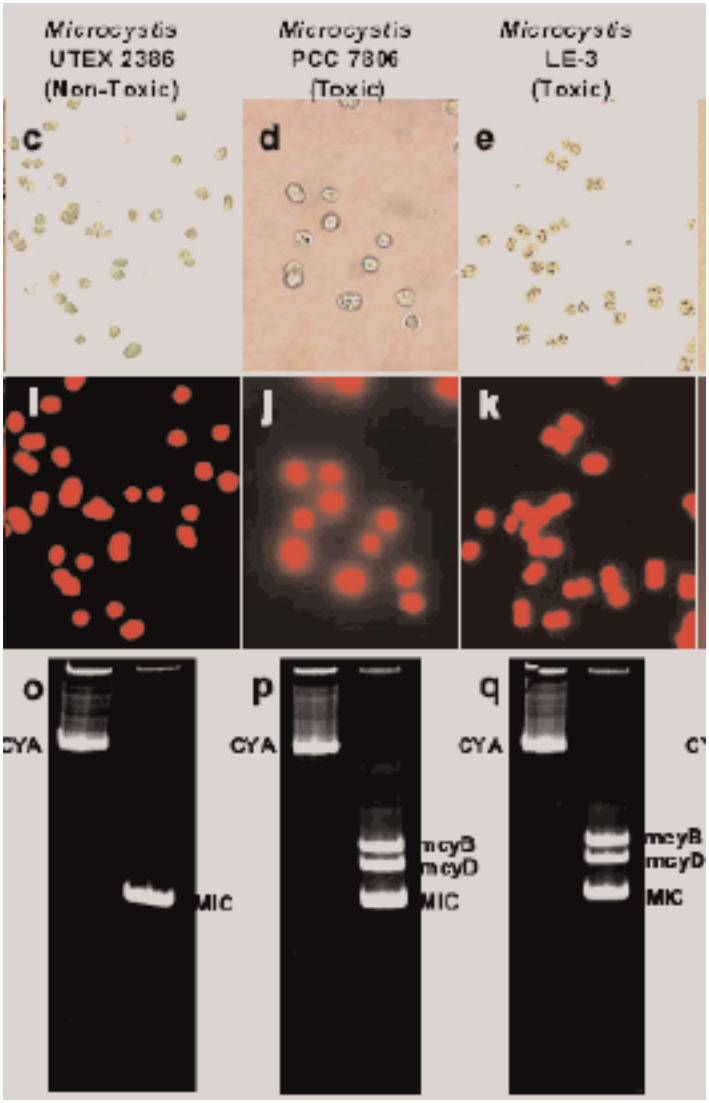


Figure 2. Two top panels: Photomicrographs illustrating morphologies of potentially toxic and non-toxic Microcystis cells from cultures. Lower panel: DNA-based detection allows distinguishing between morphologically similar toxic and non-toxic Microcystis. (adapted from Ouellette and Wilhelm 2003).

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The Occurrence of Cyanotoxins in the Nearshore and Coastal Embayments of Lake Ontario

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Lake Ontario, as many other lakes in the world, has suffered from a variety of environmental problems over the past fifty years. Massive blooms followed by die-backs of *Cladophora,* a filamentous algae, and by diebacks of the alewife, a fish, fouled beaches along the Lake Ontario shoreline in the 1960s. The phosphorus abatement program, agreed to by both Canada and the United States, successfully reduced the levels of phosphorus that stimulated the growth of algae. As a result of the reduction of phosphorus, once commonly found in detergents and sewage plant effluent, algae populations including the once ubiquitous shoreline *Cladophora* species were reduced by the late 1980s. Similarly with the introduction of the alewife-eating Pacific Salmon [11], lake alewife populations were reduced and the massive die-offs are no longer observed on the shores of Lake Ontario.

With the 1980s, came the realization that Lake Ontario fish were tainted by Persistent Bioaccumulative Toxic chemicals, including mirex and polychlorinated biphenyls, commonly known as PCBs [6]. In fact, a health advisory still exists on eating fish from Lake Ontario although there is some evidence of recent declines in mirex in salmon tissue [10]. Also, in the 1980s, came the invasion of several exotic or invasive species, such as the zebra and quagga mussel [4] and the fishhook water flea *Cercopagis* [9]. The effect of these, and other invasive species, on the food chain of Lake Ontario are still being assessed by researchers [7].

The approach of the management and research community to many environmental problems, such as those in Lake Ontario, has been reactive: that is, a problem emerges and the science and management communities react to it. Great Lakes' scientists are attempting to take a proactive approach by identifying new problems or emerging issues before they have an impact on either the lakes or the people who use them [8]. One of these emerging issues is cyanotoxins.

Cyanotoxins are produced by Cyanobacteria, among the oldest organisms on the planet. They are bacteria-like organisms that are capable of photosynthesis and at one time were commonly called blue-green algae or "pond scum". They can live in freshwater, saltwater or in mixed "brackish" water. Cyanobacteria are often considered to be nuisance organisms because they tend to occur on the surface of water in large numbers, called a bloom, affecting recreation, especially swimming.

It is known that light, temperature, and the water's nutrient content play roles in bloom formation. Under favorable conditions, slow-moving water or water rich in run-off from farms or sewage treatment plants are common places for the development of blue-green algae blooms. Some Cyanobacteria produce toxins that are harmful to humans and animals. These Cyanobacteria include *Microcystis, Cylindrospermopsis, Anabaena, Nodularia, Oscillatoria, Lyngbya* and *Aphanizomenon*. Some varieties of these algae, but not all, produce toxins within their cells which are released when the cells die or are ruptured.

There are many types of toxins, but those produced by Cyanobacteria mainly fall into three categories including hepatotoxins, neurotoxins and dermatoxins. Hepatotoxins affect the liver, neurotoxins affect the nervous system, and the dermatotoxins affect the

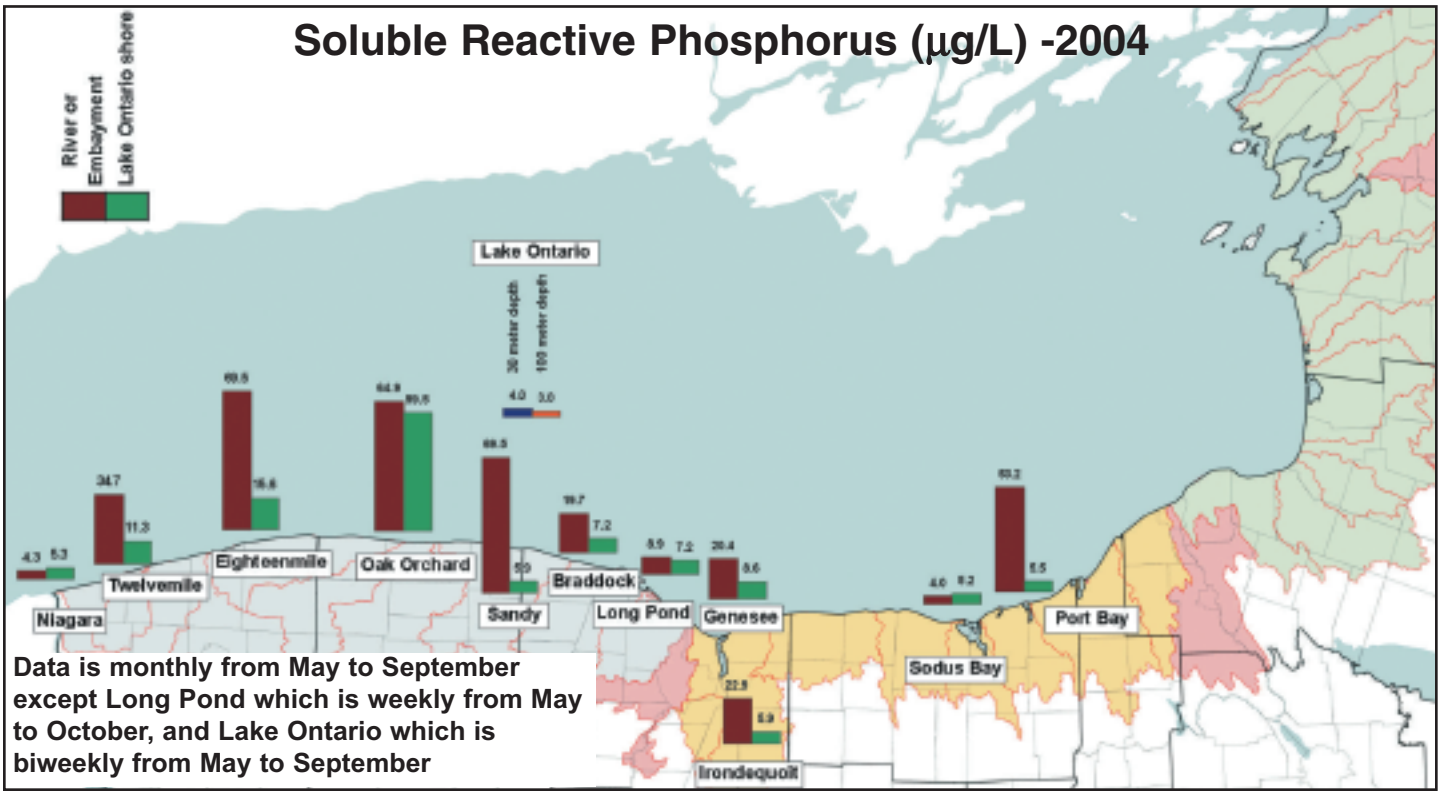

Figure 1. Levels of soluble reactive phosphorus in streams, embayments and the nearshore of Lake Ontario, 2004. Methodology follows APHA (1999).

skin and mucous membranes. Laboratory studies of these toxins indicate that at high concentrations the neurotoxins and hepatotoxins can be deadly to mice and to humans. Besides fatalities, swimming in and drinking water tainted with cyanotoxins can lead to allergic reactions on the skin, nausea, gastroenteritis, fever and headache. There have been numerous cases of people, dogs and livestock becoming ill after drinking or wading into water with cyanotoxins present. The most notorious case being in Brazil when 60 kidney patients died after drinking water contaminated with microcystin, a hepatotoxin, most likely produced by the Cyanobacteria *Microcystis* [12].

Unfortunately, it is not possible by visual inspection of an algae bloom to determine if it is a toxic bloom. At present, fairly sophisticated laboratory instrumentation is required for identification of toxins. Over time, these toxins are diluted and eventually break down and disappear. However, some of these toxins can remain in fish, which have consumed Cyanobacteria. As a result of these outbreaks, the World Health Organization [3] has placed limits, the Tolerable Daily Intake of cyanotoxins, on drinking water and recreational exposure to help prevent these outbreaks.

Why Be Concerned About Lake Ontario?

New York has the second longest shoreline of any of the Great Lakes' states. The shoreline is where the vast majority of the general public comes in contact with the lake and is also the mostly likely location of toxin-producing Cyanobacteria blooms in Lake Ontario. Over six million people per year annually visit parks along New York's Great Lakes corridor [5]. Anglers spend $134 million per year fishing on New York's Great Lakes' and despite health advisories on fish consumption, often eat fish from Lake Ontario. Most importantly, over three million New Yorkers depend on Lakes Ontario and Erie for drinking water. Clearly, New York is an important Great Lake state relying on the lakes for drinking water, recreation and economic development .

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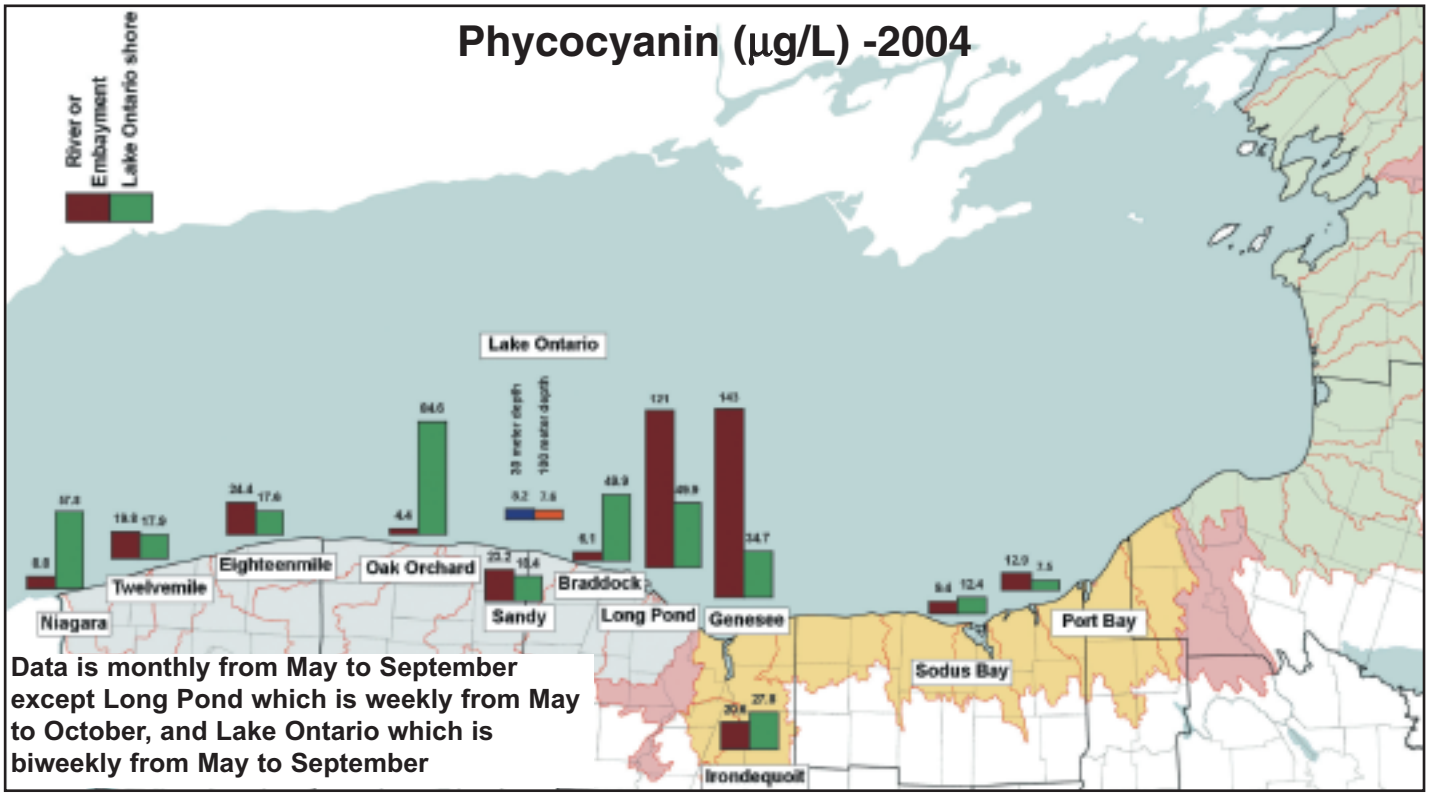

Figure 2. Levels of Cyanobacteria as indicated by phycocyanin in streams, embayments and the nearshore of Lake Ontario, 2004.

Do Cyanotoxins Exist in Lake Ontario?

Little is known about the spatial and seasonal occurrence of cyanotoxins in Lake Ontario. Recent work by the State University of New York (SUNY) at Brockport and SUNY Environmental Science and Forestry has demonstrated that the coastal waters of Lake Ontario have the necessary conditions for cyanotoxin production. In 2004, 24 sites along the south shore of Lake Ontario, as well as bays and rivers, from the Niagara River to Port Bay were sampled for phosphorus and the occurrence of the Cyanobacteria. Surprisingly, ambient levels of phosphorus in the nearshore waters often exceeded New York State's Department of Environmental Conservation guidelines of 20 µg P/L. A total of nine sites of 24 sites sampled had average concentrations greater than 20 µg P/L (Figure 1). Four sites, Eighteenmile Creek, Oak Orchard Creek, Sandy Creek and Port Bay had average concentrations exceeding 50 µg P/L. In contrast, the offshore waters of Lake Ontario averaged less than 5 µg P/L for the same time period. Since phosphorus is generally considered to be the limiting factor of plankton growth, the high phosphorus levels in the nearshore zone of Lake Ontario suggest that algal blooms in the nearshore are likely. In fact, this is the case. Phycocyanin is a measure of the amount of blue-green algae present in the water. During May, June, July, August and September of 2004, phycocyanin or blue green algae levels were exceedingly high in the nearshore region (Figure 2). For example, phycocyanin levels at Oak Orchard averaged 84.6 µg/L, while the Genesee River and Long Pond averaged 143 and 121 µg/L, respectively, compared to less than 10 µg/L at the offshore deep water sites of Lake Ontario (Figure 2). The high phosphorus and phycocyanin levels observed during the summer of 2004 suggested that cyanotoxins, such as microcystins, may be present in the nearshore of Lake Ontario. This concern became more evident when satellite imagery clearly demonstrated that a bloom of algae was "hugging" the entire southern coastline of Lake Ontario during August 2004 (Figure 3); an area of state and municipally operated beaches, private beaches, as well as the location of many municipal intake pipes of drinking water supplies of cities, towns and villages.

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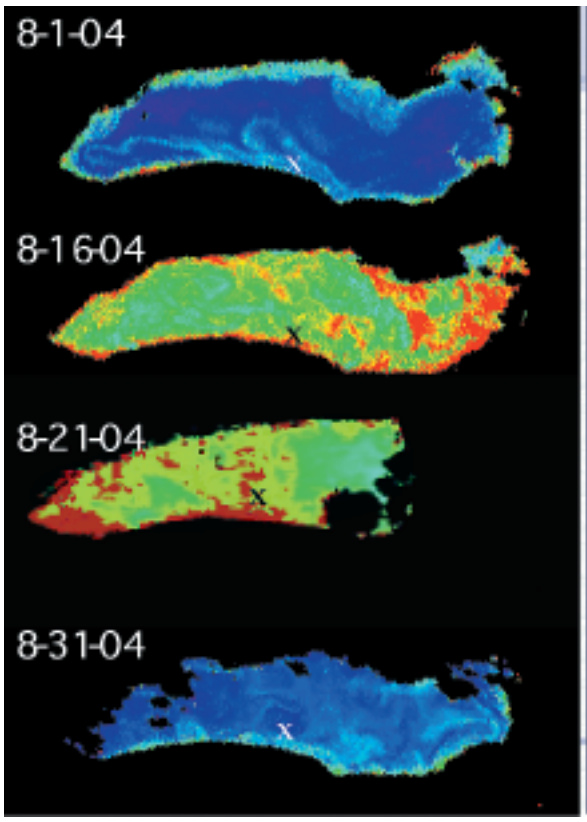

Site (Depth)	Date	MCY (µg/L)	CHLa (µg/L)	Phy (µg/L)
100m	8-3	.004	1.1	1.91
30m	8-3	.004	1.5	15.50
2m	8-3	.004	5.2	1.28
100m	8-18	.005	4.5	15.84
30m	8-18	.005	5.5	13.12
2m	8-18	.008	13.1	26.00
100m	9-2	.003	1.6	13.59
30m	9-2	.003	2.5	23.36
2m	9-2	.003	14.3	61.13

Imagery data complements of Paul Hopkins, SUNY ESF. Microcystin analysis was by protein phosphatase inhibition assay (Carmichael 1999).

Figure 3. Satellite imagery of Lake Ontario showing chlorophyll levels. Red indicates high levels of chlorophyll. Note red area, which is an algal bloom, along the south shore of Lake Ontario on 16 and 21 August 2004. Data in the table represent microcystin (MYC), phycocyanin (Phy) and chlorophyll levels (CHLa) collected from a vessel on Lake Ontario during the bloom. Imagery is SeaWiFS (Sea-viewing Wide Field-of-view Sensor). "X" indicates the general location of the sampling site.

Fortunately, analysis for the cyanotoxin microcystin during the month of August indicated that although Cyanobacteria were present in large quantities, the levels of the hepatotoxin microcystin never exceed the World Health Organization guideline of 1 $\mu g/L$ for drinking water (Figure 3). In fact, microcystin levels in the nearshore of the lake never exceeded 0.008 $\mu g/L$ while levels in the bays and rivers were often higher, by an order of magnitude (e.g., 0.076 $\mu g/L$ in Braddock Bay) (Table 1), but still significantly lower than the World Health Organization guidelines. Thus levels of the hepatotoxin microcystin were very low, near detection limits, along the south shore of Lake Ontario. However, elevated levels of microcystin exceeding WHO guidelines were observed during the summer in smaller lakes in the watershed of Lake Ontario. For example, microcystin levels were 5.07 $\mu g/L$ in Conesus Lake, and 10.7 $\mu g/L$ in Silver Lake in September 2004 and 1.59 $\mu g/L$ in Lake Neatahwanta during July of 2004 – all lakes in central and western New York that drain into Lake Ontario.

Summary

Cyanotoxins are an emerging issue that Great Lakes' scientists are conducting research on to determine occurrence, spatial and seasonal distribution, monitoring strategies and potential causes in Lake Ontario. Conditions necessary for blooms of Cyanobacteria exist along the shoreline of Lake Ontario. This is especially true in some embayments and rivers as levels of the nutrient phosphorus that stimulates the growth of Cyanobacteria is above New York State Department's of Environmental Conservation guidelines. Monitoring in 2004 demonstrated that abundance of Cyanobacteria are indeed high in streams, embayments and the nearshore compared to offshore waters of southern Lake Ontario. Initial research suggests that microcystin production along the southern shoreline of Lake Ontario is minimal and well below WHO guidelines. However, production of the microcystin toxin often exceeds World Health Organization guidelines in inland lakes and may serve as a source

to Lake Ontario. More information is required on the yearly variability of microcystin as wet and dry weather conditions appear to have affected the blooms of Cyanobacteria and the production of microcystin from year 2004 to year 2005 in both Lake Ontario and inland lakes. Vigilance by the general public utilizing the waters of Lake Ontario is still required. When visible blooms of algae are present at the surface, the general public and their animals should avoid contact with these waters.

Table 1. Microcystin (MCY), phycocyanin (Phy) and chlorophyll levels (CHLa) during the bloom of August 2004 sites along the lake proper but within the discharge plumes of creeks and bays along the south shore of Lake Ontario.

Lakeside Site in the discharge plume	Date (2004)	MCY (µg/L)	CHLa (µg/L)	Phy (µg/L)
Niagara River	8-24	.019	184.8	159.3
Twelvemile Creek	8-24	.006	2.87	19.1
Eighteenmile Creek	8-24	.037	9.69	34.9
Oak Orchard Creek	8-24	.008	3.63	14.0
Irondequoit Bay	8-25	.037	4.66	62.2
Genesee River	8-25	.063	5.65	123.4
Braddock Bay	8-25	.076	7.88	137.4
Port Bay	8-26	.006	2.36	20.5
Sodus Bay	8-26	.003	0.70	21.8

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Why do Cyanobacteria Produce Toxins? Investigating a Possible Trigger for Microcystin Production in a Harmful Algal Bloom

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The production of toxic cyanobacterial blooms is well documented in the modern scientific literature. Recent surveys from Europe and the Great Lakes suggest that as many as 50% of the natural blooms may contain either some level of toxicity, or at least the genetic potential for the production of toxicity. Other studies suggest that even within a given bloom, both toxic and non-toxic varieties of the same organism may co-exist [9]. Yet despite our increasing understanding of the molecular and biochemical basis for toxin production, the chemical nature of these toxins, and the organisms responsible for toxin formation [4, 6], we have a very poor understanding of the ecophysiological role for toxin formation. Most studies have focused on microcystin production in the colonial species *Microcystis aeruginosa*. Does microcystin production in this species provide a benefit to the toxic cyanobacteria that outweighs its cost of biosynthesis, or is it simply the vestige of an older pathway that has yet to disappear [8]? Understanding the physiological role, as well as those environmental factors that trigger microcystin production is important for the development of management plans to protect against potentially toxic harmful algal blooms.

Several hypotheses on the ecological roles for microcystin have been proposed. One of the more common hypotheses is that they serve as an anti-grazing or inhibitory compound. Ingestion of the microcystin toxins does indeed affect its consumers, suggesting that an ecological function may be related to the elimination of grazers. Experiments using mutant (non-microcystin producers) and wild type (microcystin producers) strains of *Microcystis* as food for the crustacean zooplankter *Daphnia* have shown that wild

Figure 1. Michael Twiss (Clarkson University) and Sandra Gouvêa set up a natural UV light exposure experiment onboard the USEPA vessel *Lake Guardian* in Lake Erie (August 2003), to examine the influence of UV light, copper, and zinc on the production of microcystins by cyanobacteria. Photo Credit: Nathaniel Ostrom.

type strains were toxic to the grazer whereas the mutant strains were not [10]). However, while *Daphnia* may be killed by microcystins, the *Daphnia* also showed no selection in the feeding preference between toxic and non-toxic strains, raising question as to the effectiveness of this type of approach and hence, the evolutionary advantage of producing a toxin. Microcystins have also been postulated to have an allelopathic role against other algae or even higher plants [1].

A second possibility is that microcystins serve as an intracellular regulatory compound. Careful work on

the microcystin quota of cells (e.g., the amount of microcystin per cell under changing environmental and physiological conditions) has shown microcystin production rates approximating that needed to maintain a constant concentration during cell division [7]. These findings suggest that microcystin plays an essential function in the cell and their levels are tightly regulated similar to cellular proteins and chlorophyll-*a* concentrations. Microcystin-devoid mutants of an otherwise toxic cell strain lack the ability to respond properly to light and are missing production of several key photosynthetic gene products. This has led to the suggestion that microcystins may be a quorum sensing compounds used to mediate cell density in phytoplankton blooms [4].

A third possibility is that microcystins serve an essential function in protection of the cell against external stressors such as toxic metals or UV light. Microcystins can effectively complex Cu^{2+} and Zn^{2+} ions [5] thereby reducing the toxicity of these metals. Cyanobacteria are more sensitive to metal stress than most eukaryotic algae [2, 11]. Metals such as Cu and Zn are usually complexed by dissolved organic carbon (DOC) in surface waters. However, ultraviolet radiation can oxidize these DOC complexes, releasing the metals to increase the free metal concentrations and their toxicity to phytoplankton.

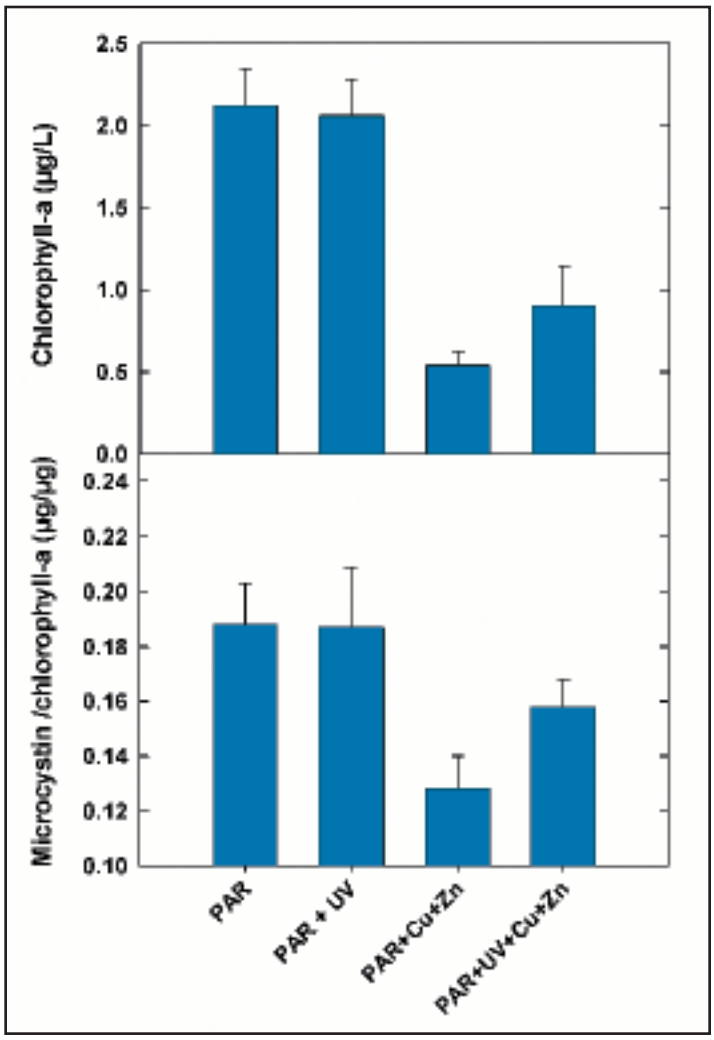

Figure 2. Effect of ultraviolet radiation (UV), 10 nM copper, and 10 nM zinc on chlorophyll-a and microcystin production in the toxic cyanbacterium *Microcystis*. The cyanobacteria were cultured in filter sterilized water from Lake Erie (Station 61) and exposed to attenuated (25% of ambient) levels of natural light (UV plus photosynthetically active radiation; PAR). Treatments in Teflon bottles received PAR and UV, treatments in polycarbonate bottles received only PAR.

To investigate if microcystins could play a role in protecting *Microcystis* from UV or toxic metal stress, we conducted a series of simple experiments in a field and laboratory to assess if exposure to UV light and trace metals (Cu and Zn) would affect growth, photosynthesis and microcystin production in *Microcystis aeruginosa*. We discovered these factors alone or combined did not increase the microcystin content (Figure 2), but that any factor that decreased biomass also decreased toxin production in *M. aeruginosa*. These results suggest that microcystin production was not induced to protect against these stressors, but that toxin production in this organism was constitutive, as suggested by Lyck [7]. This observation suggests that changes in the overall microcystin concentration in natural environments may be due to changes in the abundance of toxin-producing strains, and not due to change in toxin expression by a single strain. Given that both toxic and non-toxic strains often co-exist within a single bloom, future research will need to examine the interactions between these strains and those properties that lead one strain to dominate over the other.

We discovered that these factors (viz. sublethal concentrations of zinc or copper, and ultraviolet light) alone or combined did not increase the microcystin content, but that any factor that decreased biomass also decreased toxin production in *Microcystis aeruginosa*.

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