Technical Report No.39

Evaluation of Potential Blue Green Algal Toxins in Lake Champlain, Summer 2000

Prepared by
Barry H. Rosen, Ph. D. USDA-NRCS, Watershed Science Institute,with Angela Shambaugh, Lisa Ferber, Felicity Smith and Mary Watzin, Ph. D. University of Vermont School of Natural Resources, and Cathi Eliopoulos and Peter Stangel, VT Dept.of Environmental Conservation

for
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for the Lake Champlain Basin Program and the Centers for Disease Control and Prevention

Barry H. Rosen, Ph. D.
USDA-NRCS, Watershed Science Institute

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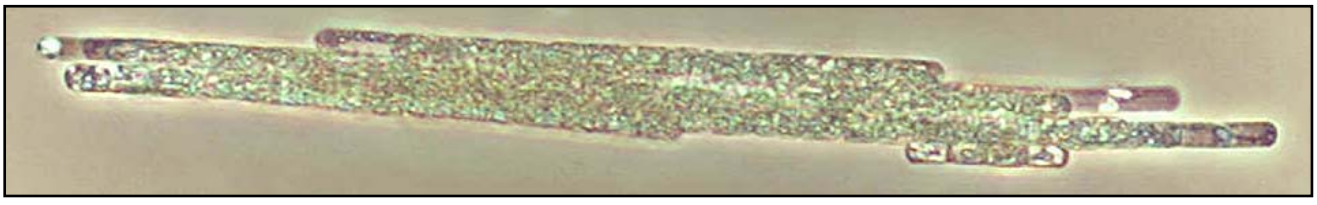
Angela Shambaugh
Lisa Ferber
Felicity Smith
Mary Watzin, Ph. D.
University of Vermont, School of Natural Resources

Cathi Eliopoulos
Peter Stangel
VT Dept. of Environmental Conservation



Executive Summary

This project examined the blue-green algae (BGA) in Lake Champlain, their potential to produce natural toxins and their consequent impact on public drinking water. This was accomplished by sampling the water from sites throughout Lake Champlain, screening them for BGA, identifying the organisms known to produce toxin, identifying the types of toxins present and determining if toxins were present in the water from 5 water treatment facilities during the summer of 2000.

At least four known toxin-producing species of BGA were identified in Lake Champlain. Phytoplankton samples were tested for toxins using 4 separate analyses. These tests confirmed that two toxins, anatoxin-a and microcystin were present, while saxitoxins were not found. Concentrations of toxins in the raw and finished drinking water samples were all below trace levels, however concentrated phytoplankton samples collected near the vicinity of the intakes were mostly positive for the presence of microcystin and anatoxin-a. Mouse bioassays performed on these samples confirmed the presence of toxins, although an acute response was not seen. The presence of toxins in samples indicates a *potentia*l for contamination during bloom conditions. No blooms of toxin-producing BGA occurred broadly in the lake in 2000 or lasted more than a few days, therefore, no human health impacts were indicated.

Anabaena flos-aquae formed a brief bloom at the end of July that was concentrated in the boat slip at the Rubenstein Ecosystem Science Laboratory (UVM). This allowed enough material to be collected for detailed analysis of this species. Water samples from the slip contained approximately 1.2 ppb anatoxin-a, and zebra mussel tissue collected in the boat slip contained 12.9µg anatoxin-a/g of tissue. Although humans do not consume zebra mussels, other shellfish with this amount of potent neurotoxin could pose a significant health risk if consumed. In August 2000, like 1999, two dogs died after they drank water from Lake Champlain with a blue-green bloom. The anatoxin-a concentration in the water in 2000 in the area the dogs drank was estimated later to be 1.4 ppb. Algal identifications were not conducted on these samples, however, the bloom happened approximately 10 days after the bloom in the boat slip at the Rubenstein Laboratory and is likely that *Anabaena flos-aquae* was the cause in this incident as well. A widespread bloom of this organism has the potential to impact drinking water and recreational users of the lake.

Future efforts should be focused on a lake-wide monitoring program for blooms of known toxin producing BGA. In the summer of 2000, the environmental conditions did not support a significant bloom, however the potential for human health risk would be present in future blooms. The monitoring program should be coupled with the development and implementation of an Action Plan designed to alert drinking water facilities and recreational users of a potentially harmful bloom. Accumulation of the toxins in the food web (fish and shellfish) may also be possible and should be examined further considering protection of human health or waterfowl.

Distribution List for Final Report

Erik Beck
Lake Champlain Coordinator
US EPA New England
One Congress Street - Suite 1100 CVT
Boston, MA 02114-2023

Eric Smeltzer
VT DEC, WQ
103 S Main St, 10 North
Waterbury VT 05671

Michaela Stickney
VT Lake Champlain Coordinator
VT DEC, WQ
103 S Main St, 10 North
Waterbury VT 05671

Fred Dunlap
NY DEC
PO Box 296
Ray Brook NY 12977

Peter Galbreith
Epidemology
Vermont Department of Health
108 Cherry St, PO Box 70
Burlington, VT 05402-0070
(802) 863-7240

Gregory L Boyer
State University of New York
College of Environmental Science and Forestry
Syracuse NY 13210
(315) 470-6825

Wayne W. Carmichael
Aquatic Biology/Toxicology
Department of Biological Sciences
Wright State University
3640 Colonel Glen Highway
Dayton, Ohio 45435 USA

Barry Gruessner
Technical Coordinator
Lake Champlain Basin Program
PO Box 204, 54 West Shore Road
Grand Isle, VT 05458

John Hurst Jr.
Maine Department of Marine Resources
PO Box 8
West Boothbay Harbor, Maine 04575

Lorrie Backer, PhD, MPH
Environmental Epidemiologist
National Center for Environmental Health
1600 Clifton Road, NE MS E-23
Atlanta, GA 30333

Bob Drawbaugh (Laboratory) &
Kim A. Cook, MD, MSPH
Infectious Disease Epidemiology
Vermont Department of Health
108 Cherry St, PO Box 70
Burlington, VT 05402-0070

Rodney Pingree
Water Supply Division
103 South Main St.
Waterbury, VT 05671-0403

John Burns
St. Johns River Water Management District
PO Box 1429
Palatka, Florida 32178

Marek Pawlowicz
Florida Department of Health
Bureau of Laboratories - Jacksonville
1217 Pearl Street
Jacksonville, Florida 32202

Doug Burnham, Program Manager
Biomonitoring and Assessment
VDEC-WQD
103 S. Main St. - 10N
Waterbury, VT 05676

Cathi Eliopoulos
VT Dept. of Environmental Conservation
103 South Main St. Bldg. 10 N.
Waterbury,VT 05671-0408

Mike Barsotti & Jim Fay
Champlain Water District
403 Queen City Park Rd.
South Burlington, VT 05403

Tom Dion
Burlington Water Department
PO Box 878
Burlington, VT 05401

George Lague
Swanton Water Village
PO Box 279
Swanton, VT 05488

Gilbert Bohannon
North Hero Water System
PO Box 38
North Hero, VT 05474

Walt Giard
Tri-town Water District
Board of Commissioners
Crown Point Rd.
Bridport, VT 05734

1. Project Description/Background

Background
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 for New York and Vermont, serving 180,000 people. Four thousand residents take water directly from the lake. It also receives point and non-point source pollution from a variety of sources and this pollution has contributed to water quality problems within the lake system. Some water quality concerns are associated with the biological communities of the lake, for example, algal blooms. These blooms are enhanced by nutrient enrichment. This project focused on determining if the algal blooms in Lake Champlain produce natural toxins, thereby posing a potential threat to recreational users and to those that use the lake for a drinking water supply.

Problem Statement
In 1999, cyanobacteria, also known as blue-green algae (BGA), bloomed in parts of Lake Champlain and may have produced toxins. The death of three dogs from two separate bloom events served as the impetus for this study of the potential human health issues associated with BGA in Lake Champlain during the summer of 2000. This project focused on detailed screening for potential toxin-producing BGA blooms in Lake Champlain. This was accomplished by identifying the organisms of concern, identifying the types of toxins present, and determining if toxins were present in the water from 5 water treatment facilities on Lake Champlain.

Specifically, the following initial set of objectives were addressed:

1) What are the species of bloom-forming BGA in Lake Champlain and how abundant are they?
2) Are these species known to produce toxins as reported by other investigators?
3) Are these species producing toxins in Lake Champlain?
4) Are these toxins in the raw and/or finished water of drinking water facilities or the recreational areas of the lake?
5) What are the types and concentrations of toxins present in the waters of Lake Champlain?
6) Would monitoring with field samples and/or satellite imaging serve as useful tools for tracking or locating and providing information on the dynamics of BGA bloom?

Partners
This project was funded by the Centers for Disease Control and Prevention (CDC) and the Lake Champlain Basin Program (LCBP). The project was supported by services and staff effort at the Watershed Science Institute of the Natural Resources Conservation Service, University of Vermont (UVM), the Vermont Department of Environmental Conservation (VT DEC), the Vermont Department of Health (VT DOH), the Florida Department of Health (FL DOH), the Maine Department of Marine Resources (ME DMR), the Champlain Water District - South Burlington, the Burlington Water Department – Burlington, the Swanton Water Department – Swanton, the North Hero Water System - North Hero, the Tri-town Water System – Addison, the State University of New York-Syracuse (SUNY-Syracuse), Wright State University, and PhycoTech, Inc.- Michigan.

Project Success and Summary of Results

Success for this project can be judged by comparing the initial objectives to the findings:

1) The species of bloom-forming BGA in Lake Champlain are identified.
   Brief Results: A list of species that were dominant during the summer of 2000 was completed. Five species with the potential to produce toxins were found, including Aphanizomenon flos-aquae, Anabaena flos-aquae, Anabaena circinalin, Anabaena planktonica, and Microcystis aeruginosa. All species of bloom-forming blue green algae found in the lake are listed in Appendix A.
2) These species are screened for toxins using 4 separate techniques.
   Brief Results: All four techniques confirmed that toxins were present in the phytoplankton.
3) Toxins are found/not found in raw and/or finished water of drinking water facilities or the recreational areas of the lake.
   *Brief Result*s: *Analyses of the raw and finished water indicated trace levels of toxin. Phytoplankton concentrated by plankton tow near the vicinity of the intakes was mostly positive for the presence of microcystin. Mouse bioassays performed on these tow samples confirmed the presence of toxins, although an acute response was not seen. No BGA blooms of toxin producing organisms lasted more than a few days this year, therefore, it was not possible to measure recreational impacts.*
4) The types and ranges in concentrations of toxins present in the waters of Lake Champlain are determined.
   Brief Results: Two toxins, anatoxin-a (HPLC-FD and LC/MS methods) and microcystin (immunoassay and PP1A methods) were confirmed, while saxitoxins were absent.
   Anabaena flos-aquae *bloomed very briefly and was the most likely source of anatoxin-a. Zebra mussels collected near the bloom contained anatoxin-a as well.*
5) Feasibility of monitoring with field samples and/or a satellite for tracking or locating a BGA bloom.
   Brief Results: No blooms that lasted more than a few days were present this year-satellite imagery not used.

2. Project/Task Technical Design (Materials and Methods)

Sampling Design and Strategy

The workflow for the project is illustrated in Figure 1. In general, qualitative samples from ongoing monitoring efforts were examined until potential toxin-producing BGA were found. Detection of these organisms triggered sampling at the water treatment plants. Both raw (untreated) and finished (treated) water were sampled. It also initiated screening for toxins in these samples as well as in the monitoring samples.

The first stage of monitoring was designed to detect a potential BGA bloom at locations throughout the lake. This project capitalized on the fieldwork already in place for monitoring Lake Champlain water quality. The VT DEC water quality monitoring staff collected phytoplankton tows (qualitative) approximately every two weeks during the initial screening (and throughout the

study). These samples were examined to determine if BGA capable of producing toxins were present. Presence initiated the toxin-screening portion of the project. Although no blooms were found, the project was fully initiated after potential toxin-producing organisms were found.

Initially, phytoplankton tows were collected throughout the lake (Figure 2, Table 1). These stations were originally selected to represent major lake segments among which distinct water quality differences exist. These samples were typically screened within 48 hours for the presence of BGA of concern. These samples provided more than adequate coverage of the lake spatially and temporarily.

Additional initial samples were collected by the Burlington Bay Project (University of Vermont). These samples were collected in Burlington Bay, which experienced a significant bloom of the potential toxin-producing BGA *Microcystis* during the summer of 1999. Samples were collected monthly in early summer and biweekly from mid-summer through the fall from nine sites.

Water Treatment Facility Sampling

The transition from winter phytoplankton communities to a spring/summer community was slow in 2000, likely because of cool temperatures. Quantitative sampling of the BGA at five water treatment facilities (listed below) was initiated as soon as potential toxin-producing BGA were detected in samples from the lake and after approval of the QAPP. Sampling at the five water treatment facilities started on July 25th and continued bi-weekly until Sept 19th:

Champlain Water District - South Burlington-(intake depth 75 ft)
Burlington Water Department - Burlington-(intake depth 43 ft)
Swanton Water Department - Swanton-(intake depth 17 ft)
North Hero Water System - North Hero-(intake depth TBD)
Tri-town Water System - Addison-(intake depth 8 ft)

At each site, 1-liter of untreated raw water and 1-liter of finished drinking water was collected and transported to UVM. Raw water is the water taken into the water treatment facility for processing. Finished water is water distributed to consumers after processing, which varies by treatment facility. A small portion of this water was removed for microcystin analysis at UVM and VT DOH. The remainder of the 1-liter samples was frozen and then transported to SUNY-Syracuse.

The VT DEC field personnel collected phytoplankton tows and whole surface water samples near the intakes of the Swanton and North Hero facilities on the same day that the water treatment facilities collected. The Burlington Bay project (UVM) collected phytoplankton tows and surface water near the intakes of the Burlington and South Burlington facilities on this same day. Other UVM staff collected phytoplankton tows and surface water near the intake of the Addison system.

The actual intake depth varied between facilities, therefore, phytoplankton tows, which are taken at the surface, are not truly representative of the water going into the a treatment facility, rather, the tows represent the potential organisms that may reach the intake. The purpose of these samples was to gather a more concentrated sample of the algae than that in the raw water sample. This maximized our chances of detecting the toxin if it was present.

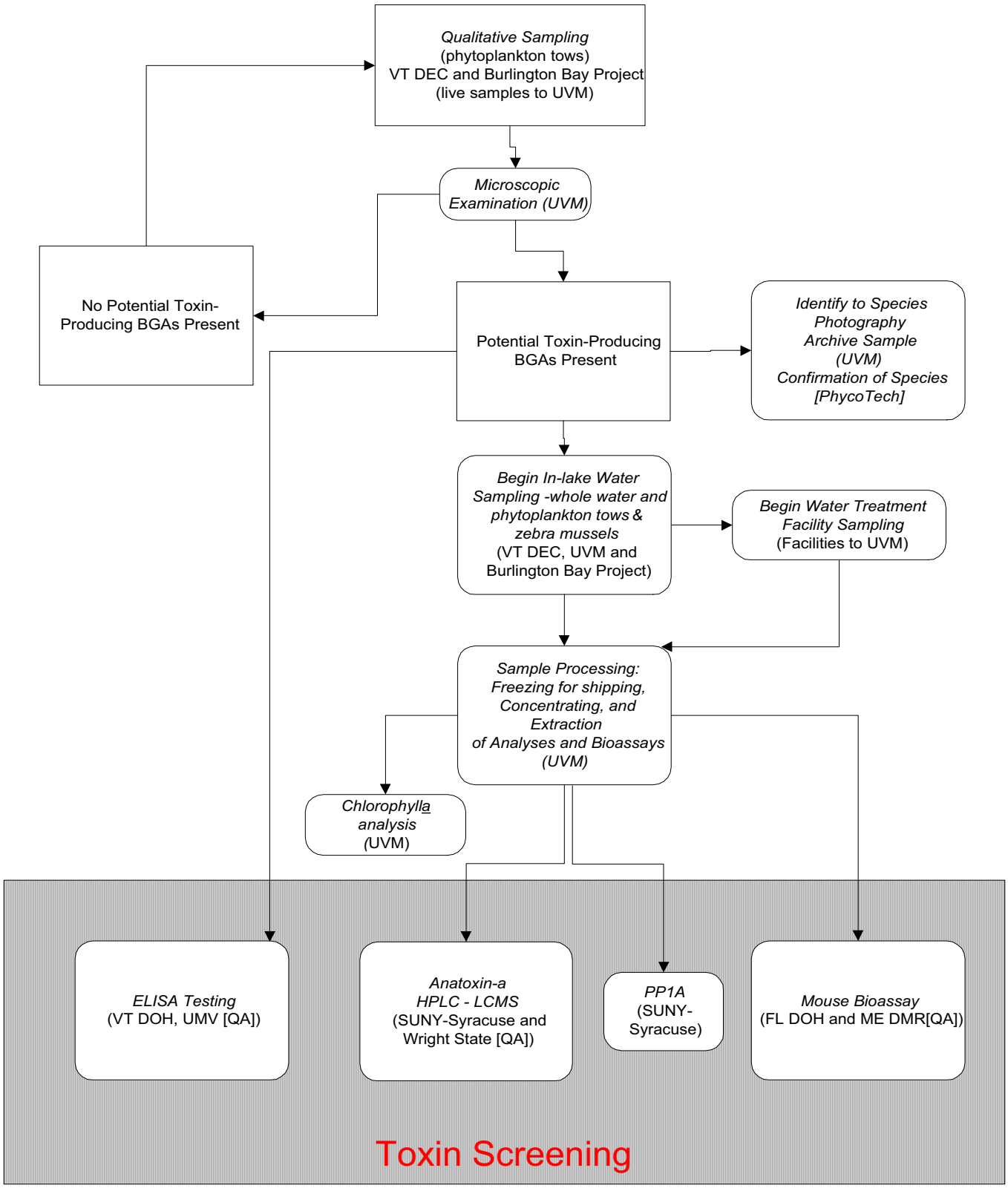


Figure 1. Work-flow for the LCBP-BGA project.

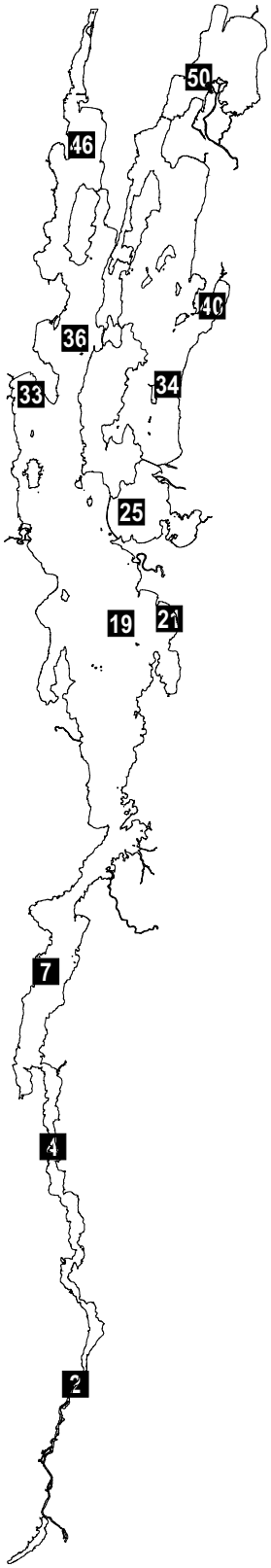


Figure 2. Sampling locations utilized for initial BGA bloom screening. Site identification corresponds to that used by the NYDEC Long-term monitoring project.

Station	Latitude	Longitude
02	43 42.89	73 22.98
04	43 57.10	73 24.47
07	44 07.56	73 24.77
10	44 18.25	73 19.32
12	44 21.42	73 19.79
19	44 28.26	73 17.95
21	44 28.49	73 13.90
25	44 34.92	73 16.87
33	44 42.07	73 25.09
34	44 42.49	73 13.61
36	44 45.37	73 21.30
40	44 47.12	73 09.73
46	44 56.90	73 20.40
50	45 00.80	73 10.43
62	44 12.30	73 22.00
65	44 16.75	73 19.75

Table 1. Lake sampling locations (from NY-VT DEC Long-term monitoring program)

Phytoplankton

Phytoplankton tow samples were placed in the dark and kept cool for transport to the laboratory at UVM. Phytoplankton were analyzed while alive (archived samples were preserved in acid Lugol's solution and/or glutaraldehyde). BGA were examined with a compound microscope at the appropriate magnification to discern the characteristics needed to identify them to species. PhycoTech Inc. confirmed the identification of key BGA. In addition, the BGA were photographed and documented in this Final Report, which serves as a permanent record of these organisms.

When toxin producing BGA were identified, the following set of analyses was performed to screen for toxins.

Microcystin Immunoassay Analysis

A Microcystin Plate Kit (Envirologix Inc.) was used for the detection of microcystins. This test is a competitive Enzyme-Linked Immuno Sorbant Assay (ELISA). Microcystin toxin in the sample competes with enzyme-labeled microcystin for a limited number of antibody binding sites on the inside surface of the test wells. After a sample wash step, the outcome of the competition is visualized with a color development step. Standards were included for calibration of the test kit. The VT DOH and UVM analyzed phytoplankton tows and water treatment facility samples (raw and finished water) for the presence of microcystin.

Microcystin Analysis with Liquid Chromatography/Mass Spectrometry (LCMS)
Samples were analyzed for the presence of microcystin (LC/MS) by Dr. Gregory Boyer, SUNY-Syracuse following the procedures in James, et al. (1997).

Microcystin Protein Phosphatase Enzyme Inhibitory Assay (PP1A) Analysis
Samples were also screened for microcystins with the protein phosphatase enzyme inhibitory assay (PP1A) by Dr. Gregory Boyer, SUNY-Syracuse. The PP1A protocol was based on the assay of An and Carmichael (1994). Microcystin LR standards (0.06 to 1000 μg/L) were prepared fresh from a 40 ug/ml stock in 50% acidified MeOH. The protein phosphatase 1, catalytic subunit (Roche), was used at a working concentration of 0.1 mU/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. Controls (no microcystin) and blanks (no microcystin, no enzyme) were run on each plate. A blank-corrected standard curve was made by plotting the % control activity (reaction rate of standard / reaction rate of control) vs. the microcystin LR concentration. A regression equation was generated using the 4-parameter, Hill sigmodal fit function in Sigma Plot, and unknown concentrations were determined from this regression.

Anatoxin-a Analysis
Anatoxin-a High Pressure Liquid Chromatography (HPLC) with fluorescent detection analyses
Greg Boyer of SUNY-Syracuse, performed these analyses. Water samples were filtered through a Whatman glass microfiber 934-AH filter and sonicated three times in 10 mL acidified 50% methanol for 20 seconds on ice under 4°C with 20 second cooling intervals. Samples were centrifuged at 15K rpm for 10 min and roughly 7 mL of the sample saved as a voucher sample. One mL of the supernatant was filtered through a 13 mm x 0.45 μ nylon syringe filter and placed in an autosampler vial for microcystin and PSP analysis. A second 1 mL supernatant was dried under vacuum for anatoxin-a analysis. Zebra mussel tissue was also extracted and analyzed with this technique.

One mL of extracted sample was dried and reconstituted with 100 μL 0.1 M sodium borate buffer and derivatized with 50 μL NBD-F. The reaction was carried out in darkness and was terminated by adding 50 μL 1 N HCl after 60 minutes. Sample was then analyzed by HPLC with fluorescent detection at 470 nm excitation and 530 nm emission wavelengths.

Anatoxin-a LC/MS
Wayne Carmichael of Wright State University performed these analyses. Phytoplankton samples were extracted with 100% MeOH w/0.1 M HAC. Samples were mixed for two hours and then centrifuged at 10,000 rpm for 15 min. Pellets were extracted in this fashion three times and the supernatants pooled. Samples were dried over nitrogen gas and resuspended in 1 mL of 100% MeOH acidified with 0.1 M acetic acid. Five hundred μL of sample (1/2 of the 1 mL extract volume) was then placed into an Amico-Microcon molecular weight cut-off filter centrifuge tube (10,000 MW cut-off). The remaining 500 μL was filtered in a 3000 MW centrifuge tube of the same type. Both tubes were centrifuged at 15,000 rpm until finished (approximately 2 hr). The filtrate was stored at –20 C and used as needed for LC/MS.

A Finnigan Thermo-Quest Micromass LCQduo benchtop LC/MS-MS with electrospray ionization (ESI) (4.0 kv spray voltage) probe was used. LC conditions were: Detector-UV6000 PDA; HPLC

column, YMC-ODS aqueous-15 cm; 0-15 min linear gradient curve using solvent A-0.10% hepafluorobutyric acid (HFBA) (ion pairing reagent), B-0.02% HFBA in acetonitrile with 5% of solvent A. MS conditions were: ESI full scan for MS/MS-m/z 50-200. Select ion mode (SIM) analyses were done on each sample to confirm anatoxin-a and the total ion concentration determined for the selected ion monitoring of 164.5-165.5 (SIM set for 165 with a +/- of 0.5 mass unit). Signal strengths of 10^5 or higher were considered significant. MS/MS were done on select (relative collision energy 40%) samples using a selected reaction monitoring of m/z 165 and its daughter ions (149 and 131 M+H). Sodium adducts were not included. The total ion concentration peak area was used to calculate the amount of anatoxin-a present as compared with standard anatoxin-a purchased from Calbiochem, California. The level of detection on column is about 100 pg.

Paralytic Shellfish Poision Toxins Analysis
Saxitoxin and neosaxitoxin were analyzed by Greg Boyer, SUNY Syracuse, using HPLC with electrochemical oxidation (ECOS) and HPLC with post-column chemical oxidation (PCRS) using the buffer systems of Oshima (Boyer and Goddard 1999). Oshima is an isocratic system specific for saxitoxin and neosaxitoxin. It does not resolve the gonyautoxins but will tell us if they are potentially present. We are currently looking at those samples that have "potential gonyautoxins" to see if they are present.

General Acute Toxicity Screening – Mouse Bioassay
Phytoplankton tow samples were concentrated onto Whatman nucleopore membrane filters, 47mm diameter, with a 8 micron pore size at UVM. The filters were frozen at –20 C before transporting to the FL DOH and the ME Department of Marine Resources (DMR). At FL DOH, Marek Pawlowicz then resuspended the samples in 1.1 mL of distilled water. Using a syringe, 0.5 mL was injected into duplicate mice. This gave the maximum potential response that could be expected from these concentrated samples. Observations were made through the first day and the following morning. Livers from mice that had died were examined by FL DOH.

3. Results

Organisms of concern
Five species of cyanobacteria identified as known toxin producers from the literature (Chorus and Bartram 1999) were commonly found in Lake Champlain during the summer of 2000 (Table 2). Two other species, *Gloeotrichia echinulata* and *Coelosphaerium Naegelianum*, produce compounds that cause irritation or gastrointestinal problems but not toxicity. These species were tested and no toxins were detected in this study. Images of these organisms are shown in Figures 3-10.

Aphanizomenon flos-aquae was moderately abundant in the northern part of the lake. Certain strains of this species are known toxin producers. Filaments of this organism cluster together to form visible colonies that resemble grass clippings. Several clusters of filaments were frozen and shipped to SUNY-Syracuse for more detailed analysis. None of these tests were positive for toxins.

Table 2. Key cyanobacteria found in Lake Champlain (summer of 2000) known to produce toxins based on the literature (Chorus and Bartram 1999, NHMR 1994).

Organisms	Potential Toxins
Aphanizomenon flos-aquae	saxitoxins, anatoxin-a
Anabaena flos-aquae	anatoxin-a, & (s), microcystins
Anabaena circinalis	saxitoxins, microcystins
Anabaena planktonica	anatoxin-a
Microcystis aeruginosa	microcystins, lipopolysaccharide endotoxins
Coelosphaerium Naegelianum	*Unknown type (Scott 1991)*
Gloeotrichia echinulata	*Unknown type (Leeuwangh et al. 1983)*

Anabaena flos-aquae was the most obvious source of the anatoxin-a detected. It formed a brief bloom at the end of July that was concentrated in the boat slip at the Rubenstein Ecosystem Science Laboratory (UVM), although in-lake populations were relatively diffuse. This bloom allowed enough material to be collected for detailed analysis of this species. Water samples from the slip contained approximately 1.2 μg/L (= ppb) anatoxin-a, and zebra mussel tissue collected in the boat slip contained 12.9 μg anatoxin-a/g of tissue. This concentration of toxin potentially could affect any vertebrate consuming these mussels. The LD_{50} (the lethal dose at which 50 percent of the test organisms die) for anatoxin-a is 200-250 μg kg^{-1} body weight (Skulberg et al. 1992).

No effort was made to determine if the anatoxin-a in the zebra mussels was in the gut (passing through) or the tissue itself. This would be useful information in order to understand the significance of this initial finding. Most predators eating zebra mussels would consume the whole organism; if most of the toxin was in the gut, and not incorporated into tissue, then the treat would end as the bloom subsided.

Two dog deaths occurred in Lake Champlain during the summer 2000 that were attributed by a veterinarian to anatoxin-a. Apparently the two dogs drank water from a New York boat slip with a blue-green bloom. Algal identifications were not conducted on these samples, however, it happened approximately 10 days after the bloom in the boat slip at the Rubenstein Ecosystem Laboratory in Vermont, which may indicate that *Anabaena flos-aquae* was responsible. Dr. Boyer analyzed water collected by the owner of the dogs in NY and anatoxin-a was detected at approximately 1.4 μg/L or ppb.

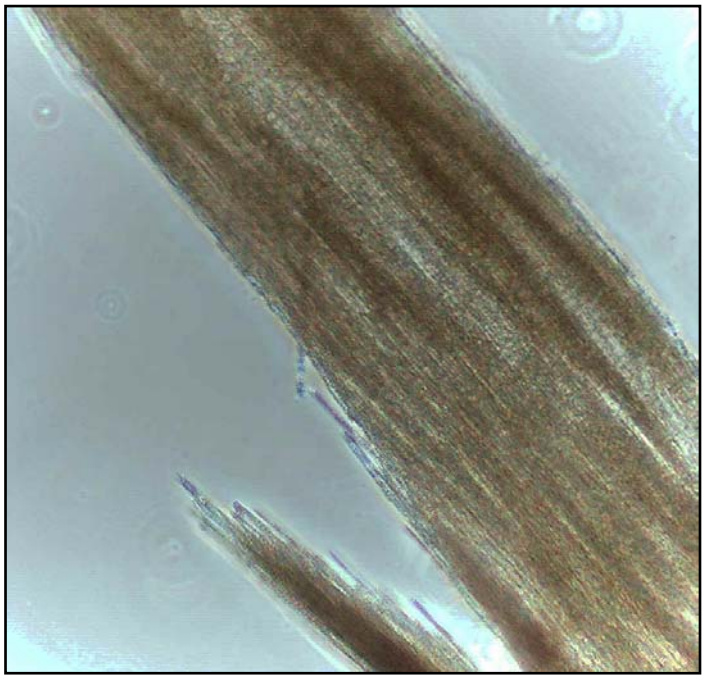


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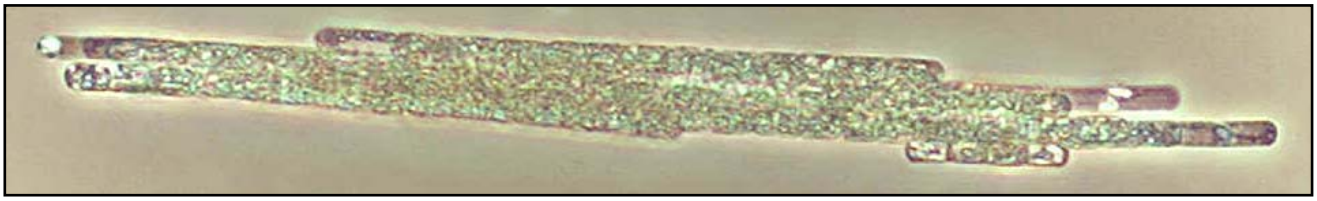
Figure 3. *Aphanizomenon flos-aquae,* as viewed at 100x magnification. Note the cluster of filaments that give it the appearance of grass clippings. Color is normally grass-green.

Figure 4. *Aphanizomenon flos-aquae,* as viewed at 400x magnification. ↓



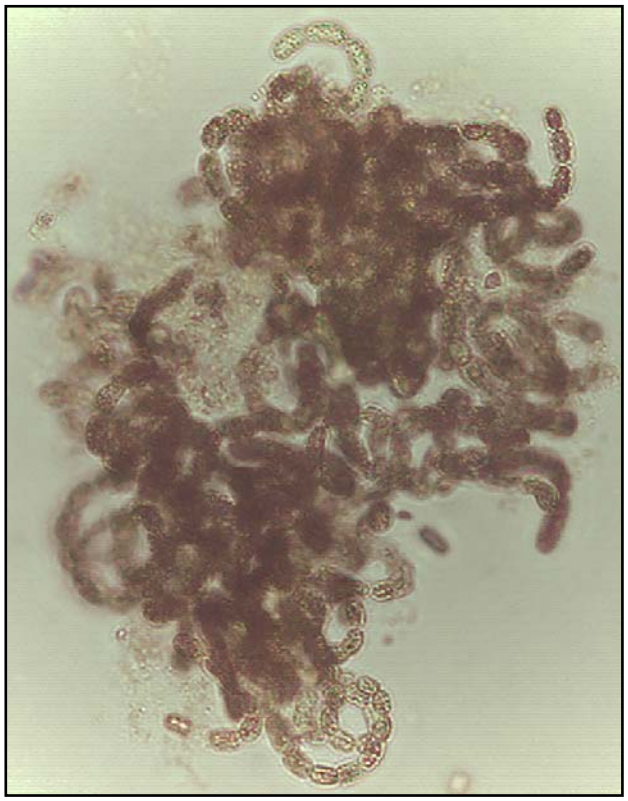
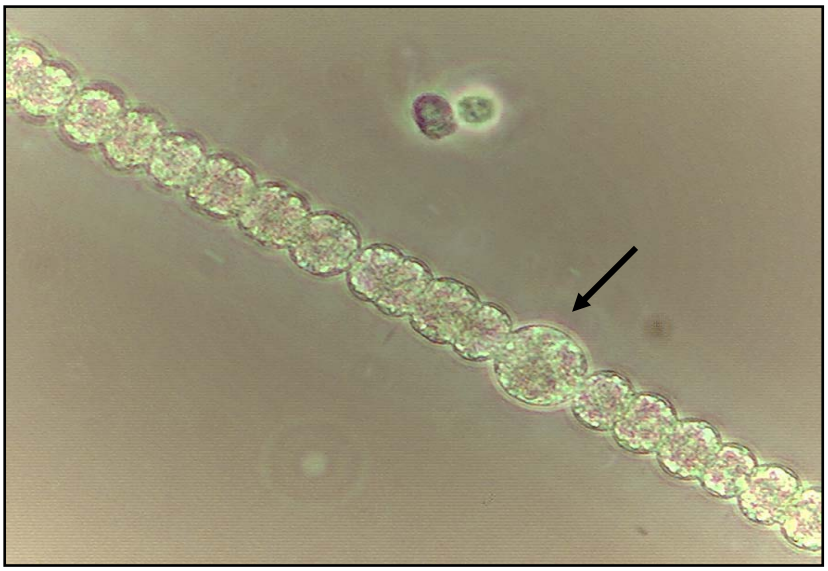


Figure 5. *Anabaena flos-aquae* as viewed at 200x magnification.



← Figure 6. *Anabaena planktonica* as viewed at 400x magnification. Arrow points to the heterocyst, the site of atmospheric nitrogen fixation.

Figure 7. *Anabaena circinalis* as viewed at 200x magnification.

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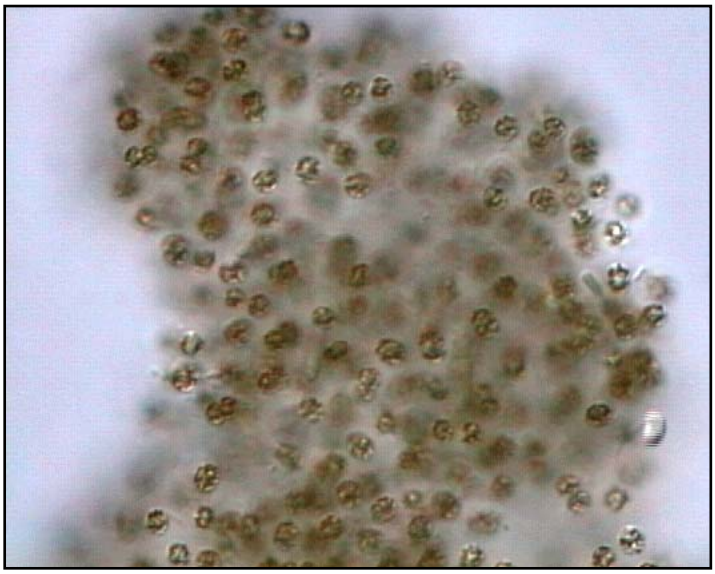

Figure 8. *Microcystis aeruginosa as viewed at* 400x.

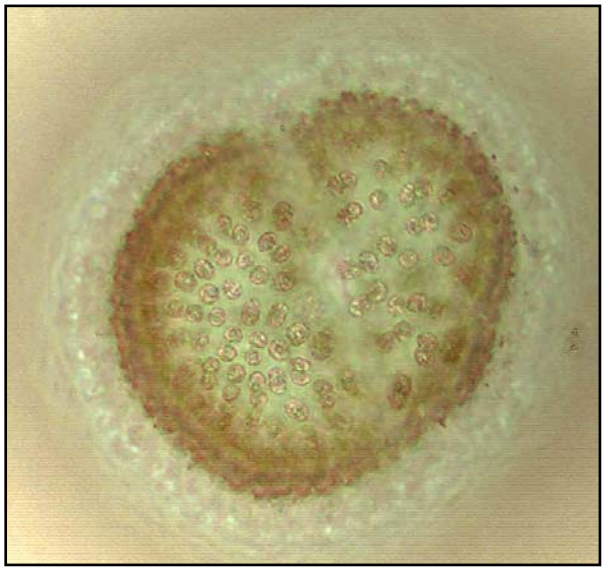

Figure 9. *Coelosphaerium Naegelianum* as viewed at 200x.

Figure 10. *Gloeotrichia echinulata* viewed at 100x. This organism forms a round colony that is the size of a pinhead and easily visible in the water.

Microcystin was also detected in the boat slip sample collected near the Rubenstein Ecosystem Laboratory. Although *Anabaena flos-aquae* can produce microcystin, a minor phytoplankton constituent, such as *Microcystis*, could also have produced it.

Anabaena circinalis was not abundant in the samples, but it was found in several of them. It has not been demonstrated to produce toxin in the US but has been documented as doing so in several other countries. It also is known to produce the off-flavor (earthy-musty) compound geosmin (Rosen et al. 1992).

Anabaena planktonica was occasionally abundant in the samples and it was found in several of them. It has been demonstrated to produce toxin in Italy.

Microcystis aeruginosa was found in almost all samples and was considered the likely source of microcystin detected. This is the organism that bloomed in 1999; however, it did not bloom in the summer of 2000. Two other similar species of *Microcystis* were noted and rare.

Algal Abundance

Surface samples were collected for this study even though the water treatment facility intake structures were at greater depths. This method was chosen because several species of bloom-

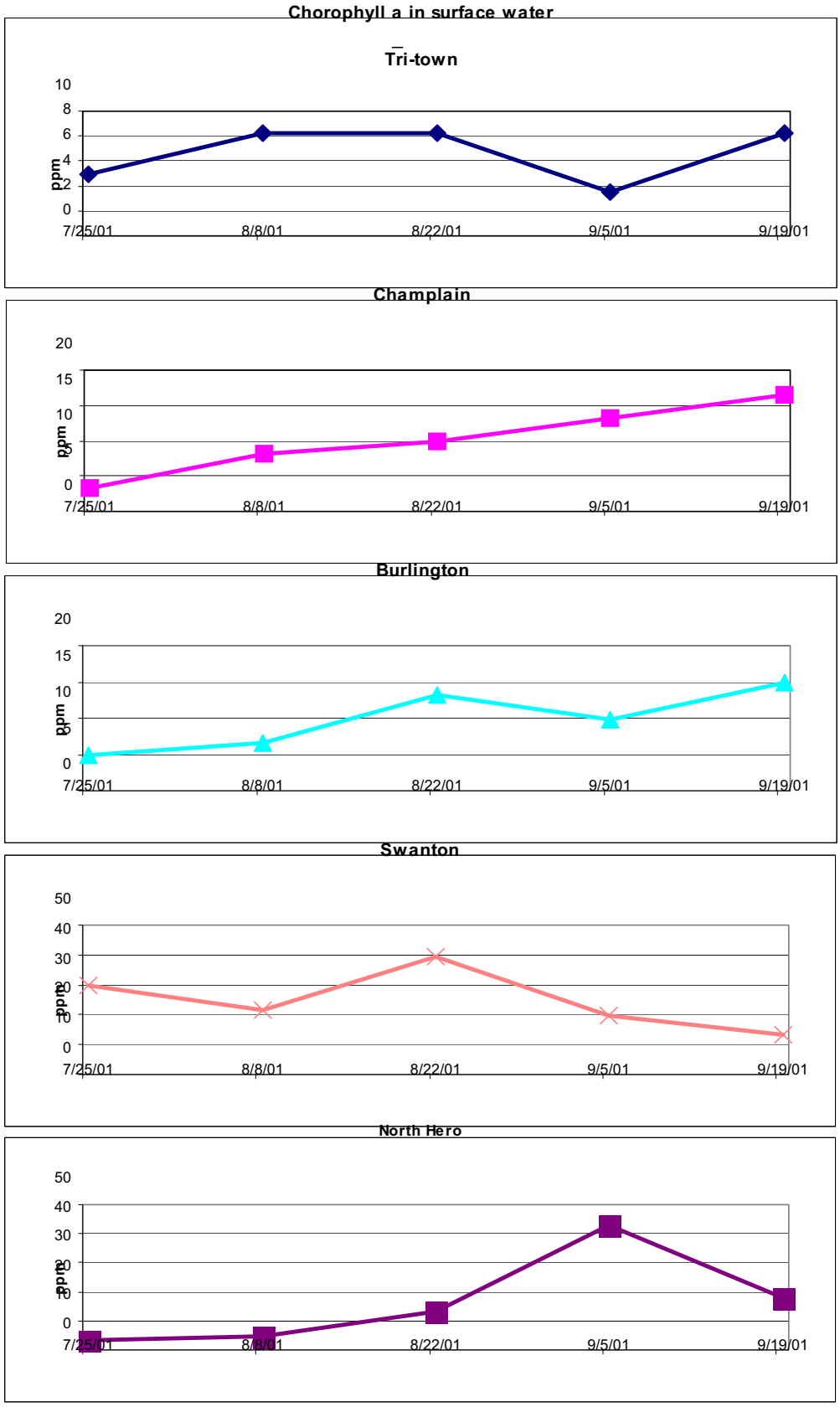

Figure 11. Chlorophyll a from surface water near each treatment facility intake.

forming BGA can regulate their buoyancy and rise to the surface to optimize photosynthesis. Once at the surface, wind often causes an accumulation of organisms in shallow areas of a lake.

Algal Biomass

Chlorophyll a was used to estimate the biomass of phytoplankton. Figure 11 illustrates the chlorophyll a concentration in surface water near each water treatment facility intake. These data show that Swanton and North Hero waters had the greatest phytoplankton biomass, with peaks occurring in mid-August and early September, respectively. These data are consistent with the visual observations made at these locations documenting large, visible colonies of *Gloeotrichia* for much of the summer.

It could not be determined from this study if the changes in chlorophyll a are a result of changes in growth rate of the organisms at a site or if recruitment/export of organisms is also occurring. A combination of growth and recruitment/export is the most probable explanation of the changes in chlorophyll a.

Immunoassay for Microcystins

The standards provided with the immunoassay range from 0.16 to 1.6 ppb. The most accurate values from this assay fall within this range. A predictive equation was used for readings above and below these concentrations. Only trace amounts of microcystin were found in raw or finished drinking water using immunoassays (Table 3 and 4, respectively). Similar data (not shown) was generated by UVM. All of these data are below the World Health Organization provisional guideline of 1.0 ppb (Chorus and Bartram 1999) for protection of human health.

Table 3. Microcystin analyses for raw drinking water samples-VT Department of Health. Mean +/- standard deviation.

Microcystin in Raw Water (as tested by immunoassay-µg/L or ppb)					
	25-Jul	8-Aug	22-Aug	5-Sep	19-Sep
Tri-Town	0.091 +/- 0.005	0.059 +/- 0.017	0.088 +/- 0.004	0.064 +/- 0.005	0.104 +/- 0.022
Champlain	0.097 +/- 0.008	0.073 +/- 0.013	0.072 +/- 0.016	0.076 +/- 0.003	0.140 +/- 0.015
Burlington	0.073 +/- 0.010	0.094 +/- 0.021	0.058 +/- 0.006	0.060 +/- 0.013	0.103 +/- 0.027
Swanton	0.083 +/- 0.021	0.096 +/- 0.009	0.150 +/- 0.001	0.059 +/- 0.003	0.128 +/- 0.003
North Hero	0.063 +/- 0.003	*not analyzed	0.129 +/- 0.032	0.125 +/- 0.010	0.144 +/- 0.026

*sample not collected

Table 4. Microcystin analyses for finished drinking water samples-VT Department of Health. Mean +/- standard deviation.

Microcystin in Finished Water (as tested by immunoassay-µg/L or ppb)					
	25-Jul	8-Aug	22-Aug	5-Sep	19-Sep
Tri-Town	not analyzed	not analyzed	0.070 +/- 0.018	0.051 +/- 0.027	0.056 +/- 0.008
Champlain	not analyzed	not analyzed	0.069 +/- 0.001	0.098 +/- 0.001	0.116 +/- 0.022
Burlington	not analyzed	not analyzed	0.121 +/- 0.009	0.086 +/- 0.001	0.082 +/- 0.010
Swanton	not analyzed	not analyzed	0.093 +/- 0.004	0.050 +/- 0.013	0.087 +/- 0.026
North Hero	not analyzed	not analyzed	0.114 +/- 0.018	0.103 +/- 0.009	<0.16

Based on these data, microcystin concentration was generally lower in finished water compared to raw water, with a few exceptions. However, because these values are very low, well below the quantification range of the assay, these data should be considered very preliminary. In the future, water samples should be concentrated for greater analytical accuracy, which normally is accomplished by freeze drying the samples and reconstituting them in a smaller volume of water. More complete research is needed to determine how the treatment processes at the five facilities change microcystin concentration. Microcystin is contained within the cells of BGA and is not released until cell death. However, if BGA are trapped and broken on treatment plant filters, their microcystin would be released into the water, elevating the concentration in the finished water.

The data from phytoplankton tows taken above the water intakes provided additional evidence that microcystin was being produced. Phytoplankton tows concentrated the BGA. Because of this increase in biomass the potential detection in the immunoassays is enhanced (Table 5). To estimate the concentration factor of the phytoplankton tows relative to the natural phytoplankton

Table 5. Phytoplankton tow samples microcystin concentrations based on immunoassay.

Phytoplankton Tow Microcystin Concentration (as tested by immunoassay-µg/L or ppb)					
	25-Jul	8-Aug	22-Aug	5-Sep	19-Sep
Tri-Town	0.235	0.114	0.177	0.110	0.757
Champlain	0.096	0.219	1.412	0.855	1.209
Burlington	0.256	0.129	1.296	0.296	1.702
Swanton	3.261	3.248	2.198	1.764	1.830
North Hero	3.099	2.969	1.939	1.998	2.013

Note: these numbers represent concentrated samples, they are **not** the concentrations found in ambient water.

density of the lake water, chlorophyll a was measured in whole water samples from the lake and in the phytoplankton tow samples. The same concentration factor derived from the chlorophyll a analysis was applied to the microcystin analysis. This allowed the estimation of the ambient (natural) microcystin concentration with greater accuracy than using whole water samples that are below the detection limit. Table 6 shows the results of these calculations.

Table 6. Estimated microcystin concentrations in ambient lake water based on immunoassay.

Ambient Microcystin Concentration (as tested by immunoassay-µg/L or ppb)					
	25-Jul	8-Aug	22-Aug	5-Sep	19-Sep
Tri-Town	0.001	0.005	0.003	0.002	0.020
Champlain	0.001	0.005	0.020	0.014	0.043
Burlington	0.005	0.002	0.035	0.003	0.062
Swanton	0.007	0.009	0.009	0.009	0.004
North Hero	0.002	0.014	0.005	0.011	0.019

PP1A for Microcystin

PP1A analyses on raw and finished water showed no detectable microcystin toxins. Because the PP1A and immunoassay are based on different chemical principles, it is not possible to quantitatively compare the results of the two tests at this time. However, a high concentration in one should generally correspond to a high concentration the other.

Estimates of the concentration of microcystin in surface water using PP1A were also developed using phytoplankon tow samples in same way described for immunoassay (Table 7 and 8).

Table 7. Phytoplankton tow samples microcystin concentrations based on PP1A analysis.

Phytoplankton Tow Microcystin Concentration (as tested by PP1A-µg/L or ppb)					
	25-Jul	8-Aug	22-Aug	5-Sep	19-Sep
Tri-Town	0.000	0.000	0.220	0.230	0.120
Champlain	0.000	0.000	1.090	2.979	3.460
Burlington	0.000	0.254	1.790	0.361	2.030
Swanton	2.705	35.421	8.733	9.700	3.766
North Hero	3.303	10.286	12.020	13.960	7.716

Note: these numbers represent concentrated samples, they are **not** the concentrations found in ambient water.

Table 8. Estimated microcystin concentrations in ambient lake water based on PP1A analysis.

Ambient Microcystin Concentration (as tested by PP1A-µg/L or ppb)					
	25-Jul	8-Aug	22-Aug	5-Sep	19-Sep
Tri-Town	0.000	0.000	0.004	0.046	0.003
Champlain	0.000	0.000	0.015	0.047	0.124
Burlington	0.000	0.005	0.048	0.004	0.073
Swanton	0.005	0.096	0.035	0.047	0.008
North Hero	0.002	0.048	0.029	0.077	0.071

Mouse Bioassay

Mouse bioassays were performed to determine general acute toxicity upon exposure to samples. Because of the low number of algae in the whole water samples, only the concentrated phytoplankton tow samples were analyzed. Both the ME DMR and the FL DOH used a new, shared protocol for all samples beginning with the August 8 sample (Table 9). In this protocol, the normal response period was extended to 24 hours (overnight) to measure the full toxin potential. No samples showed an acute response, defined as mortality occurring within 1 to 60 minutes after injection.

The FL DOH observed 100% mouse fatality in duplicate injections after 6-12 hours for this date. ME DMR had mixed results for this date, with one mouse (of a duplicate) death in both the Tri-town and the North Hero samples, and no mouse death with the other three samples. No obvious reason can be given for the discrepancy between the two laboratories. Variation in mice susceptibility and natural variation in the amount or kind of phytoplankton in the extract are probable causes.

FL DOH followed-up the mouse bioassays they conducted by performing autopsies. All mice from all stations on the four sample dates showed liver damage (bloody, swollen livers). This indicates that microcystin was present in these samples. A sub-sample of the extract used in the injections also tested positive for the presence of microcystin (immunoassay method performed by the FL DOH).

Microcystin Analysis Summary

Three separate methodologies, immunoassay, PP1A, and mouse bioassay, confirmed the presence of microcystin in the phytoplankton of Lake Champlain. Ambient concentrations were all well below the World Health Organization human health guideline of 1.0 pbb. In most samples, the ambient concentrations were at least one order of magnitude less than this guideline.

Table 9. Mouse bioassay performed on phytoplankton concentrates.

Mouse Bioassays (time until death)					
	25-Jul	*8-Aug	22-Aug	5-Sep	19-Sep
Tri-Town	no data	**6-12 hours	6-12 hours	6-12 hours	6-12 hours
Champlain	no data	*6-12 hours	6-12 hours	6-12 hours	6-12 hours
Burlington	no data	*6-12 hours	6-12 hours	6-12 hours	6-12 hours
Swanton	no data	*6-12 hours	6-12 hours	6-12 hours	6-12 hours
North Hero	no data	**6-12 hours	6-12 hours	6-12 hours	6-12 hours

*QA performed on 8/8 samples. * indicates conflicting data between labs (mice lived with ME DMR, died in the FL DOH lab). ** indicates single mouse death with ME DMR and duplicate mice death in the FL DOH lab.

In the 2000 season, there was no bloom of *Microcystis*, therefore, it was necessary to concentrate phytoplankton by plankton tow to obtain enough biomass for microcystin analyses. Based on these results, no drinking water or recreational impacts resulting from toxins would be expected. When a bloom of *Microcystis* does occur in Lake Champlain, there is the distinct possibility that biomass will accumulate on or near shore due to wind and wave action. Accumulation of *Microcystis* on shorelines is very common. Such an accumulation must be examined for the presence of microcystin. If microcystin is found, notification for recreational users would be a prudent. In addition, water treatment facilities should test for the presence of microcystin during a bloom of *Microcystis*. Prolonged exposure of the public to drinking water above the 1.0 ppb World Health Organization guideline should be avoided because of the potential heath issues.

Anatoxin-a analysis by HPLC-FD

All raw and finished drinking water samples tested for anatoxin-a were below the detection limit of 0.1 µg/L. The plankton tow samples were also negative with the possible exception of two samples from September 22. Upon re-examination, these samples were also judged negative.

Anatoxin-a was detected during a surface bloom of *Anabaena flos-aquae* collected from the boat slip at the Rubenstein Ecosystem Science Laboratory (UVM) (as described earlier in this report for this species). The deaths of two dogs in New York were also attributable to anatoxin-a.

Anatoxin-a analysis by LC/MS

Wright State University analyzed the phytoplankton tows from the August 8th collection as part of the QA for this project. The data (Table 10) suggest that anatoxin-a was more commonly found than indicated by the HPLC-FD technique, although the concentrations were quite low.

Table 10. Anatoxin-a detected by LC/MS conducted by Wright State University. Duplicate data points were averaged and standard deviations calculated.

Anatoxin-a in Phytoplankton Tows (μg/L or ppb for the August 8th samples)		
	avg.	std. dev.
Tri-Town	0.066	0.023
Champlain	0.008	0.011
Burlington	0.000	0.000
Swanton	0.028	0.006
North Hero	0.037	0.007

4. Summary and Recommendations for Future Direction

Despite lake conditions that were not conducive to development of BGA blooms across wide portions of Lake Champlain in 2000, this project has contributed significantly to available information regarding cyanobacteria in the lake.

1) Known toxin producing taxa were commonly observed in many areas of Lake Champlain, though rarely in bloom concentrations during the summer 2000.
2) Trace levels of microcystin were detected in raw and finished drinking water using the ELISA assay procedures and confirmed by PP1A.
3) Raw and finished drinking water samples, as well as phytoplankton tow samples, tested negative for anatoxin-a using HPLC methodology. One small bloom of *Anabaena flos-aquae* tested positive. Zebra mussels from the same location also tested positive for this toxin.
4) Mouse bioassays conducted with concentrated samples of Lake Champlain phytoplankton confirmed the presence of toxins at a sub-acute level.
5) The ELISA assay shows promise of being a simple and rapid assessment method for the presence of microcystin.

The human health risks associated with the BGA toxins in Lake Champlain needs additional assessment. Concentrations of toxins in 2000 were very low, but bloom conditions were not encountered. At least two key organisms need to be followed in future studies of the lake, *Anabaena flos-aquae* and *Microcystis aeruginosa*.

Future research should concentrate on understanding the BGA in a bloom. This would consist of the amount of toxin per colony and the natural variation in toxin production by these colonies. These data are needed throughout the different stages (rapid growth to death) of a bloom, as it is important to understand how much toxin is staying in the cells versus leaking into the water during

the different stages. When a bloom dies, leaking is known to occur and would have direct management implications.

A strategy for managing human health risks should also be developed. This could be based on "Alert Levels Framework" that triggers a graduated response to a potentially harmful bloom (Chorus and Bartram 1999). A framework could contain Vigilance and Alert levels that are triggered by the number of organisms present in the water. For example, if 10 colonies per mL colonies of *Anabaena flos-aquae* were found, it would trigger a Vigilance Level response, while 100 colonies per mL would trigger an Alert Level. When counts exceed 5000 colonies per mL we may go to a higher alert status, etc. An important piece of information is required for this approach. Determining the timing and condition of a bloom would enhance the Alert Level Framework precision.

It would also be useful to add a BGA quantification component to the water that is actually brought into the treatment facilities for processing, especially during the bloom period. Water intake depth, for example, might make a big difference in which BGA enter the facility. Surface water samples may not represent the BGA actually entering the plant in the raw water stream. In a similar area, limited information is available on the removal of toxins by drinking water facilities. Additional data on how each process in water treatment plant affects toxins would be important information for operators seeking to mitigate the effects of a harmful BGA bloom.

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