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Monitoring and Evaluation of Cyanobacteria in Lake Champlain

Summer 2005

Prepared by
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MONITORING AND EVALUATION OF CYANOBACTERIA IN LAKE CHAMPLAIN

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Report to

Lake Champlain Basin Program

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EXECUTIVE SUMMARY

In 2005, monitoring for potential toxin-producing cyanobacteria continued on Lake Champlain with the following specific objectives:

- Continue monitoring of blue-green algae (BGA) at the Long-term Water Quality and Biological Monitoring Project sites, selected stations in the greater Burlington area, St. Albans Bay and Missisquoi Bay by UVM and the Vermont DEC.
- Continue to work with volunteer lay monitors in Missisquoi Bay and add lay monitors in other locations, especially in the north lake and on the New York side of the lake.
- Continue screening for the presence of toxins when potential toxin-producing BGA are observed.
- Continue to use and refine a tiered BGA alert system framework, incorporating data and knowledge gained during 2003 and 2004.
- Enhance the communication network among state and provincial agencies in Vermont, New York and Quebec to facilitate regular exchange of information about current BGA conditions and the potential for human exposure to toxins. Continue to work towards a lake-wide standard for reporting this information.

Collections of net and whole water plankton began in June in most locations, and continued through October. Sample sites encompassed all of Lake Champlain, but a special effort was made in Missisquoi Bay, St. Albans Bay, and the north lake, areas known to have problems with toxic blooms in the past. Citizen monitors living around the lake at 15 specific sites were recruited to collect samples from shoreline locations where algae accumulated.

In 2005, potential toxin-producing cyanobacteria species remained a common part of Lake Champlain's plankton, but bloom intensity and toxicity were lower than in 2004. As in the past years, highest abundances were found in Missisquoi Bay and St. Albans Bay, where total phosphorus and total nitrogen concentrations were also high. As in past years, there was a high degree of spatial variability in toxin concentrations, and most high toxin concentrations were found in dense surface accumulations of algae or in scums. The seasonal median concentration of microcystin in Missisquoi Bay was 0.8 µg/L; in St. Albans Bay, it was 0.4 µg/L. The median concentration in duplicate samples collected in October in Burlington Bay also contained elevated concentrations of microcsytin, about 7.4 µg/L. In 2005, anatoxin-a was not detected at any site.

The e-mail notification system worked well to keep public health officials informed about algal and toxin conditions. In order to improve communication with the users of Lake Champlain, in 2005, we partnered with Vermont Department of Health to post information about blue-green algae and the weekly results of our testing on their web site. This information included information from all sites and all locations where testing was done.

INTRODUCTION

Lake Champlain is one of the largest lakes in the United States and is often called the "Sixth Great Lake." Although primarily a recreational lake, it also serves as a source of drinking water and for the disposal of municipal wastes. It also receives non-point runoff from agricultural areas. These activities, among others, have been identified as contributing to recognized, or potential, water quality problems within the lake system. Current issues of concern include eutrophication, toxics, and nuisance plants. The problems of concern are associated with the biological communities of the lake, e.g., algal blooms and their potential toxins, depletion of dissolved oxygen, low transparency, contaminated fish flesh, declining fisheries, and invasions of exotics.

In response to a dog-poisoning attributed to cyanobacteria toxins in 1999, the LCBP initiated a study to investigate the occurrence of potential toxin-producing cyanobacteria and their toxins in Lake Champlain. Over the next five years, this monitoring program has evolved to document the presence and extent of toxic cyanobacteria blooms in Lake Champlain, and the levels of cyanotoxins that have occurred.

In addition, a project supported through NOAA's MERHAB program began in 2002 and continues on Lake Champlain, through a partnership between UVM, SUNY-ESF and SUNY-Plattsburgh. The project has multiple objectives, including documenting the distribution of cyanotoxins in the lake, developing a rapid screening method for anatoxin, and developing methods for monitoring throughout the lower Great Lakes (Lakes Erie, Ontario and Champlain). Data collected from this project are not available rapidly enough to drive the weekly public alert system, but data are regularly shared among the project investigators.

This project will continue to add to the data gathered about BGA in Lake Champlain since 2000. It will continue to refine and test a tiered monitoring and alert system framework to inform health officials and the general public about the occurrence of, and potential risks associated with, the development of BGA blooms.

Beginning in 2003, regular monitoring has been conducted by UVM in partnership with the LCBP long-term monitoring program and with citizen monitors recruited with the assistance of the Lake Champlain Committee. In 2005 we continued this effort with the following specific objectives:

Objectives:

- Continue monitoring of BGA at the Long-term Water Quality and Biological Monitoring Project sites, selected stations in the greater Burlington area, St. Albans Bay and Missisquoi Bay by UVM and the Vermont DEC.
- Continue to work with volunteer lay monitors in Missisquoi Bay and add lay monitors in other locations, especially in the north lake and on the New York side of the lake.
- Continue screening for the presence of toxins when potential toxin-producing BGA are observed.
- Continue to use and refine a tiered BGA alert system framework, incorporating data and knowledge gained during 2003 and 2004.

- Enhance the communication network among state and provincial agencies in Vermont, New York and Quebec to facilitate regular exchange of information about current BGA conditions and the potential for human exposure to toxins. Continue to work towards a lake-wide standard for reporting this information.

METHODS

Field Collection

To survey plankton populations lakewide, we established partnerships with the VT DEC staff conducting the LCBP long-term monitoring program. VT DEC staff collected plankton samples from the 14 Long-Term Monitoring Program sites during their routine collections (Figure 1). Working with the Lake Champlain Committee, we also recruited volunteers to sample shoreline locations in Missisquoi Bay Maquam Bay, and other areas of the lake (Figure 1). We also sampled sites in Missisquoi Bay, St. Albans Bay, and Burlington Bay, where the highest population density of basin residents live and two large water supply systems draw their water.

Frequency. Monitoring for the presence of BGA began in June at the VT DEC sites and at the UVM sites, and in early July at the citizen monitoring sites. The VT DEC sites were sampled approximately biweekly regardless of bloom conditions, as dictated by the state's regular program activities. Frequency of sample collection in Burlington Bay, Missisquoi Bay, and St. Albans Bay was bi-weekly or weekly, as determined following the tiered alert system framework (Table 1). This framework, based on recommendations in Chorus and Bartram (1999) calls for less frequent sampling initially, then weekly sampling once bloom conditions appear. The lay monitors in Missisquoi Bay and Maquam Bay sampled weekly from July through August. In Burlington Bay, cyanobacteria levels remained low throughout the season, and we remained at a bi-weekly sampling interval for the entire period. In Missisquoi and St. Albans Bay, algal densities were much higher, and weekly sampling was initiated in early July and continued until September, when cell densities indicated the decline of the bloom.

Analytical Parameters. The following types of samples were collected in Burlington Bay, St. Albans Bay and Missisquoi Bay during 2005:

- whole water and net plankton
- whole water for total nitrogen
- whole water for total phosphorus
- whole water for chlorophyll *a*
- whole water and net plankton for toxins (the analysis of this parameter began when microscopic analysis indicated potential toxin-producing taxa were present)

At the VT DEC sites, only net plankton samples were collected for this project; however, total nitrogen, total phosphorus, and chlorophyll samples were collected as part of the Long-Term Biomonitoring Project.

Figure 1. Location of the blue-green algae monitoring stations. Gray boxes delineate areas sampled by UVM and citizen monitors.

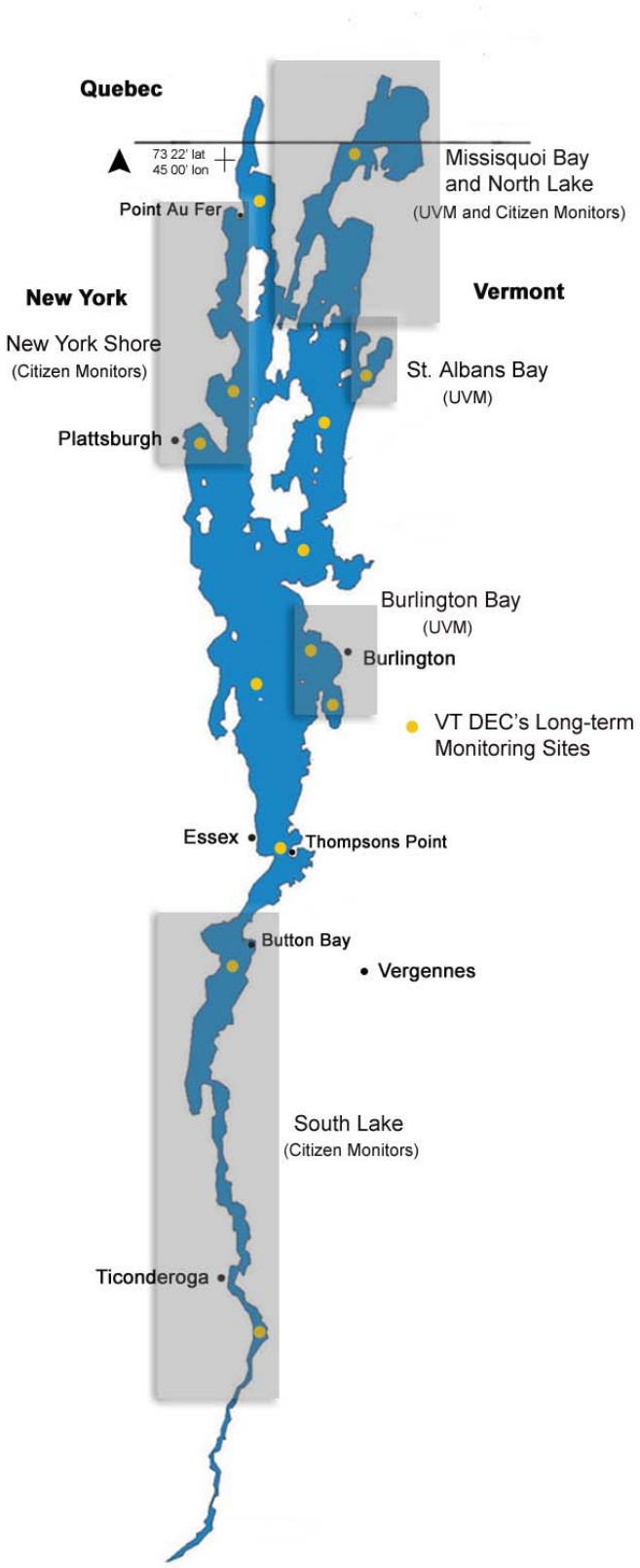


Table 1. Outline of our prototype tiered sampling and alert framework.

Qualitative sampling	
Frequency:	2/month
Collect:	Vertical plankton tows (63 µm net, upper 3 m) Screened within 48 hours
Conclusions:	If potential toxin-producing taxa observed, proceed to *Quantitative sampling*
Quantitative sampling	
Frequency:	2/month
Collect:	Vertical plankton tow (63 µm net, upper 3m) Full enumeration within 48 hours
Conclusions:	If BGA reaches densities reach 2000 cells/mL, proceed to V*igilance level*
Vigilance level	
Frequency:	1/wk at mid-day
Collect:	Vertical plankton tow (63 µm net, upper 3m) Full enumeration within 48 hours
Conclusions:	If BGA exceed 4,000 cells/mL with greater than 80% of natural units BGA, proceed to *Alert Level 1* Return to *Quantitative sampling* if densities fall below 2,000 BGA cells/mL Notify public health officials that BGA are abundant and blooms could form
Alert Level 1	
Frequency:	1/wk at mid-day (or more frequently as needed)
Collect:	Whole water phytoplankton samples Whole water chlorophyll *a* Whole water toxin samples
Conclusions:	If microcystin concentration exceeds 6 µg/L (VT DOH recreational standard), proceed to *Alert Level 2* Notify public health officials of potential risks to humans and animals
Alert Level 2	
Frequency:	1/wk at mid-day (or more frequently as weather conditions dictate)
Collect:	As for alert level 1
Conclusions:	Return to *Alert Level 1* if microcystin concentration drops below 6 µg/L Notify public health officials that significant risk to humans and animals exists. Public Health Advisories should be issued by appropriate agencies.

In Burlington Bay, temperature, conductivity and oxygen were measured using a Seabird profiler from 0.5 m below the surface to 0.5 m above the bottom on some dates. Secchi depths were measured at all locations in Burlington Bay, Missisquoi Bay, St. Albans Bay, and at the Long-Term Biomonitoring Project sites.

Sample Collection. Net plankton, net chlorophyll and net toxin samples were obtained using a 63µm Wisconsin net. A single 3m tow was collected, placed in a cooler, and transported back to the laboratory where the total volume was recorded and the sample was subdivided for the analyses.

Total nitrogen, total phosphorus, whole water chlorophyll, and whole water plankton samples were collected by surface grab sampling. Two replicates were collected for each parameter.

Preservation and storage. Nalgene bottles were used for all samples. Nutrient and toxin containers were cleaned with 20% hydrochloric acid solution prior to use. Nitrogen samples were preserved with sulfuric acid to a pH less than 2 and stored until analysis. Total phosphorus samples were frozen until analysis. Plankton samples were preserved with 1% Lugols iodine solution and stored in the dark until analysis. Chlorophyll samples were filtered and frozen for analysis within 24 hours. Lake water samples for toxin analysis were preserved in one of three ways: filtered and frozen upon return to the lab, filtered and shipped for analysis at SUNY within 24 hours, or shipped as whole water samples for analysis at SUNY within 24 hours.

Sample Analysis

Chlorophyll. All samples were thoroughly mixed and then filtered onto 1.2 µm glass fiber filters (Whatman 934-AH) under low pressure. After sufficient material was filtered to leave a visible green layer, filters were placed in clean 15mL plastic centrifuge tubes and frozen. For chlorophyll extraction, 8mL of 95% ethanol was added to each tube, all tubes were placed in an 80°C water bath for 8 minutes, covered in foil, and placed in a refrigerator overnight. After extraction, the samples were brought to room temperature, shaken to homogenize the extract, and centrifuged at 3000 rpm for 10 minutes. Non-acidified and acidified extract absorbance was measured at 665 and 750 nm. Chlorophyll concentrations in the net plankton samples were extrapolated to reflect actual chlorophyll concentrations in the original lake water. About 10% of the samples were run in duplicate.

Net plankton. Net plankton were analyzed either as qualitative or quantitative samples. Initial samples were evaluated qualitatively, noting and recording the taxa present. Once potentially toxic cyanobacteria were identified in the samples, evaluation became quantitative; individual algal units in the samples were identified and enumerated, and densities were calculated for each taxon.

An aliquot of well-mixed sample was placed in a Sedgwick Rafter cell and allowed to settle for 10 minutes. Cells were examined at 100X with phase contrast using an inverted Olympus IX70 microscope. For qualitative samples, the entire chamber was scanned and algal taxa present were recorded. For quantitative samples, algal units were identified and enumerated. Counting continued until 100 cells of the most abundant genus had been observed or at least 10 fields had

been examined. Algal units were categorized by size (single cells, fragments of colonies or filaments, small, medium, or large colonies or filaments). The enumerated natural units were multiplied by a cell factor to estimate cell densities (Table 2). Cell densities were extrapolated to reflect plankton populations in the original lake water.

Table 2. Cell factors used to estimate field densities of colonial algae.

Taxon	Unit Category	Estimated Cells/Unit	Cell Factor
Anabaena spp., Aulocoseira, Fragilaria	fragment	1 – 20	10
	small	20 – 100	60
	medium	100 – 1000	500
	large	>1000	1000
Microcystis, Coelosphaerium	small	<100	50
	medium	100 - 1000	500
	large	>1000	1000
Gloeotrichia spp.	fragment	single trichome	20
	small	quarter of a colony	2500
	medium	half of a colony	5000
	large	entire colony	10,000
Aphanizomenon spp.	fragment	single trichome	measured
	small	small flake	200
	medium	medium flake	500
	large	large flake	1000

Whole water plankton. Whole water plankton were examined using Ütermohl settling chambers. Aliquots of well-mixed samples were allowed to settle for a minimum of 4 days, then counted using an Olympus IX70 inverted microscope with phase contrast at 400x. Counting continued until 100 individuals of the most abundant taxa had been observed or 100 fields had been evaluated. Natural units and cell densities were determined as described above.

Phosphorus. Total phosphorus samples were thawed and mixed thoroughly. An aliquot (generally 50mL) was digested using ammonium persulfate (APHA 1995) and analyzed following Quikchem™ Method 10-115-01-1-F using a Lachet Quikchem™ 8000 Series Flow Injection Analyzer.

Total Nitrogen. Total nitrogen samples were analyzed using persulfate digestion (APHA 1995) and cadmium reduction following Quikchem™ Method 10-107-06-2-H using a Lachet Quikchem™ 8000 Series Flow Injection Analyzer.

Toxin Sample Preparation. To prepare net plankton for analysis, a well-mixed aliquot of plankton concentrate was filtered onto Whatman 934-AH glass fiber filters. Filters for analysis of toxins by high performance liquid chromatography (HPLC) and protein phosphatase

inhibition assay (PPIA) at SUNY were placed on ice and shipped by overnight carrier. Filters for enyzme-linked immunosorbant assay (ELISA) analysis by the VT DOH were placed on ice and delivered to their laboratory in Burlington within 24 hours. Filters for ELISA assay by UVM were placed in 15mL glass centrifuge tubes with Teflon-lined caps with 8 mL of 50% methanol, shaken well and stored at –80°C until analysis.

Whole water samples for analysis by HPLC and PPIA at SUNY were kept cold and sent by overnight carrier within 24 hours.

Microcystin (s) by ELISA. Toxin samples in 50% methanol were thawed, shaken and re-frozen two times before beginning analysis. Extracted samples were diluted with deionized water until methanol represented less than 5% of the total volume, following recommendations to improve the accuracy of the method (Metcalf et al. 2000). Microcystin plate kits were purchased from Envirologix Inc. (Portland ME). UVM and VT DOH used kits from the same production lot. Analyses at VT DOH were used as a QA/QC check.

Samples were run in duplicate following manufacturer's instructions on a KC Jr. plate reader (Biotek Instruments), utilizing standards provided in the kit. Mean values were used to determine the toxin concentration of each pair of samples. Samples exceeding the range recommended by the kit were diluted and re-analyzed. Samples below the range were also re-analyzed using manufacturer recommended dilution procedures for the standards. Laboratory blanks were run with each sample batch using deionized water.

Microcystin (s) by PPIA. PPIA analysis followed a modification of Carmichael and An (1999). Microcystin LR standards (0.06 – 1000 µg/L) were prepared fresh from a 40 µg/L stock in 50% acidified methanol. The protein phosphatase 1, catalytic subunit Roche, was used at a working concentration of 0.1mU/200 µL. All assays were done in 96 well plates in a 37°C incubator. Readings at 405nm were taken every 5 minutes for 60 minutes using an E-max plate reader.

Anatoxin-a by HPLC. At SUNY, algal material was freeze-dried and then extracted with acidified methanol. Solid phase extraction cartridges were eluted with 100% methanol. Samples were analyzed in a Zorbax ACE C18 column with C-18 Phenomenex guard disk following James et al. (1997). Several duplicate samples were analyzed at Wright State University by Dr. Wayne Carmichael, using the same procedures, as a QA/QC check.

RESULTS

Cyanobacteria and Toxins at the Monitoring Sites

While many of the samples collected at the Long Term Monitoring Sites were analyzed qualitatively until mid to late summer, almost all of the samples collected by UVM and the citizen monitors were analyzed quantitatively. The total number of samples collected for each type of analysis (quantitative plankton, toxin and chlorophyll) was about 500 in 2005 (Table 3).

Table 3. Number of quantitative samples collected and analyzed in the Cyanobacteria Monitoring Program in 2005.

Type of Sample	Phytoplankton		Microcystin		Chlorophyll *a*	
	net	water[1]	net plankton	whole water plankton	net plankton	whole water plankton
Number of Samples Collected	324	144	214	271	214	346
Number of Samples Analyzed	324	140	2*	136*	202	335
water[1] - whole water samples to be counted by net protocol						
*meeting QA						

The alert status reached and the maximum density of potentially toxic cyanobacteria cells at each site monitored are listed in Table 4. *Aphanizomenon* spp., *Microcystis* spp. and *Anabaena flos-aquae* were all widely distributed at sites across Lake Champlain.

At the Long-term Biomonitoring sites, cyanobacteria densities did not reach the Alert Level at any site except Station 50, in Missisquoi Bay. At the UVM Sites, Alert Level was reached in mid-July in Missisquoi Bay and in early August in St. Albans Bay. At the citizen monitoring sites, all of which were located along the shoreline, Alert Level was reached in early to mid-July in most locations in Missisquoi Bay. The Burlington Bay sites did not reach Alert Level at any of the regular sites, but did along the shoreline briefly in October. Three South Lake sites reached Alert Level briefly during the season (Long Point, Littlefield Shore, and Button Bay).

Table 4. Summary of plankton sample status at cyanobacteria monitoring stations in 2005. All microcystin concentrations are based on ELISA tests conducted at UVM.

VT DEC Monitoring

Region	Station/Location	Monitoring Status	Date Achieved	Cyanobacteria Present	Maximum Density of Potentially Toxic Cells/mL
South	2. Benson Landing	Qualitative	Jul 11	None observed	0
	4. Crown Point	Quantitative	Jul 11	*Anabaena*, *Aphanizomenon*, *Microcystis*	1130 (Aug 12)
	7. Cole Bay	Quantitative	Jul 21	*Anabaena*, *Aphanizomenon*, *Microcystis*	860 (Aug 15)
	9. Diamond Island	Quantitative	Jul 21	*Anabaena*, *Aphanizomenon*, *Microcystis*	262 (Aug 03)
Main	16. Shelburne Bay	Quantitative	Jul 13	*Anabaena*, *Aphanizomenon*, *Microcystis*	157 (Aug 18)

	19. Main Lake	Quantitative	Jul 13	*Anabaena*, *Aphanizomenon*, *Microcystis*	785 (Sep 16)
	21. Burlington Harbor	Quantitative	Jul 13	*Anabaena*, *Aphanizomenon*, *Microcystis*	203 (Aug 04)
	25. Malletts Bay	Quantitative	Jun 29	*Anabaena*, *Aphanizomenon*, *Coelosphaerium*, *Microcystis*	73 (Sep 06)
Northwest	33. Cumberland Bay	Quantitative	Jun 29	*Anabaena*, *Aphanizomenon*, *Coelosphaerium*, *Microcystis*	107 (Aug 29)
	36. Point au Roche	Quantitative	Jun 29	*Anabaena*, *Aphanizomenon*, *Microcystis*	545 (Aug 19)
	46. Alburg Center	Quantitative	Jul 18	*Anabaena*, *Aphanizomenon*, *Coelosphaerium*, *Microcystis*	90 (Sep 13)
Northeast	34. Inland Sea	Quantitative	Aug 18	*Anabaena*, *Aphanizomenon*, *Microcystis*	676
	40. St. Albans Bay	Quantitative	Jul 06	*Anabaena*, *Aphanizomenon*, *Microcystis*	1304 (Aug 02)
Missisquoi Bay	50. Missisquoi Bay	Alert 1	Aug 22	*Anabaena*, *Aphanizomenon*, *Microcystis*	6607 (Aug 22)

UVM Monitoring

Region	Location	Monitoring Status	Date Achieved	Highest Microcystin (µg/L) Observed (wwp)	Cyanobacteria Present	Maximum Density of Potentially Toxic Cells/mL (net plankton)
South	Long Point N. Ferrisburgh	Alert 1	Oct 24	Not measured	*Anabaena*, *Aphanizomenon*	1,085,600 (Oct 24)
Main	Burlington Waterfront	Vigilance	Sep 30	Not measured	*Anabaena*, *Aphanizomenon*, *Microcystis*	3246 (Sep 30)
	Burlington Water Bay	Vigilance	Sep 14	Not measured	*Anabaena*, *Aphanizomenon*, *Microcystis*	1446 (Sep 14)
	Champlain Water Bay	Quantitative	Jul 20	Not measured	*Anabaena*, *Aphanizomenon*, *Coelosphaerium*, *Microcystis*	653 (Aug 29)

	Melosira Boat Slip	Alert 1, 2	Sep 27	8.80 (Oct 21)	*Anabaena*, *Aphanizomenon*, *Microcystis*	207,886 Oct 21
	North Beach	Quantitative	Jul 20	Not measured	*Anabaena*, *Aphanizomenon*, *Microcystis*	1197 (Aug 29)
	Red Rocks Beach	Quantitative	Jul 05	Not measured	*Anabaena*, *Aphanizomenon*, *Microcystis*	805 (Aug 17)
Northeast	Ransom Bay	Qualitative	Aug 23	Not measured	None observed n=1	0
	St. Albans Boatlaunch	Alert 1	Aug 02	0.76 (Aug 16)	*Anabaena*, *Aphanizomenon*, *Coelosphaerium*, *Microcystis*	12,227 (Aug 09)
Mississquoi Bay	Alburg	Vigilance	Aug 02	0.54 (Aug 09)	*Anabaena*, *Aphanizomenon*, *Coelosphaerium*, *Microcystis*	3775 (Aug 02)
	Highgate Cliffs	Alert 2	Jul 12	21.28 (Jul 20)	*Anabaena*, *Aphanizomenon*, *Gleotrichia*, *Microcystis*	50,646 (Sep 13)
	Highgate Springs	Alert 2	Jul 20	14.57 (Jul 20)	*Anabaena*, *Aphanizomenon*, *Coelosphaerium*, *Microcystis*	10,171 (Aug 02)
	Rock River Access	Alert 1	Aug 16	2.98 (Aug 16)	*Anabaena*, *Aphanizomenon*, *Microcystis*	39,200 (Aug 16
	Rte. 78 Access	Alert 1	Jul 26	1.35 (Jul 20)	*Anabaena*, *Aphanizomenon*, *Microcystis*	9,615 (Jul 26-shoreline)

Citizen Monitoring Samples – Whole water samples, analyzed by Sedgewick-Rafter cell following net sample protocol.

Region	Location	Monitoring Status	Date Achieved	Highest Microcystin (µg/L) Observed	Cyanobacteria Present	Maximum Density of Potentially Toxic Cells/mL
South	Button Bay	Alert 1	Aug 09	0.07 (Aug 09)	*Anabaena*, *Aphanizomenon*	5088 (Aug 09)
	Littlefield Shore	Alert 1	Aug 22	0.01 (Aug 22)	*Anabaena*, *Aphanizomenon*, *Microcystis*	4066 (Aug 22)
Northwest	Cumberland Bay State Park	Quantitative	Jul 04	Not measured	*Anabaena*, *Aphanizomenon*,	395 (Jul 25)
	Monty's Bay	Quantitative	Jul 25	Not measured	*Aphanizomenon*	386 (Aug 15)

	Point Au Roche	Quantitative	Jul 11	Not measured	*Anabaena*	211 (Aug 22)
Northeast	Carry Bay	Alert 1	Aug 15	0.07 (Aug 15)	*Anabaena, Aphanizomenon, Microcystis*	14,000 (Aug 15)
	Maquam Bay	Quantitative	Jul 11	Not measured	*Anabaena, Aphanizomenon, Microcystis*	772 (Aug 01)
	North Hero State Park	Alert 1	Jul 18	0.11 (Aug 15)	*Anabaena, Aphanizomenon, Microcystis*	7368 (Aug 15)
	Pelot's Bay	Alert 1	Aug 08	0.19 (Aug 16)	*Anabaena, Aphanizomenon, Microcystis*	26,552 (Aug 08)
Mississquoi Bay	Chapman Bay	Alert 1	Aug 02	0.77 (Aug 08)	*Anabaena, Aphanizomenon, Microcystis*	8947 (Aug 29)
	Comolli Campsite	Alert 1	Jul 18	0.45 (Aug 08)	*Anabaena, Aphanizomenon, Microcystis*	23,421 (Sep 05)
	High Rocks	Alert 2	Jul 19	14.99 (Jul 19)	*Anabaena, Aphanizomenon, Microcystis*	50,526 (Aug 08)
	Highgate Springs Shipyard	Alert 1,2	Jul 06	21.86 (Sep 05)	*Anabaena, Aphanizomenon, Microcystis*	507,600 (Sep 05)
	Raake's Point	Alert 2	Jul 19	6.78 (Jul 19)	*Anabaena, Aphanizomenon, Microcystis*	18,684 (Jul 25)

The highest concentrations of microcystins were found in Missisquoi Bay (Table 5). Most sites in the bay showed concentrations above 1 µg/L, the WHO level of human health concern, on several dates in July and August. Highest concentrations were found on the east side of the bay, at the Highgate Cliffs, Highgate Springs and Shipyard sites; all of these sites exceeded the Vermont Department of Health recreational threshold of 6 µg/L on a few dates during the summer season.

Other sites in Lake Champlain showing elevated concentration of microcystin included St. Albans Bay, and, in late fall, Burlington Bay (*Melosira* boat slip). As in previous years, the citizen monitoring stations along the shoreline captured a significant number of the locations that attained alert level status. The citizen monitoring stations in the northeastern lake and Missisquoi Bay had much higher densities of potential toxin producing taxa and more measurable microcystin concentrations than the citizen monitoring stations in the south lake or northwestern side of the lake.

No measurable concentrations of anatoxin were detected at any sites in 2005.

Table 5. Number of samples tested by ELISA at UVM and the maximum concentrations of microcystin measured in 2005.

Lake Section	Collected by	Location	Number of Samples Tested	Maximum Microcystin Concentration (µg/L)
Missisquoi Bay	Lay monitor	Chapman Bay	5	0.8
		Comolli Campsite	7	1.5
		High Rocks	5	15.0
		Highgate Springs Shipyard	12	21.9
		Raake's Point	5	6.8
	UVM	Alburg	8	0.6
		Highgate Cliffs	30	22.1
		Highgate Springs	25	14.6
		Highgate Springs Shipyard-shoreline	2	1.4
		Rock River Access	1	3.0
		Rte 78 Access	10	1.4
		Rte 78 Access- shore	2	0.8
St. Albans Bay	UVM	St. Albans Boatlaunch	12	0.8
Other sections of Lake Champlain	Lay monitor	Basin Harbor	0	
		Button Bay	2	0.1
		Carry Bay	2	0.1
		Cumberland Bay State Park	0	
		Littlefield Shore	1	0.0
		Maquam Bay	0	
		Monty's Bay	0	
		N. Hero State Park	2	0.1
		Pelot's Bay	3	0.2
		Point au Roche State Park	0	
		Thompson's Point	0	
	UVM	Melosira boat slip	2	8.8
		Champlain Water Bay	0	
		North Beach	0	
		Ransom Bay	1	1.2
		Red Rocks	0	
Total Number of Samples Tested			**137**	

As examples of the seasonal variation at sites, Figures 2-4 show the season pattern of cell density and alert level status in St. Albans Bay, at the Rte 78 boatlaunch site, and at Highgate Cliffs.

Figure 2. Alert status in St. Albans Bay over the summer 2005.

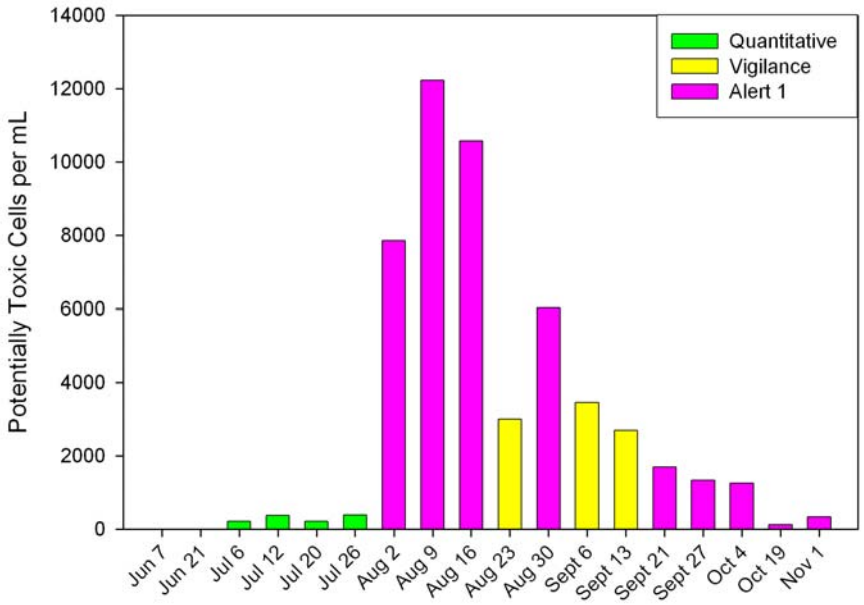


Figure 3. Alert status at the Rte 78 Boatlaunch over the summer 2005.

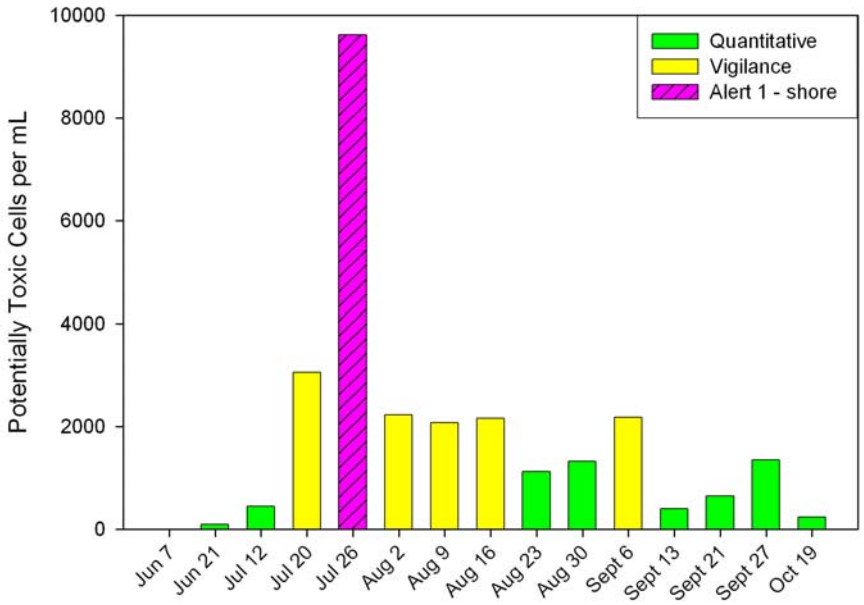
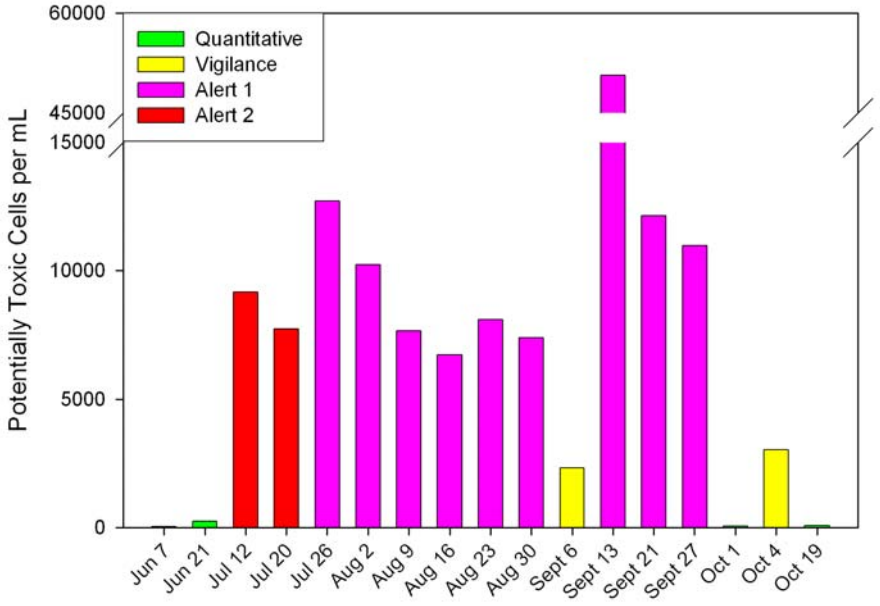


Figure 4. Alert status at Highgate Cliffs over the summer 2005.



The samples collected by VT DEC at Station 50 in Missisquoi Bay generally showed much lower densities of potentially toxic cells than the other sites in Missisquoi Bay, as they did in 2003 and 2004. August 22 was the only date when cell counts at this station even approached the Alert Level (Figure 5).

Nutrients at the Cyanobacteria Monitoring Sites

Concentrations of total phosphorus (TP) and total nitrogen (TN) were averaged by date for monitoring sites in Burlington Bay, St. Albans Bay and Missisquoi Bay. Mean concentrations of both nutrients were almost always highest in Missisquoi Bay, intermediate in St. Albans Bay, and lowest at Burlington Bay (Figure 6).

We also looked at the variability in TP and TN concentrations across selected study sites in Missisquoi Bay and at Station 50, the LCBP/Vermont DEC long term monitoring site in Missisquoi Bay. For Station 50, we used the data collected by Vermont DEC: although there were fewer samples at this site, we found nine TN and TP samples at Station 50.

Figure 5. Cyanobacteria densities in net plankton samples collected at Station 50 and at the regular UVM sites in Missisquoi Bay in 2005.

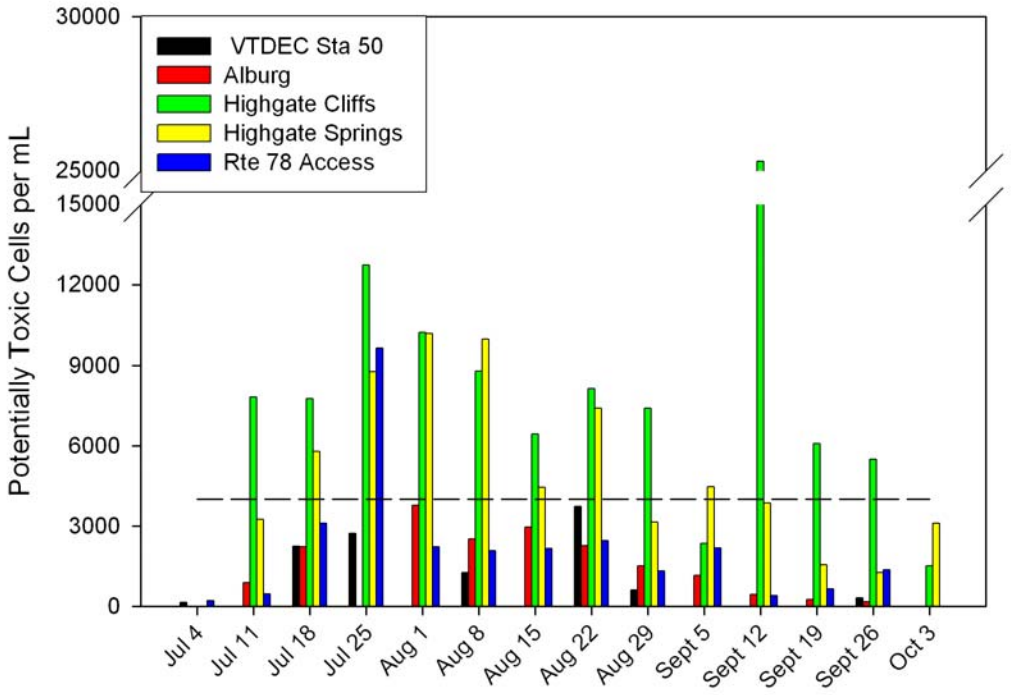
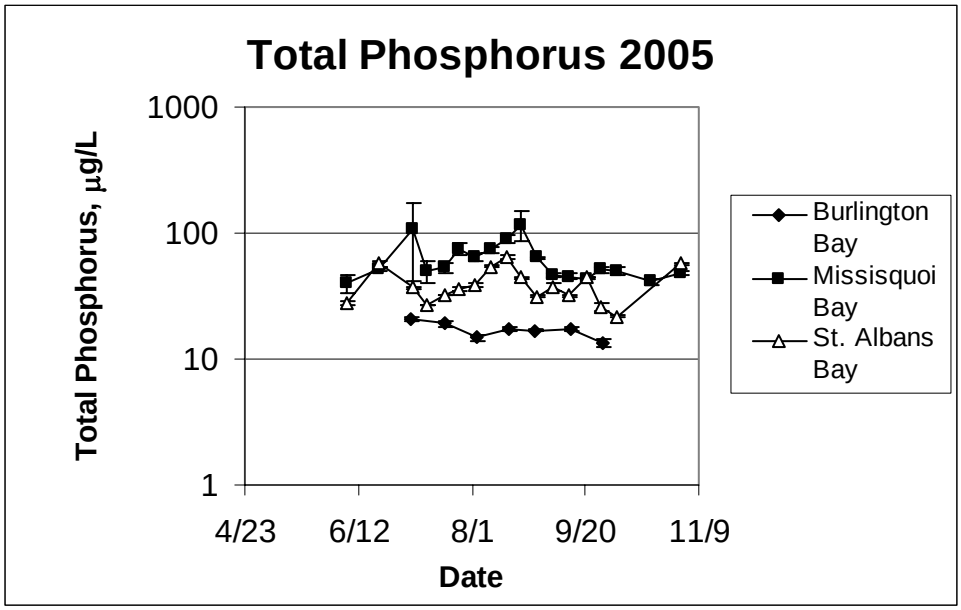


Figure 6. Mean total phosphorus concentrations (± one standard error) in Burlington Bay, Missisquoi Bay and St. Albans Bay, 2005.



There were statistically significant differences in both nutrients among sites in Missisquoi Bay (ANOVA on log-transformed data, $p \leq 0.0001$). Concentrations of TP (Figure 7) were higher at our Highgate Cliffs site than at either our Alburg site on the west side of the bay or at Station 50 (SNK, $P \leq 0.05$). Similarly, concentrations of TN (Figure 8) were also higher at our Highgate Cliffs site than at our Alburg site on the west side of the bay or at Station 50 (SNK, $P \leq 0.05$).

Figure 7. Mean concentrations of total phosphorus across sample sites in Missisquoi Bay in 2005.

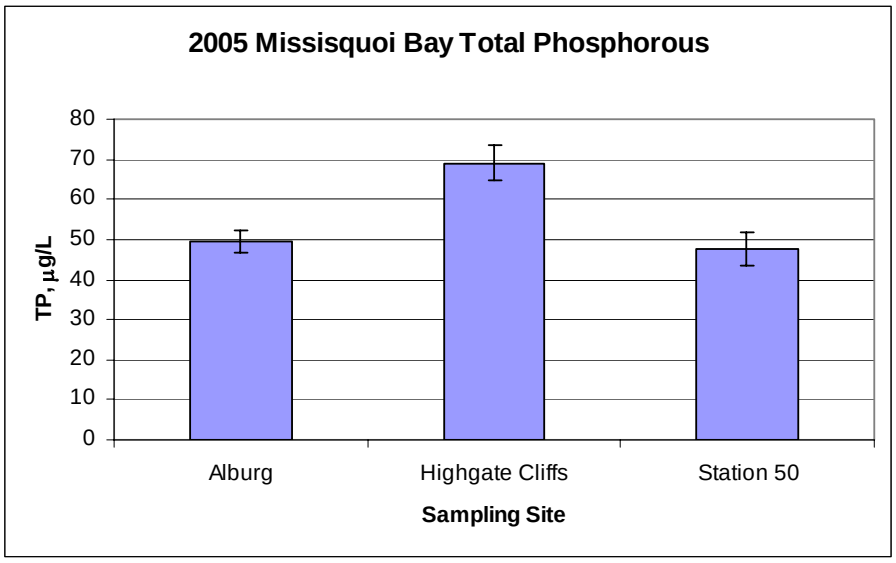
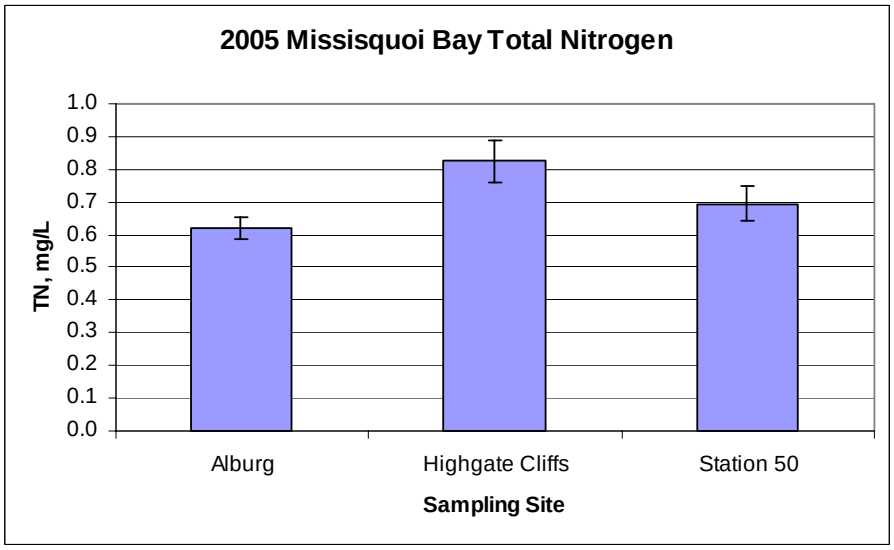
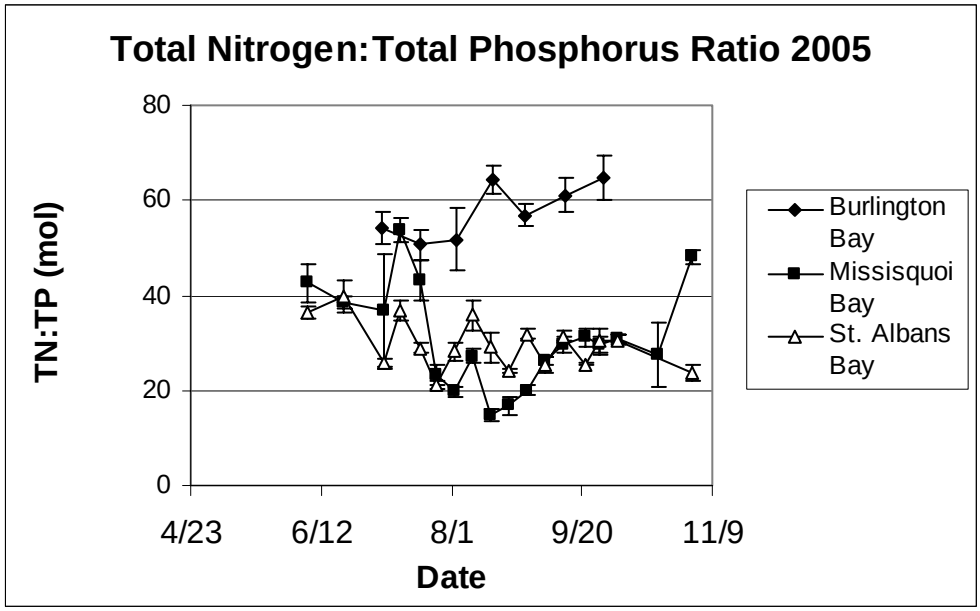


Figure 8. Mean concentrations of total nitrogen across sample sites in Missisquoi Bay in 2005.



We also calculated the ratio of TN:TP in Burlington Bay, St. Albans Bay, and Missisquoi Bay (Figure 9). We found significantly lower ratios at Missisquoi Bay and St. Albans Bay compared to Burlington Bay from mid-summer through the fall.

Figure 9. TN:TP ratios in Burlington Bay, St. Albans Bay, and Missisquoi Bay.



Coordination

Coordination meetings were held with Vermont Health Department officials in May 2005, and an e-mail distribution list that included 40 partner organizations and individuals was established for regular information sharing over the summer season. Beginning in June, weekly or bi-weekly e-mail updates on monitoring results were distributed to these officials and to other professionals with an interest in bloom conditions and public health. Working with the Vermont Department of Health, we also posted background information about cyanobacteria and cyanotoxins, and provided information for a map depicting bloom conditions across the lake on their "Healthy Vermonter's" website. Information on bloom conditions was updated on a weekly basis from early July through September.

DISCUSSION AND CONCLUSIONS

Comparison of Patterns of Cyanobacteria and Toxins 2003-2005

From 2003 – 2005, the cyanobacteria monitoring program conducted on Lake Champlain has been fairly consistent, allowing us to compare patterns of cell density and toxin concentrations across the years and among sites.

The median and range of toxin concentrations in the various lake segments over the last three years is summarized in Table 6. Although there was very little difference in median concentration between 2004 and 2005 in Missisquoi Bay, the range of concentrations found in 2004 was two orders of magnitude greater than 2005. In St. Albans Bay, the median concentration of microcystin increased by an order of magnitude in 2005 compared to 2004. In the Northeastern Lake (Maquam, Carry, and Pelots Bay and North Hero), both the median microcystin concentration and the range was significantly lower in 2005 compared to 2004.

Table 6. Microcystin concentrations (µg/L) in various lake segments from 2003 – 2005.

Lake Region		2003	2004	2005
Burlington Bay	Median	0.014		7.419
	Range	ND - 0.083		6.04-8.797
	# of Samples	8		2
Mississquoi Bay	Median	0.199	0.883	0.802
	Range	ND - 23.9	ND - 6490	ND - 22.1
	# of Samples	160	142	125
Northeast Lake	Median	0.049	0.509	0.079
	Range	ND - 0.18	ND - 17.5	ND - 0.193
	# of Samples	6	8	7
South Lake	Median	0.532		0.048
	Range	ND - 1.4		ND - .067
	# of Samples	3		3
St. Albans Bay	Median	0.053	0.044	0.440
	Range	ND - 0.46	ND - 22.5	0.057 - 0.942
	# of Samples	16	22	15
Main Lake	Median	0.117		
	Range			
	# of Samples	1		

In Missisquoi Bay, across all sites, 2005 generally had lower cell densities and lower toxin concentrations that the previous two years (Figures 10-13). The reasons for these differences between years are not clear. Certainly, the weather (especially precipitation), and thus the nutrient loading differed from year to year. Summer TP averaged ~100 µg/L in Missisquoi Bay in 2004, but was only 62.7 µg/L in 2005. As part of another project, was also measured and calculated the seasonal mean SRP in Missisquoi Bay and found that the mean concentration in 2003 was 5.3 µg/L, the mean in 2004 was 7.4 µg/L, and the mean in 2005 was 5.4 µg/L (Watzin, unpublished data).

Figure 10. Alert status at the Rte 78 Boatlaunch from 2003 - 2005. Each bar represents a sample date between June and early October.

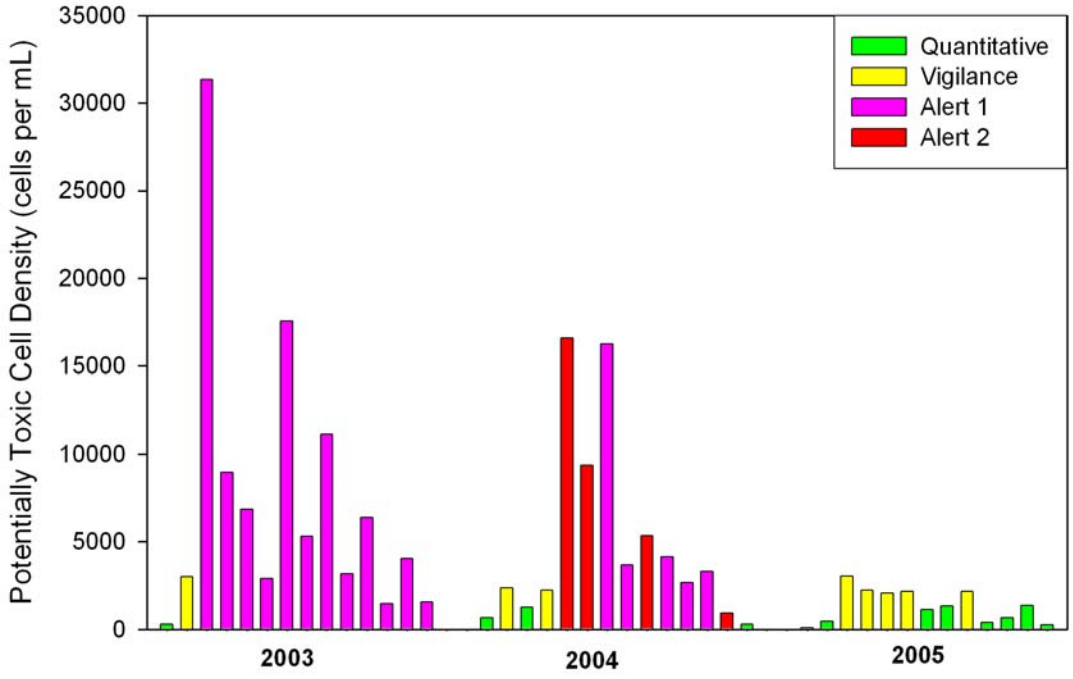


Figure 11. Alert status along the west side of Missisquoi Bay (Chapman Bay/Alburg) 2003 - 2005. Each bar represents a sample date between June and early October.

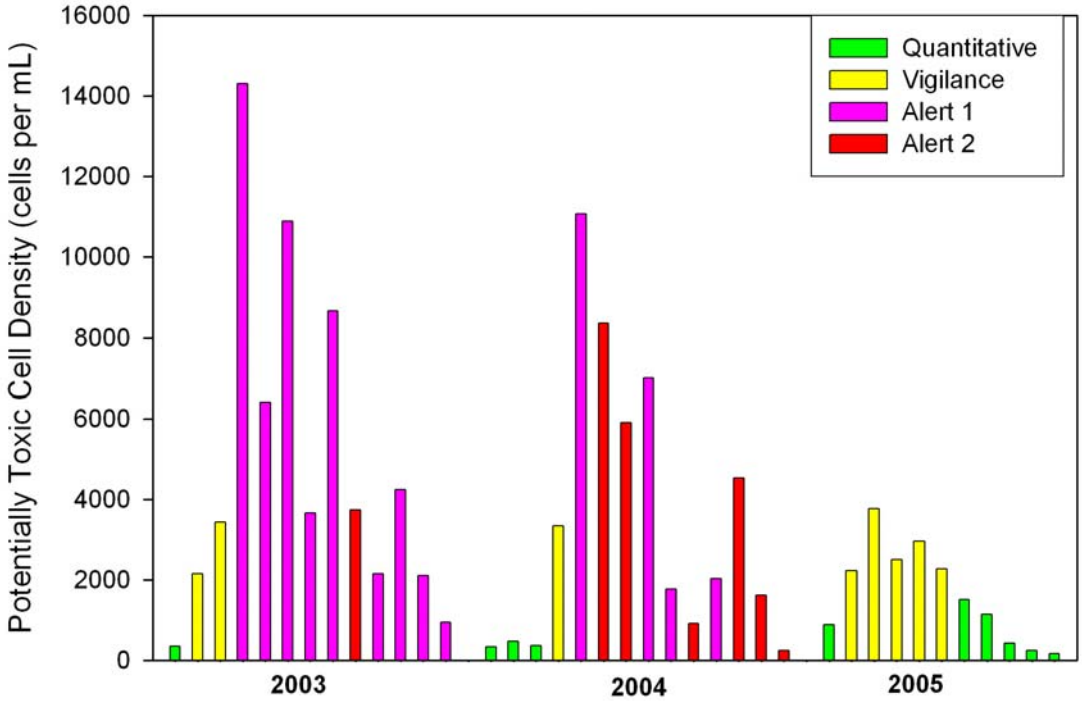


Figure 12. Alert status at Highgate Springs 2003 - 2005. Each bar represents a sample date between June and early October.

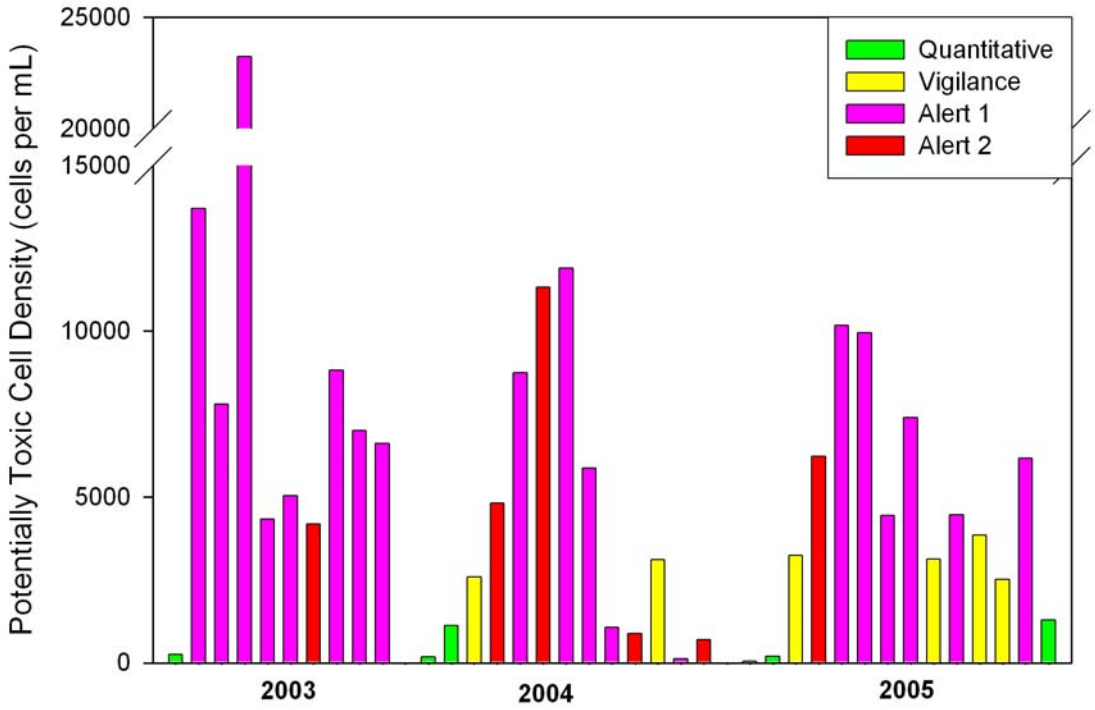
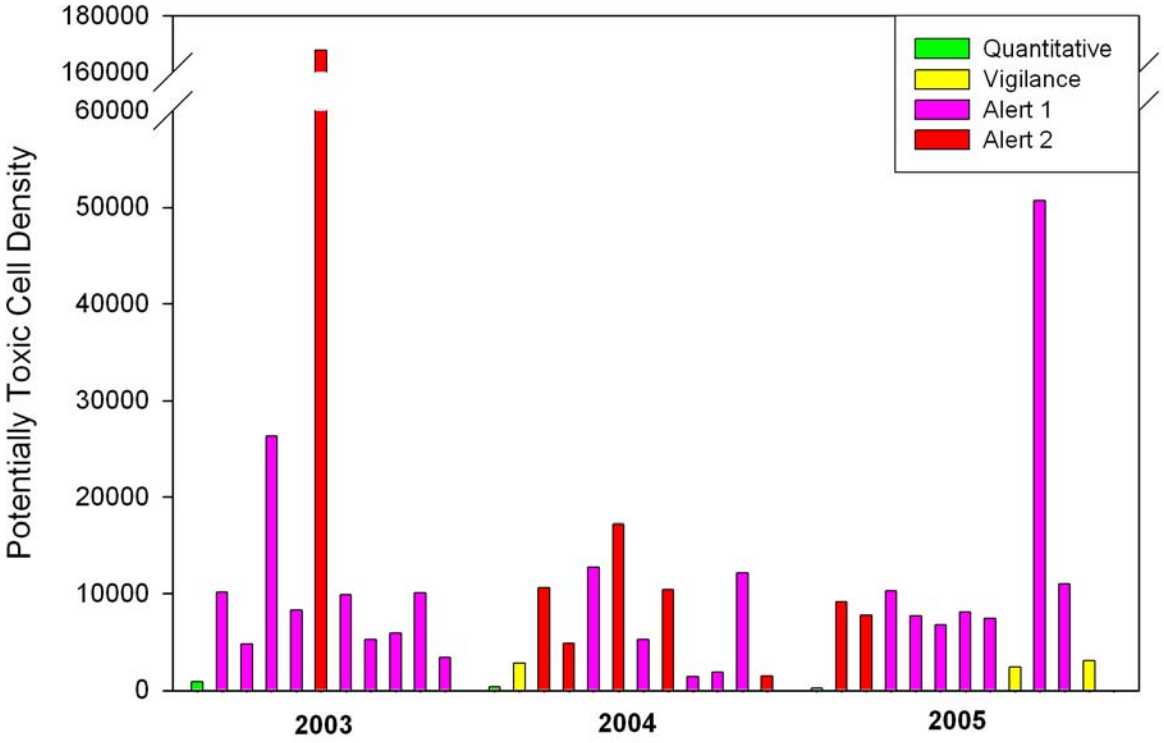
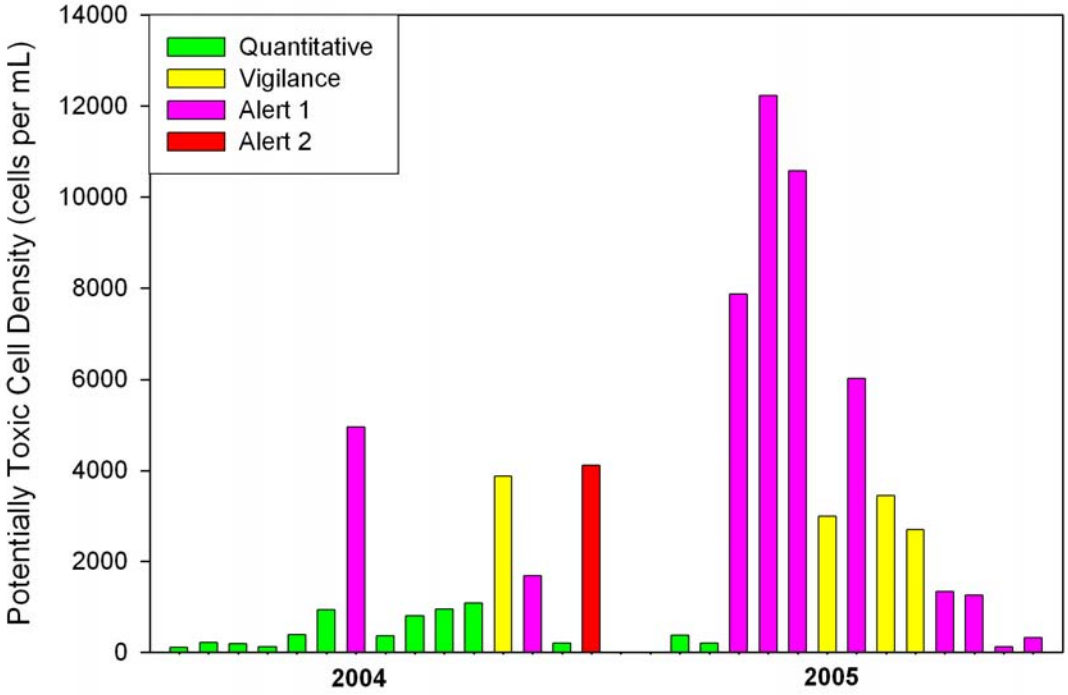


Figure 13. Alert status at Highgate Cliffs 2003 - 2005. Each bar represents a sample date between June and early October.



In contrast, in St. Albans Bay, where we have just two years of comparable data, 2005 showed higher cell densities and higher toxin concentrations than 2004 (Figure 14). Summer TP in St. Albans Bay averaged ~32 µg/L in 2004 and ~39 µg/L in 2005. We have also measured and calculated the seasonal mean SRP in St. Albans Bay, and found that the mean concentration in 2004 was 2.2 µg/L, and the mean in 2005 was 3.5 µg/L (Watzin, unpublished data).

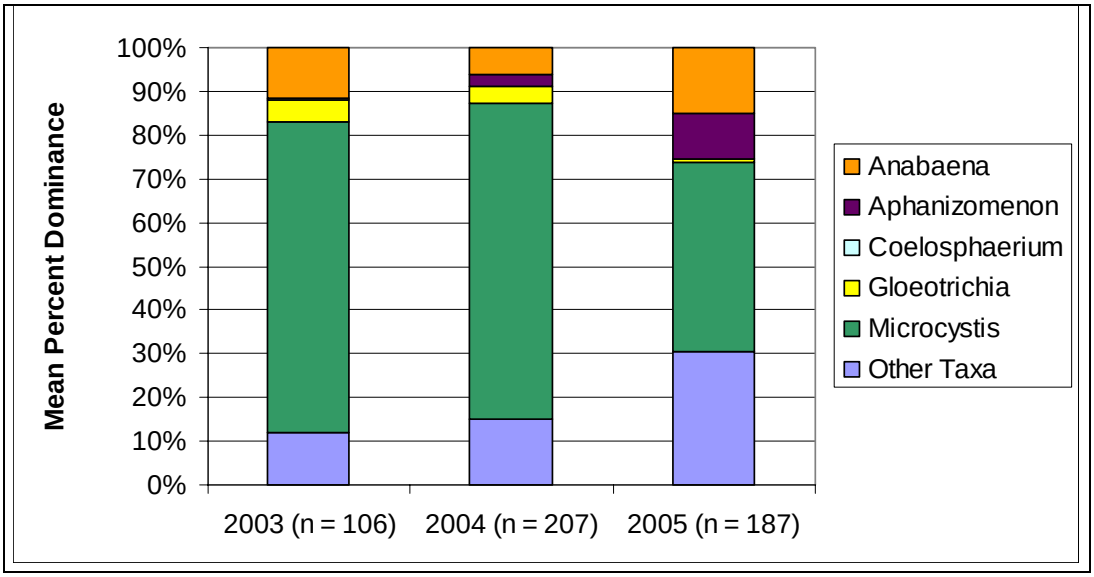
Figure 14. Alert status in St. Albans Bay, 2004 - 2005. Each bar represents a sample date between June and early October.



Several studies have shown a positive relationship between cyanobacterial relative abundance and total phosphorus (e.g., Huszar and Caraco 1998, Trimbee and Prepas 1987), however, cyanobacteria have also been found in high abundances at lower phosphorus concentrations (Chang and Rossmann 1988, Downing et al. 2001, Raikow et al. 2004), and can dominate when nitrogen is limiting compared to phosphorus, that is at low nitrogen to phosphorus (N:P) ratios (e.g., Smith 1983, Findlay et al. 1994, Hyenstrand et al. 1998a, Elser 1999). Smith (1983) found that cyanobacterial blooms tended to occur when epilimnetic N:P ratios fell below 64:1, but others have not found the same relationship between N:P ratios and cyanobacterial abundance (Jensen et al. 1994; Scheffer et al. 1997, Downing et al. 2001, Scheffer 2005). Ratios of TN:TP in Missisquoi Bay and St. Albans Bay are generally well below 64 during the summer bloom season (Figure 9).

The taxonomic composition of the phytoplankton has also varied considerably over the three years of our monitoring effort. In Missisquoi Bay, the strong dominance by *Microcystis* seen in 2003 and 2004 was not nearly as strong in 2005 (Figure 15).

Figure 15. Percent composition of the genera of cyanobacteria in Missisquoi Bay, 2003-2005.



In St. Albans Bay, the abundance of *Aphanizomenon* seems to be increasing, while *Gloeotrichia* has been declining in relative abundance (Figure 16). In Burlington Bay, both *Aphanizomenon* and Anabaena seem to be increasing in relative abundance (Figure 17).

Figure 16. Percent composition of the genera of cyanobacteria in St. Albans Bay, 2003-2005.

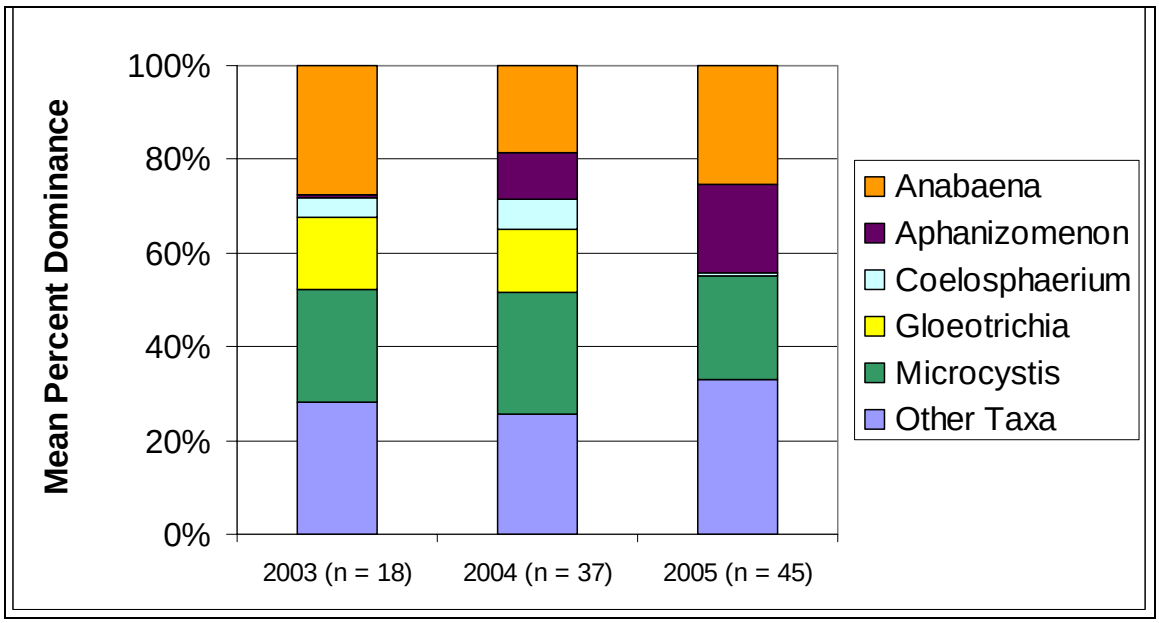
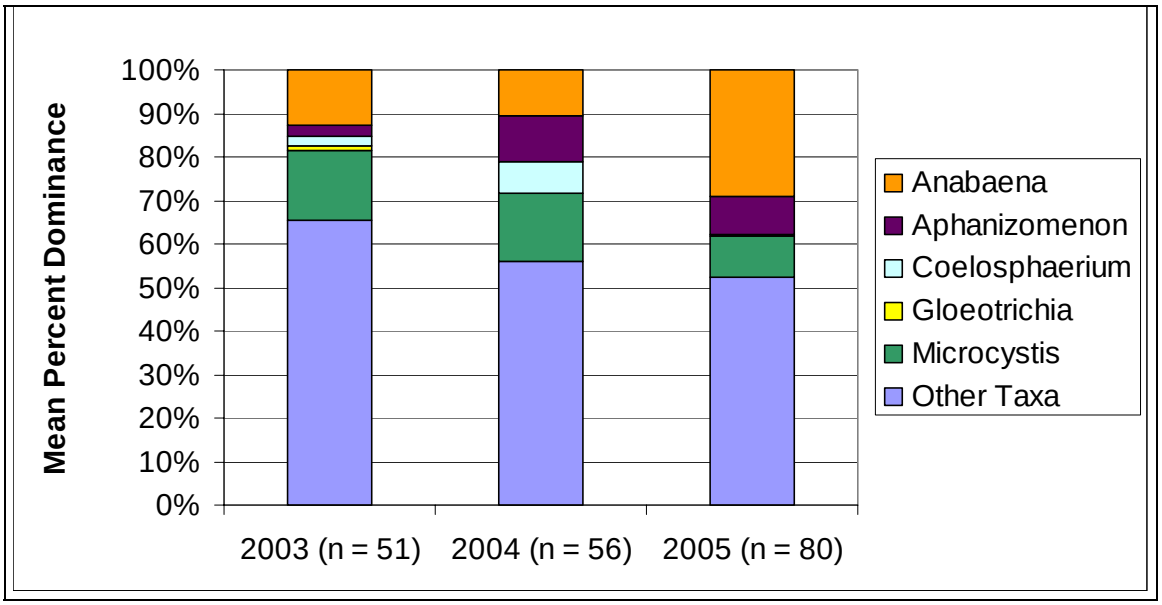


Figure 17. Percent composition of the genera of cyanobacteria in Burlington Bay, 2003-2005.



Although some investigators have suggested that low N:P ratios only favor heterocystous cyanobacteria (Levine and Schindler 1999), others suggest that nitrogen fixation may not be the only prerequisite for cyanobacteria dominance at lower N:P ratios (Smith 1983). One explanation for the success of non-heterocystous cyanobacteria at low N:P ratios may be related to their ability to regulate their buoyancy, allowing them to gain nutrients that are not available to other phytoplankton. For example, *Microcystis* can sink to the bottom in shallower waters to exploit nutrients diffusing from the sediments and return to the surface waters to photosynthesize during the day (Hyenstrand et al. 1998a). Because microcystin is a nitrogenous compound (Carmichael et al. 1998), production of this toxin may also provide a mechanism for species like *Microcystis* spp. to sequester nitrogen when it is available (Kotek et al. 2000, Lee et al. 2000, Downing, et al. 2005).

The speciation of nitrogen may also play a critical role in determining cyanobacterial success at low N:P ratios. Cyanobacteria can outcompete eukaryotic phytoplankton for ammonium but cannot compete as well for nitrate, perhaps because of less efficient induction of nitrate reductase (Hyenstrand et al. 1998b; Blomqvist et al.. 1994). Smaller cyanobacterial cells may outcompete larger eukaryotic cells for ammonium in part because of their higher surface to volume ratios which allows for increased diffusion and active uptake (Stolte and Reigman 1996). In a recent study of *Microcystis*, Downing et al. (2005) found that when nitrogen uptake exceeded relative growth rate, microcystin production also increased. Increases in intracellular ammonium were also associated with microcystin production in this study. Therefore, the flux of ammonium from the sediments in shallow and waters like Missisquoi Bay may provide a unique advantage to vertically migrating *Microcystis*.

Another potential explanation for cyanobacterial dominance in Missisquoi Bay is that these species are grazer resistant (Haney 1987). The toxins many cyanobacteria produce may render them undesirable or even acutely toxic to zooplankton, and Jang et al. (2003) have demonstrated that microcystin production increased in each of four different cultured strains of *Microcystis* following exposure to *Daphnia* spp. or their filtrate. Others have also documented reduced *Daphnia* feeding activity with toxin-producing *Microcystis* (Reinikainen 1997, Claska and Gilbert 1998, Demott 1999) and lower fecundity and population growth of *Daphnia* cultures with a higher percentage of toxic cyanobacteria cells (Ferrao-Filho et al. 2000). But cyanobacteria dominance is not always associated with toxin production, and even in bloom conditions both toxin-producing strains and non-toxin producing strains can be located in close proximity (Rinta-Kanto et al. 2005, Wilson et al. 2005). Increased colony size may also be an anti-grazing strategy (Jang et al. 2003, Sarnelle et al. 2005), as larger colonies are less easily ingested.

Dominance by zooplanktivorous fish has been associated with decreasing large zooplankton and increasing cyanobacterial dominance in other lakes (Hunt et al. 2003, Stephen et al. 2004). Once cyanobacteria dominance occurs, it may not be easily reversed in the absence of planktivorous fish because large cyanobacteria colonies impose a mechanical barrier to efficient grazing (DeBernardi and Giussani 1990, Scheffer et al. 1997, Stephen et al. 2004). Some large cladocerans can feed directly on toxic strains of cyanobacteria (Gustafsson and Hansson 2004). Even though the nutritional quality of cyanobacteria is less than green algae, the increase in food availability of blooms may compensate for the lack of quality for large cladoceran grazers (Kurmayer 2001).

The second mechanism through which large cladocerans may control cyanobacteria is through nutrient cycling. Large fast growing cladocerans have a high phosphorus content and recycle phosphorus at a much lower rate than they do nitrogen (Acharya et al. 2004). As a result, it is possible that a large population of cladocerans with high N:P nutrient cycling may effectively limit the stoichiometric ratios that favor cyanobacteria dominance. When grazing removes these cladocerans, lower N:P ratios may result, favoring cyanobacteria dominance (Smith 1983, Elser 1999, Levine and Schindler 1999, Elser et al. 2000, Xie et al. 2003).

We hope in the coming years to be able to evaluate the relative contribution of some of these other factors to cyanobacteria dominance and bloom dynamics in Lake Champlain.

Coordination

The e-mail notitfication system has evolved into a useful tool for rapid communication among the professional community. Our partnership with the Vermont Department of Health to post weekly information about bloom conditions on their “Healthy Vermonter’s” website was highly successful.

Our volunteer citizen monitoring effort was also highly successful. In 2005, our volunteer effort was expanded to include 15 volunteers, increasing our shoreline monitoring coverage in New York and other areas in the northern sections of the lake. The Lake Champlain Committee, a local nonprofit organization, assisted with volunteer recruitment, sample pick-up and

coordination. Volunteer monitoring at some sites in the north lake continued weekly into October.

Other outreach activities included coordination and informational sessions with water suppliers, public beach managers, watershed organizations, and others. In response to the high level of public concern about toxic cyanobacteria blooms, we gave several public presentations on the status of our research in association with the Vermont Department of Health, Friends of the Missisquoi River, and others. We also designed and staffed an exhibit for ECHO, a science center and aquarium on Burlington's waterfront, during their two-day public education workshop entitled, "Voices of the Lake." We offered blue-green algae microscope viewing, identification and Q&A opportunities. For ECHO's revolving "workbench" exhibit, we designed and produced a video slide show describing the blue-green algae problem on Lake Champlain and our monitoring efforts.

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