

SUPREME COURT OF THE STATE OF NEW YORK  
COUNTY OF YATES

In the Matter of the Application of

SIERRA CLUB, COMMITTEE TO PRESERVE THE FINGER  
LAKES by and in the name of PETER GAMBA, its President;  
and COALITION TO PROTECT NEW YORK by and in the  
name of KATHRYN BARTHOLOMEW, its Treasurer,

Petitioners,

For a Judgment Pursuant to Article 78 of the  
Civil Practice Law and Rules,

–against–

NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL  
CONSERVATION, BASIL SEGGOS, COMMISSIONER,  
GREENIDGE GENERATION, LLC, GREENIDGE PIPELINE,  
LLC, GREENIDGE PIPELINE PROPERTIES CORPORATION  
and LOCKWOOD HILLS, LLC,

Respondents.

State of New York,  
County of Onondaga, ss.:

GREGORY BOYER, being duly sworn, deposes and says:

1. I am Professor of Biochemistry and Director, Great Lakes Research Consortium at the State University of New York, College of Environmental Science and Forestry in Syracuse, New York.

2. I received my B.A. Degree in Biochemistry from the University of California at Berkeley and my Ph.D. degree in Biochemistry from the University of Wisconsin. After postdoctoral fellowships at the Plant Research Labs at Michigan State University and in the Department of Oceanography at the University of British Columbia, I joined the Faculty of Chemistry at SUNY-ESF in 1985. My expertise is with toxins and other compounds produced by marine and freshwater algae. I was director of NOAA's

AFFIDAVIT OF  
GREGORY BOYER IN  
SUPPORT OF  
PETITIONERS'  
MEMORANDUM OF  
LAW IN OPPOSITION  
TO THE MOTIONS TO  
DISMISS

Index No. 2016-0165

Lower Great Lakes project to develop monitoring strategies for toxic blue-green algae in the lower great lakes and currently assist the NYS Department of Environmental Conservation with their Citizen State Lake Assessment Program with algal issues. I have authored over 130 publications on bioactive natural products, served as a principal investigator on over \$10 million in research funding, served as chair of the Department of Chemistry at SUNY-ESF for four years and am an advisor to the International Joint Commission on issues regarding Harmful Algal Blooms (HABs) within the Great Lakes. I am the current director of New York's Great Lakes Research Consortium. The GLRC consists of 18 New York Universities and nine Canadian Universities, almost 300 scientists in total, working on all aspects of Great Lakes Science, education and outreach. My educational background and professional experience are more fully set forth in my Curriculum Vitae, attached as Exhibit A.

3. This affidavit addresses the impact increasing water temperature at the mouth of the Keuka Outlet might have on occurrences of HABs in Seneca Lake near the Outlet.

4. HABs are algal blooms composed of cyanobacteria known to naturally produce biotoxins. They can occur when certain types of microscopic algae grow quickly in water.

5. Toxic cyanobacteria are a global public health and environmental concern.

6. Toxic blooms are most commonly formed by *Microcystis*, a well-known producer of the hepatotoxin, microcystin

7. Long term exposure to microcystin has been associated with severe human health effects, including liver and colorectal cancers.

8. While it is clear that the occurrence of toxic cyanobacteria blooms around the world have increased during recent decades, the underlying causes of such blooms and

the factors influencing the dynamics of toxic and non-toxic strains within them are poorly understood.

9. My colleagues and I have studied the effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms in Lake Agawam, Lake Ronkonkoma, Lake Champlain, and Mill Pond, Long Island. In a research paper published in 2009, Timothy W. Davis, Dianna L. Berry, Christopher J. Gobler and I described the results of our study. Davies et al, *Harmful Algae* 8 (2009) 715–725. A copy of this paper is attached as Exhibit B. Our study found that in Mill Pond, Long Island in June 2006 temperature was a significant treatment effect which increased the growth rate of toxic *Microcystis* by 22% relative to the control. Higher phosphorus concentrations also increased the growth rate of toxic *Microcystis* (by 33% relative to the control). However, the additive interaction between temperature and phosphorus yielded a growth rate for toxic *Microcystis* that was 63% higher than the control and was the highest growth rate of either population in any treatment. The other measurements reported in the study showed similarly that the highest levels of toxic *Microcystis* were measured when the highest levels of both temperature and nutrients were observed.

10. Reports of HABs are increasing in New York State as a whole.

11. In the last few years, reports of HABs in the Finger Lakes have increased.

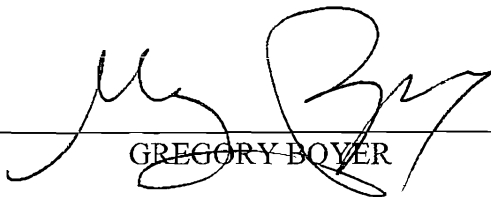
12. HABs outbreaks were discovered in Seneca Lake in 2015 and 2016, including one just south of the Keuka Outlet on Perry Point that was analyzed by my lab in August 2016. The concentration of the liver toxin microcystins in two bloom samples collected from Seneca Lake in September of 2016 exceeded the US-EPA guideline values for microcystin in drinking water by more than 10-fold, and exceeded the proposed US-EPA guidelines for recreational contact with microcystin containing blooms by 5-8 fold.

13. It is my understanding that the Greenidge Generating Station in Dresden, New York is scheduled to resume operations this spring after a six year hiatus and that when those operations begin the plant will begin to discharge very large volumes of heated condenser cooling water into the Keuka Outlet a short distance before the Outlet discharges into Seneca Lake.

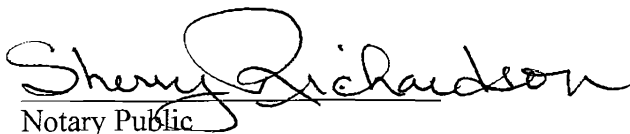
14. These discharges are likely to result in increases in the water temperature of the Dresden bay area of Seneca Lake surrounding the mouth the Outlet.

15. It is known that the Keuka Outlet carries agricultural runoff and discharges from the Penn Yan wastewater treatment plant which may result in significant increased nutrient concentrations in the Outlet. See the results of sampling conducted by members of the Seneca Lake Pure Waters Association (SLPWA) at various sites along the Outlet in July, August and September 2015 attached as Exhibit C. These results are posted on the SLPWA website at <http://senecalake.org/wp-content/uploads/2016/06/KeukaOutletFSD-052816.pdf>.

16. In these circumstances, I am of the opinion that increasing water temperatures in the Dresden bay area of Seneca Lake could result in increased HABs outbreaks in that area and that this issue deserves further study before proceeding forward.

  
GREGORY BOYER

Sworn to before me this  
14 day of January, 2017

  
Notary Public

**SHERRY RICHARDSON**  
Notary Public, State of New York  
Qualified in Onondaga County  
Reg. No. 01RI6223246  
Commission Expires June 7, 2018

# **Exhibit A**

## **Gregory L. Boyer**

Faculty of Chemistry, State University of New York  
College of Environmental Science and Forestry  
Syracuse, NY 13210

Contact: Email: glboyer@esf.edu

Telephone: (315) 470-6825  
FAX: (315) 470-6855

### **RESEARCH INTERESTS**

The chemistry and biochemistry of biologically active natural products from plants and algae including toxins, siderophores, allelopathic agents, and growth regulators. Special interests include the chemistry / ecology of marine and freshwater harmful algal blooms such as cyanobacteria in large lake ecosystems, automated monitoring systems for these blooms, rapid detection methods for toxic cyanobacteria and paralytic shellfish poisoning (PSP) toxins, large scale algal culture facilities for bioproducts productions, and the biochemistry of iron in forest and aquatic (marine and freshwater) ecosystems.

### **EDUCATION:**

Ph.D.,	University of Wisconsin - Madison,	1980,	(Biochemistry).
A.B.,	University of California - Berkeley,	1975,	(Biochemistry).
A.S.,	Reedley College (Reedley, Calif.),	1973,	(Chemistry).

### **PROFESSIONAL EXPERIENCE:**

2007-present: Director, Great Lakes Research Consortium, Syracuse, NY 13210  
1998-present: Professor of BioChemistry, State University of New York, College of Environmental Science and Forestry, (SUNY-ESF) Syracuse NY 13210.  
2011-2014: Chair, Department of Chemistry, SUNY-ESF.  
2010 E.T.S. Walton Visiting Professor, Cork Institute of Technology, Cork, Ireland  
1991-1998 Associate Professor of Chemistry, SUNY-ESF.  
1994 Visiting Scientist, Biology Dept., Woods Hole Oceanographic Institute, Woods Hole, MA  
1986-1991 Joint Academic Appointment in the Faculty of Environmental Sciences, SUNY-ESF.  
1985-1990 Assistant Professor of Chemistry, SUNY-ESF.  
1983-1984 Research Associate, Dept. of Oceanography, Univ. of British Columbia, Vancouver, BC.  
1980-1982 Research Associate, Michigan State Univ. - DOE, Plant Research Labs. East Lansing, MI.  
1975-1980 Research Assistant, Department of Biochemistry, University of Wisconsin, Madison, WI.

### **HONORS and Supporting Activities:**

Life member; Phi Beta Kappa - Alpha (UC-Berkeley Honor Soc.) and Alpha Gamma Sigma (Reedley College Honor Soc.), International Expert for IAEA (International Atomic Energy Agency) on PSP toxins (1999), Participant in EPA's "Creating a Cyanotoxin Target List for the Unregulated Contaminant Monitoring Rule" taskforce (2001), Participant in NOAA workshop entitled "Developing a National Plan for Remediation of Harmful Algal Blooms", Steering committee for "National Plan for Marine Biotoxins-2004; Elected member of the US National Harmful Algal Bloom Advisory Committee (2005-2008), Invited External Reviewer for EPA Toxicological Reviews of Cyanobacteria Toxins (2007), Elected to the Governing Body of the Northeast Algal Society 1999-2017; Recipient; State University of New York Research Foundations 2003 Award for Excellence in the Pursuit on Knowledge. Recipient; 2005 ESF award for integrating outreach activities with Teaching and Research. Recipient; ESF 2009 Exemplary Researcher Award ; Recipient; 2010 Science Foundation of Ireland E.T.S. Walton Visiting Professorship, Chief Scientist, Centers for Excellent in Ocean Science Education (COSEE) research cruises 2005, 2008 and 2009, Participate in more than 25 International Research Cruises (eight times as Senior or lead Scientist), Member of International Joint Commission's Council of Research Managers and New York's Great Lakes Basin Advisory Committee. Recipient; 2015 Lake Tear of the Clouds Award for outstanding service to the New York State Federal of Lake Associations. Recipient: 2016 Chancellors Award for Excellence in Faculty Service.

**SELECTED PROFESSIONAL SOCIETIES:** American Society of Limnology and Oceanography, North East Algal Society, International Association for Great Lakes Research, International, Society for the Study of Harmful Algal Blooms, North American Lake Management Society.

## **PUBLICATIONS OF GREGORY L. BOYER:**

1. Boyer, G.L., E.J. Schantz, and H.K. Schnoes (1978) Characterization of 11-hydroxysaxitoxin sulfate, a major toxin in scallops exposed to blooms of the poisonous dinoflagellate, *Gonyaulax tamarensis*. J.C.S. Chem. Comm. 20: 889-890.
2. Boyer, G.L., C.F. Wichmann, J. Mosser, E.J. Schantz, and H.K. Schnoes (1979) Toxins isolated from Bay of Fundy Scallops. In: Toxic Dinoflagellate Blooms, D.L. Taylor, H.H. Seliger, eds. Elsevier-North Holland, New York. pp. 373-376.
3. Wichmann, C.F., C.L. Divan, G.L. Boyer, E.J. Schantz, and H.K. Schnoes (1981) Neurotoxins from *Gonyaulax excavata* and the Bay of Fundy scallops. Tetrahedron Letters 22(21): 1941-1944.
4. Boyer, G.L. and J.A.D. Zeevaart (1982) Isolation and quantitation of B-D-glucopyranosyl abscisate from leaves of *Xanthium* and Spinach. Plant Physiol. 70: 227-231.
5. Zeevaart, J.A.D. and G.L. Boyer (1982) Metabolism of abscisic acid in *Xanthium strumarium* and *Ricinus communis*. In: Plant Growth Substances 1982, P.F. Wareing, ed. Springer Verlag, New York, pp. 335-342.
6. Zeevaart, J.A.D. and G.L. Boyer (1984) Accumulation and transport of abscisic acid and its metabolites in *Ricinus* and *Xanthium*. Plant Physiol. 74: 934-939.
7. Hall, S., S.D. Darling, G.L. Boyer, P.B. Reichardt, and H.-W. Liu (1984) Dinoflagellate neurotoxins related to saxitoxin: Structures of toxins C3 and C4, and confirmation of the structure of neosaxitoxin. Tetrahedron Letters 25(33): 3537-3538.
8. Boyer, G.L. and J.A.D. Zeevaart (1986) 7'-hydroxy abscisic acid; an artifact of feeding racemic abscisic acid to *Xanthium strumarium*. Phytochemistry 25: 1103-1105.
9. Boyer, G.L., J.J. Sullivan, R.J. Andersen, P.J. Harrison, and F.J.R. Taylor (1986) Toxin production in three isolates of *Protogonyaulax* sp. In: Toxic Dinoflagellates, D.M. Anderson, A.W. White, and D.G. Baden, eds., Elsevier, pp 281-286.
10. \*Boyer, G.L., J.J. Sullivan, M. LeBlanc, and R.J. Andersen (1986) The assimilation of PSP toxins by the copepod *Tigriopus* from dietary *Protogonyaulax tamarensis*. In: Toxic Dinoflagellates, D.M. Anderson, A.W. White, and D.G. Baden, eds., Elsevier, pp 407-412.
11. Zeevaart, J.A.D., G.L. Boyer, K. Cornish, and R. Creelman (1986) Metabolism of abscisic acid. In: Plant Growth Substances 1985, M. Bopp, ed., Springer-Verlag, pp. 101-107.
12. Boyer, G.L., J.J. Sullivan, R.J. Andersen, F.J.R. Taylor, P.J. Harrison, and A.D. Cembella (1986) The use of HPLC to investigate the production of paralytic shellfish toxins by *Protogonyaulax* sp. in culture. Mar. Biol. 93: 361-369.
13. Boyer, G.L., B.V. Milborrow, P.F. Wareing, and J.A.D. Zeevaart (1986) The nomenclature of abscisic acid and its metabolites. In; Plant Growth Substances 1985, M. Bopp, ed., Springer-Verlag, pp. 99-100.
14. Boyer, G.L., A.H. Gillam, and C.G. Trick (1987) Iron Ecology and Uptake. In: The Cyanobacteria - A Comprehensive Review, P. Fay and C. van Baalen, eds., Elsevier Biomedical, pp. 415-436.
15. Zeevaart, J.A.D., and G.L. Boyer (1987) Photoperiodic Induction and the Floral Stimulus in *Perilla*. In: The Manipulation of Flowering, J.G. Atherton, ed. Butterworths, London, pp. 269-277.
16. Cembella, A.D., J.J. Sullivan, G.L. Boyer, F.J.R. Taylor, and R.J. Andersen (1987) Toxin variability within the *Protogonyaulax tamarensis* / *catenella* species complex. Biochem. Syst. Ecol. 15:171-186.
17. Whitefleet, J., G.L. Boyer, and H.K. Schnoes (1987) Isolation and spectral characteristics of four toxins from the dinoflagellate *Ptychodiscus brevis*. Toxicon 24:1075-1090.
18. Boyer, G.L., J.J. Sullivan, R.J. Andersen, P.J. Harrison, and F.J.R. Taylor (1987) The effects of nutrient limitation on toxin production in *Protogonyaulax tamarensis*. Marine Biol. 96: 123-128.

19. Boyer, G.L. and S.S. Dougherty (1988) Identification of abscisic acid in the seaweed *Ascophyllum nodosum*. *Phytochemistry*, 27: 1521-1522.
20. Omholt, T.E. and G.L. Boyer (1988) The reduction of iron in squash callus cultures. *J. Plant Nutr.*, 11(6-11): 1227-1235.
21. Doucette, G.J., A.D. Cembella, and G.L. Boyer (1989) Cyst formation in the red tide dinoflagellate *Alexandrium tamarensis* (Dinophyceae): Effects of iron stress. *J. Phycol.*, 25: 721-731.
22. Boyer, G.L. (1989) Chlorophyll and phytoplankton dynamics of Beaver Lake. In: "Beaver Lake 1988" J.M. Hassett, Ed. SUNY College of Environmental Science and Forestry, Syracuse pp 104-124.
23. Speirs, R.J., and G.L. Boyer (1991) Analysis of <sup>55</sup>-Fe Labeled hydroxamate siderophores by high-performance liquid chromatography. *J. Chrom.* 537:259-267.
24. Boyer, G.L. (1991) HPLC separation of Iron Chelators. *J. Chromato. Science.* 29:319
25. Boyer, G.L., and D.J. Nicholson (1991) Occurrence of abscisic acid in unicellular green algae. *Plant Physiol.* 96s:76.
26. Aronson, D.B., and G.L. Boyer (1992) *Frankia* produces a hydroxamate siderophore under iron limitation. *J. Plant Nutr.* 15:2193-2201.
27. Freeman, R.A. and G.L. Boyer (1992) Solid phase extraction techniques for the isolation of siderophores from aquatic environments. *J. Plant Nutr.* 15:2263-2275.
28. Janiszewski, J. and G.L. Boyer (1993) The electrochemical oxidation of saxitoxin and derivatives: its application to the HPLC analysis of PSP toxins. In "Toxic Phytoplankton Blooms in the Sea" T. Symeda and Y. Shimizu, (Eds.), Elsevier Science Publishers, pages 889-894.
29. Boyer, G. L., and D. B. Aronson (1994) Iron uptake and siderophore formation in the actinorhizal symbiont *Frankia*. In: "The Biochemistry of Metal Micronutrients in the Rhizosphere" J.A. Manthey, D.E. Crowley, D.G. Luster, Eds., Lewis Publ., Chelsea, MI., pp. 41-54.
30. Aronson, D. B., and G. L. Boyer (1994) Growth and Siderophore formation in six iron-limited strains of *Frankia*. *Soil Biol. Biochem.* 26: 561-567.
31. Hu, X., and G. L. Boyer (1995) Isolation and characterization of the siderophore N-deoxyschizokinen from *Bacillus megaterium* ATCC 19213. *BioMetals.* 8:357-364.
32. Prabhu, V., H. Wilcox, and G. Boyer (1995) Properties of Nitrate Reductase from the mycorrhizal ascomycete *Wilcoxina mikolae* var *mikaloe*. *Mycol. Res.* 99:1356-1360.
33. Boyer, G. L. (1995) Role of Iron in Brown Tides: An Overview. In: "Proceedings of the Brown Tide Summit, October 20-21, 1995", A. McElroy, (ed.), New York Sea Grant Institute; Stony Brook, NY. pp. 49-51.
34. Prabhu, V., P. Biolchini, and G. L. Boyer (1996) Detection and identification of ferricrocin produced by ectendomycorrhizal fungi in the genus *Wilcoxina*. *Biometals.* 9:229-234.
35. Hu, X., and G. L. Boyer (1996) Effect of metal ions on the quantitative determination of hydroxamic acids. *Anal. Chem.* 68:1812-1815.
36. Prabhu, V., H. Wilcox, and G. Boyer (1996) Regulation of nitrate reductase in the mycorrhizal ascomycete fungus *Wilcoxina mikolae* var *mikaloe*. *Mycol. Res.* 100: 333-336.
37. Hu, X., and G. L. Boyer (1996) Siderophore-mediated aluminum uptake by *Bacillus megaterium* ATCC 19213. *Appl. Environ. Microbiol.* 62:4044-4048.
38. Boyer, G. L., and X. Hu (1996) The electrochemical detection of PSP toxins. *Can. Tech. Rep. Fish. Aquat. Sci.* #2138:1-5.



39. Boyer, G. L., J. J. Janiszewski, and X. Hu (1997) A comparison of Electrochemical Methods for the HPLC analysis of PSP toxins. In: "Harmful Microalgae" B. Reguera, J. Blanco, M. L. Fernandez, and T. Wyatt, (eds.), Xunta del Galicia, Vigo Spain., pp 515-518.
40. Boyer, G. L., and L. Brand (1998) Micro nutrient availability and trace metal chelator interactions. NATO ASI Physiological Ecology of Harmful Algal Blooms, pp. 489-508.
41. Giner, J.-L., and G. L. Boyer (1998) Sterols of the Brown Tide Alga *Aureococcus anophagefferens*. *Phytochemistry*. 48:479-484.
42. Boyer, G. L., and G. D. Goddard (1999) High performance liquid chromatography (HPLC) coupled with post-column electrochemical oxidation (ECOS) for the detection of PSP toxins. In: "Advances in Detection methods for Fungal and Algal Toxins" F. M. Van Dolah, and J. L. Richard, Eds., Gordon Research Satellite Conference, Mt Desert Island, June 17-19.
43. Boyer, G. L., S. A. Kane, J. A. Alexander, and D. B. Aronson (1999) Siderophore formation in iron-limited cultures of *Frankia* sp. 52065 and *Frankia* sp. CeSI5. *Can. J. Bot.* 77:1316-1320.
44. Boyer, G. L., D. B. Szmyr, and J. A. Alexander (1999) Iron and Nitrogen nutrition in the Brown tide organism *Aureococcus anophagefferens*. In: J.L. Martin and K. Haya (eds) Proceedings of the Sixth Canadian Workshop on Harmful Marine Algae. *Can Tech Rep. Fish. Aquat. Sci.* 2261:11-13.
45. Goddard, G., and G. L. Boyer (1999) High performance liquid chromatography (HPLC) coupled with post-column electrochemical oxidation (ECOS) for the detection of PSP toxins. *Natural Toxins*. 7:353-359.
46. Goddard, G., K. Haya, and G. L. Boyer (1999) Evaluation of the electrochemical oxidation system for the analysis of PSP toxins in natural shellfish samples. In: J.L. Martin and K. Haya (eds.) Proceedings of the Sixth Canadian Workshop on Harmful Marine Algae *Can. Tech. Rep. Fish. Aquat. Sci.* 2261:58-62
47. Bates, S. S., C. Leger, M. Satchwell, and G. L. Boyer (2001) The effects of iron on domoic acid production by *Pseudo-nitzschia multiseries*. In: "Harmful Algal Blooms 2000" S.I. Blackburn G.M. Hallegraeff, C.J. Bolch, R.J. Lewis, ed., p. 320-323.
48. Giner, J.-L., X. Li, and G. L. Boyer (2001) Sterol composition of *Aureoumbra legunensis*, the Texas brown tide alga. *Phytochemistry*, 57:787-789.
49. Goddard, G. D., and G. L. Boyer (2001) A comparison of HPLC with electrochemical oxidation, HPLC with chemical oxidation, and the mouse bioassay for the analysis of PSP toxins in shellfish. In: "Harmful Algal Blooms 2000", G.M. Hallegraeff, S.I. Blackburn, C.J. Bolch, R.J. Lewis, ed., p. 261-265.
50. Nichols, D. B., M. F. Satchwell, J. E. Alexander, N. M. Martin, M. T. Baesl, and G. L. Boyer (2001) Iron nutrition in the brown tide algae, *Aureococcus anophagefferens*: Characterization of a ferric chelate reductase activity. In: "Harmful Algal Blooms 2000", G.M. Hallegraeff, S.I. Blackburn, C.J. Bolch, R.J. Lewis, ed., p. 340-343.
51. Baker, T. R., G. J. Doucette, C. L. Powell, G. L. Boyer, and F. G. Plumley (2003) Characterization of fluorescent compounds from *Pseudomonas stutzeri* SF/PS and *Pseudomonas/Altermonas* PTB-1, bacteria associated with *Alexandrium* spp. and paralytic Shellfish Poisoning. *Toxicon* 41:339-347.
52. Giner, J.-L., J.A. Faridos, G.L. Boyer (2003) Unique sterols of the toxic dinoflagellate *Gymnodinium breve* and a proposed defensive function for unusual marine sterols, *J. Phycol.* 39:1-6
53. Satchwell, M. F., and G. L. Boyer (2003) Comparison of three methods for the detection of microcystin cyanobacterial toxins: In "Harmful Algae 2002", K. A. Steidinger, J.H. Landsberg, C.R.Tomas, G. A. Vargo, eds, p 169-171.

54. Boyer, G., M. C. Watzin, A. D. Shambaugh, M. F. Satchwell, B. R. Rosen, and T. Mihuc (2004) The occurrence of cyanobacterial toxins in Lake Champlain. In: "Lake Champlain: partnerships and Research in the New Millennium. (Proceedings of the Lake Champlain Research Consortium, May 20th 2002, Saint-Jean-sur-Richelieu, Quebec" T. Manley, Ed., p 241-257.
55. Lehman, P. W., S. Waller, G. Boyer, and K. Gehrts (2004) Distribution and toxicity of a new *Microcystis aeruginosa* bloom in the upper San Francisco Bay region. Report prepared for NOAA Coastal Ocean Program Monitoring and Event Response for Harmful Algal Blooms. 17 p.
56. Lehman, P., G. Boyer, C. Hall, S. Waller, and K. Gerhrt (2004) Distribution and toxicity of a new colonial *Microcystis aeruginosa* bloom in San Francisco Estuary, California. *Hydrobiology*. 541:87-99.
57. Patchett, E.A. M.F. Satchwell, J. Alexander, and G.L. Boyer (2004) The effects of Iron Limitation on Growth and PSP toxin Production in *Alexandrium fundyense*. In "Harmful Algae 2002", K. A. Steidinger, J.H. Landsberg, C.R.Tomas, G. A. Vargo, eds, p 213-215.
58. Atkinson, J. F., M. L. Green, M. Sultan, R. Becker, and G. L. Boyer (2005) Monitoring algal blooms in the Great Lakes. In: "Proceedings, 8th International Conference of Remote Sensing for Marine and Coastal Environments, Halifax, Nova Scotia, Canada, May 17-19, 2005" missing page numbers
59. Becker, R., M. Sultan, J. Atkinson, G. Boyer, and E. Konopko (2005) Spatial and temporal variations of algal blooms in the lower Great Lakes. In: "Proceedings, 8th International Conference on Remote Sensing for marine and Coastal Environments, Halifax, Nova Scotia, Canada, May 17-19, 2005 missing page numbers
60. Hotto, A., M. Satchwell, and G. Boyer (2005) Seasonal Production and Molecular Characterization of Microcystins in Oneida Lake, New York, USA. *Environmental Toxicology*. 20:243-248.
61. Gobler, C. J., D. J. Lonsdale, and G. L. Boyer (2005) A review of causes, effects, and potential management of harmful brown tide blooms caused by the alga *Aureococcus anophagefferens* (Hargraves et Sieburth). *Estuaries*. 28:726-749
62. Mihuc, T. B., G. L. Boyer, M. F. Satchwell, M. Pellam, J. Jones, J. Vasile, A. Bouchard, and R. Bonham (2005) 2002 Phytoplankton community composition and cyanobacterial toxins in Lake Champlain, U.S.A. *Verh. Internat. Verein. Limnol.* 39:328-333
63. Rinta-Kanto, J. M., A. J. A. Ouellette, M. R. Twiss, G. L. Boyer, T. Bridgeman, and S. W. Wilhelm (2005) Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in western Lake Erie using quantitative real-time PCR. *Environ. Sci. Technol.* 39:4198-4205.
64. Zou, G., and G. L. Boyer (2005) Synthesis and Properties of different metal Complexes of the siderophore desferri-ferricrocin. *Biometals*, 18:63-74
65. Boyer, G. (2006) Toxic Cyanobacteria in Large Lake Ecosystems. *LakeLine*.26(2):36-39
66. Boyer, G. L. (2006) Introduction. In: GLRC Great Lakes Research Review. 7:1.
67. Boyer, G. L. (2006) Toxic Cyanobacteria in the Great Lakes: More than just the Western Basin of Lake Erie. *GLRC Great Lakes Research Review*. 7:2-7.
68. Becker, R., M. Sultan, G. Boyer, and E. Konopko (2006) Mapping variations of algal blooms in the Lower Great Lakes. *GLRC Great Lakes Research Review*. 7:14-17.
69. Makarewicz, J. C., G. L. Boyer, W. Guenther, M. Arnold, and T. W. Lewis (2006) The occurrence of cyanotoxins in the nearshore and coastal embayments of Lake Ontario. *GLRC Great Lakes Research Review*. 7:25-31.
70. Mihuc, T. B., G. L. Boyer, J. Jones, M. F. Satchwell, and M. C. Watzin (2006) Lake Champlain phytoplankton and algal toxins: past and present. *GLRC Great Lakes Research Review*. 7:18-21.

71. Boyer, G. L. (2007) The occurrence of Cyanobacterial toxins in New York lakes: Lessons for the MERHAB-Lower Great lakes program. *Lake Reservoir Management*. 23: 153-160.
72. Boyer, G. L., E. Konopko, and H. Gilbert (2007) Rapid field-based monitoring systems for the detection of Toxic cyanobacteria blooms, ImmunoStrips and Fluorescence-based monitoring systems. In: "Proceedings, 12th International Conference Harmful Algae" Moestrup et al., Ed., Springer, pp. 341-343.
73. Gobler, C. J., T. W. Davis, K. J. Coyne, and G. L. Boyer (2007) Interactive influences of toxin expression, nutrient loading and zooplankton grazing on the growth and toxicity of cyanobacteria blooms in eutrophic Lake Agawam, New York. *Harmful Algae*, 6:119-133.
74. Hotto, A. M., M. F. Satchwell, and G. L. Boyer (2007) Characterization of Lake Ontario Embayments for potential microcystin production reveals a unique mcyA genotype. *Appl. Environ. Microbiol.* 73(14):4570-4578
75. Howard, K. L., and G. L. Boyer (2007) Adduct simplification in the analysis of cyanobacterial toxins by matrix-assisted laser desorption/ionization mass spectrometry. *Rapid Comm. Mass Spectrom.* 21:699-706
76. Howard, K. L., and G. L. Boyer (2007) Quantitative analysis of cyanobacterial toxins by Matrix-assisted Laser Desorption/Ionization mass spectrometry. *Analytical Chemistry*. 79:5980-5986
77. Richardson, L. L., R. Sekar, J. L. Myers, M. Gantar, J. D. Voss, L. Kaczmarek, E. R. Remily, G. L. Boyer, and R. V. Zimba (2007) Production of the cyanobacterial toxin microcystin in black band disease of corals. *FEMS Microbiology Letters*. 272: 182-187
78. Boyer, G. L. (2008) Cyanobacterial Toxins in New York and the Lower Great Lakes Ecosystems. In: "Proceedings of the Interagency International Symposium on Cyanobacterial Harmful Algal Blooms" H. K. Hudnell, Ed., *Adv. Exp. Med. Biol.*, Vol 619 pp 151-163.
79. Fristachi, A., J. L. Sinclair, J. A. Hambrook-Berkman, G. Boyer, J. Burkholder, J. Burns, W. Carmichael, A. du Four, W. Frazier, S. L. Morton, E. O'Brien, and S. Walker (2008) Occurrence of Cyanobacterial Harmful Algal Blooms working group report. In: "Proceedings of the Interagency International Symposium on Cyanobacterial Harmful Algal Blooms" H. K. Hudnell, Ed., *Adv. Exp. Med. Biol.*, Vol 619 pp. 37-97
80. Gouvea, S. P., G. L. Boyer, and M. R. Twiss (2008) Influence of ultraviolet radiation, copper, and zinc on microcystin content in *Microcystis aeruginosa* (Cyanobacteria). *Harmful Algae*.7:194-205.
81. Hotto, A. M., M. F. Satchwell, D. L. Berry, C. J. Gobler, and G. L. Boyer (2008) Spatial and temporal diversity of microcystins and microcystin-producing genotypes in Oneida Lake, NY. *Harmful Algae*. 7: 671-681.
82. Lehman, P., G. L. Boyer, M. F. Satchwell, and S. Waller (2008) The influence of environmental conditions on the seasonal variation of *Microcystis aeruginosa* cell density and microcystins concentration in the San Francisco Estuary. *Hydrobiology*, 600:187-204.
83. Mihuc, T. B., C. Pershyn, S. Thomas, G. Boyer, M. Satchwell, J. Jones, E. Allen, and M. Greene (2008) Cyanobacteria and the sixth Great lake: community dynamics of toxic algal blooms in Lake Champlain, USA. *Verh. Internat. Verein. Limnol.* 30:312-317.
84. Smith, J. L., G. L. Boyer, E. Mills, and K. L. Schulz (2008) Toxicity of microcystin-LR, a cyanobacterial toxin, to multiple life stages of the burrowing mayfly, *Hexagenia*, and possible implications for recruitment. *Aquat. Toxicol.*, 23(4) 499-506
85. Smith, J. L., G. L. Boyer, and P. V. Zimba (2008) A review of cyanobacterial odorous and bioactive metabolites: Impacts and management alternatives in aquaculture. *Aquaculture*. 280:5-20.

86. Watson, S. B., G. Boyer, and J. Ridal (2008) Taste and odour and cyanobacterial toxins: Impairment, prediction and management in the Great Lakes. *Can J Fish Aquat Sci.*, 65(8): 1779-1796.
87. Allender, C. J., G. R. LeCleir, J. M. Rinta-Kanto, R. L. Smith, M. F. Satchwell, G. L. Boyer, and S. W. Wilhelm (2009) Identifying the source of unknown microcystin genes and predicting microcystin variants by linking multiple genes within uncultured cyanobacterial cells. *Appl. Environ. Microbiol.* 75:3598-3604.
88. Becker, R.H., Sultan, M.I., Boyer, G.L., Twiss, M.R., and Konopko, E. (2009) Mapping cyanobacterial blooms in the Great Lakes using MODIS, *J. Great Lakes Res.* 35(3):447-453.
89. Boyer, G. L. (2009) Algal Toxins and their Detection. In: "Shellfish Safety" S. Shumway and G.E. Rodrick, Eds., Woodhead Publishing, New York NY, p. 129-161.
90. Cusick, K. D., G. L. Boyer, S. W. Wilhelm, and G. S. Sayler (2009) Transcriptional profiling of *Saccharomyces cerevisiae* upon exposure to saxitoxin. *Environ. Sci. Technol.* 43(15) 6039-6045.
91. Davis, T. W., D. L. Berry, G. L. Boyer, and C. J. Gobler (2009) The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae*, 9:715-725
92. Giner, J., H. Zhao, G. L. Boyer, M. F. Satchwell, and R. A. Anderson (2009) Sterol chemotaxonomy of marine Pelagophyte algae. *Chemistry & Biodiversity*, 6:1111-1130.
93. Makarewicz, J. C., G. L. Boyer, T. W. Lewis, W. Guenther, J. Atkinson and M. Arnold (2009) Spatial and temporal distribution of the cyanotoxin microcystin in Lake Ontario ecosystem: Coastal embayments, rivers, nearshore and offshore and upland lakes. *J. Great Lakes Res.* 35(supp 1) 83-89
94. Rinta-Kanto, J. M., E. A. Konopko, J. M. DeBruyn, R. A. Bourbonniere, G. L. Boyer, and S. W. Wilhelm (2009) Lake Erie *Microcystis*: relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. *Harmful Algae* 8:665-673
95. Rinta-Kanto, J. M., M. A. Saxton, J. M. DeBruyn, J. L. Smith, C. H. Marvin, K. A. Krieger, G. L. Boyer, and S. W. Wilhelm (2009) The diversity and distribution of toxigenic *Microcystis* spp. in present day and archived pelagic and sediment samples from Lake Erie. *Harmful Algae*. 8:385-394.
96. Smith, J. L., and G. L. Boyer (2009) Standardization of microcystin extraction from fish tissues: A novel internal standard as a surrogate for polar and non-polar variants. *Toxicol.* 53(2) 238-245.
97. Lehman, P. W., S. J. Teh, G. L. Boyer, M. L. Nobriga, E. Bass, and C. Hogle (2009) Initial impacts of *Microcystis aeruginosa* blooms on the aquatic food web in the San Francisco Estuary. *Hydrobiologia*. 637:229-248.
98. Smith, J. L., K. L. Schulz, P. V. Zimba, and G. L. Boyer (2010) Possible mechanism for the foodweb transfer of covalently-bound microcystins. *Ecotoxicology and Environm. Safety*. 73:757-761
99. Rogers, E. D., T. B. Henry, M. J. Twiner, J. S. Gouffon, J. T. McPherson, G. L. Boyer, G. S. Sayler, and S. W. Wilhelm (2011) Global gene expression profiling in larval zebrafish exposed to microcystin-LR and *Microcystis* reveals endocrine disrupting effects of cyanobacteria. *Environ. Sci. Technol.* 45(5) 1962-1969.
100. Wilhelm, S. W., S. E. Farnsley, G. R. LeCleir, A. C. Layton, M. F. Satchwell, J. M. DeBruyn, G. L. Boyer, G. Zhu, and H. W. Paerl (2011) The relationships between nutrients, cyanobacterial toxins and the microbial community in Taihu (Lake Tai), China. *Harmful Algae*, 10:207-215.
101. Wilhelm SW and GL Boyer (2011) Healthy competition. *Nature Climate Change* 1: 300-301.
102. Thessen, A. E., L. Clough, A. Whiting, H. Bowers and G. L. Boyer (2012) A recurring bloom of toxic marine cyanobacteria above the Arctic Circle. *Harmful Algal News*, 46 (June):12-15.

103. Pavlac, M. M., T. T. Smith, S. P. Thomas, J. C. Makarewicz, T. W. Lewis, W. J. Edwards, C. M. Pennuto, C. P. Basiliko, J. F. Atkinson, and G. L. Boyer (2012) Assessment of phytoplankton distribution in the nearshore zone using continuous *in situ* fluorometry. *J. Great Lakes Res.* 38(suppl 4) 78-84.
104. Makarewicz, J. C., T. W. Lewis, W. J. Edwards, C. Pennuto, G. L. Boyer, and G. Thomas (2012) Physical and chemical characteristics of the nearshore zone of Lake Ontario. *J. Great Lakes Res.* 38(suppl 4) 21-3.
105. Makarewicz, J. C., T. W. Lewis, G. L. Boyer and W.J. Edwards (2012) The influence of streams on nearshore water chemistry, Lake Ontario. *J. Great Lakes Res.* 38(suppl 4) 62-71.
106. Makarewicz, J. C., T. W. Lewis, and G. L. Boyer (2012) Nutrient enrichment and depletion on the shoreside of the spring thermal front. *J. Great Lakes Res.* 38(suppl 4) 72-76.
107. Steffen, M. M., Z. Li., T. C. Effler, L. J. Hauser, G. L. Boyer, and S. Wilhelm (2012) Comparative metagenomics of toxic freshwater cyanobacteria bloom communities on two continents. *PLoS ONE.* 7(8) e44002.
108. Allan, J. D., P. B. McIntyre, S. D. P. Smith, B. S. Halpern, G. Boyer, A. Buchsbaum, A. Burton, L. Campbell, L. Chadderton, J. Ciborowski, P. Doran, T. Eder, D. M. Infante, L. B. Johnson, C. G. Joseph, A. L. Marino, A. Prusevich, J. Read, J. Rose, E. Rutherford, S. Sowa, and A. Steinman (2013) Joint analysis of stressors and ecosystems services to enhance restoration effectiveness. *Proc. Natl Acad. Sci (USA).* 110(1) 372-377.
109. Schmidt, J.R., M. Shaskus, F. F. Estenik, C. Oesch, R. Khidekel, and G. L. Boyer (2013) Variations in the microcystin content of different fish species collected from a eutrophic lake. *Toxins* 5:992-1009
110. Lehman, P. W., K. Marr, G.L. Boyer, S. Acuna, and S. J. Teh (2013) Long term trends and causal factors associated with *Microcystis* abundance and toxicity in San Francisco Estuary and implications for climate change impacts. *Hydrobiologia* 718:141-158.
111. Kring, S. A. S.E. Figary, G. L. Boyer, S. B. Watson, and M. R. Twiss (2014) Rapid *in situ* measures of phytoplankton communities using the bbe FluoroProbe: evaluation of spectral calibration, instrument intercompatibility and performance range. *Can J Fish Aquat Sci* 71(7): 1087-1095. *doi 10.1139/cjfas-2013-0599*
112. Steffen, M. M., B. S. Belisle, S. B. Watson, G. L. Boyer, and S.W. Wilhelm (2014) Status, Causes, and Controls of Cyanobacterial Blooms in Lake Erie. *J. Great Lakes Research* 40 (2014) 215–225. (Corrigendum 40:466-467)
113. Davis, T.W., P. T. Orr, G. L. Boyer, M. A. Burford (2014) Investigating the production and release of cylindrospermopsin and deoxy-cylindrospermopsin by *Cylindrospermopsis raciborskii* over a natural growth cycle. *Harmful Algae*, 31:18-25.
114. Lehman, P. W., C. Kendall, M. A. Guerin, M. B. Young, S. R. Silva, G. L. Boyer and S. J. Teh. (2014) Characterization of the *Microcystis* bloom and its nitrogen supply in San Francisco Estuary using stable isotopes. *Estuaries and Coasts*, *doi 10.0007/S12237-014098811*
115. Kapuscinski, K. L., J. M. Farrell, S. V. Stehman, G. L. Boyer, D. D. Fernando, M. A. Teece and T. J. Tschaplinski (2014) Selective herbivory by an invasive cyprinid, the Rudd *Scardinius erythrophthalmus*. *Freshwater Biology*, 59: 2315-2327. *doi 10.1111/fwb.12433*.
116. Smith S. D.P., P.B. McIntyre, B. S. Halpern, R. M. Cooke, A.L. Marino, G. L. Boyer, A. Buchsbaum, G.A. Burton Jr., L. M. Campbell, J.J.H. Ciborowski, P. J. Doran, D. M. Infante, L. B. Johnson, J. G. Read, J. B. Rose, E. S. Rutherford, A. D. Steinman, and J. D. Allan (2014) Rating

- impacts in a multi-stressor world: a quantitative assessment of 50 stressors affecting the Great Lakes, *Ecological Applications*, 25(3), 717–728. <http://dx.doi.org/10.1890/14-0366.1>
117. Schmidt, J.R., S.W. Wilhelm and G.L. Boyer (2014) The fate of microcystins in the environment and challenges for monitoring. *Toxins* 6:3354-3387.
  118. Steffen, M.M., B S Belisle, S.B. Watson, G.L. Boyer, R.A. Bourbonniere, and S.W. Wilhem (2015) Metatranscriptomic evidence for co-occurring top-down and bottom-up controls on toxic cyanobacterial communities. *Appl. Environ. Microbiol.* 81(9):3268-3276.
  119. Cale, J.A., S.A. Teale, M.T. Johnson, G.L. Boyer, K.A. Perri and J.D. Castello (2015) New ecological and physiological dimensions of beech bark disease development in aftermath forests. *Forest Ecology and Management* 336:99–108.
  120. Perri, K. A., J. M. Sullivan and G. L. Boyer (2015) Harmful Algal blooms in Sodus Bay, Lake Ontario: a comparison of nutrients, shoreline use and cyanobacterial toxins. *J Great Lake Res.* 41(2), 326-337.
  121. Boyer, G, S. Kishbaugh, M. Perkins and N. Mueller (2015) The New York State citizen-based monitoring program for cyanobacteria toxins. *In: A. Lincoln MacKenzie [Ed]. Marine and Freshwater Harmful Algae 2014. Proceedings of the 16th International Conference on Harmful Algae, October 24th, 2014, Wellington New Zealand, pp250-253.*
  122. Perri K, S. B. Watson and G.L. Boyer (2015) Production of Siderophores by Freshwater Cyanobacteria in the Lower Laurentian Great Lakes. *In: A. Lincoln MacKenzie [Ed]. Marine and Freshwater Harmful Algae 2014. Proceedings of the 16th International Conference on Harmful Algae, October 24th, 2014, Wellington New Zealand, pp122-125.*
  123. Bullerjahn, G. S., R.M. McKay, T.W. Davis, D.B. Baker, G.L. Boyer, L.V. D’Anglada, G.J. Doucette, J.C. Ho, E.G. Irwin, C.L. Kling, R.M. Kudela, R.Kurmayer, J.D. Ortiz, T.G. Otten, H.W. Paerl, B. Qin, B.L. Sohngen, R.P. Stumpf, P.M. Visser and S.W. Wilhelm (2016) Global solutions for regional problems: collecting global expertise to address the problem of harmful algal blooms. A Lake Erie case study. *Harmful Algae*, 54:223-238
  124. Carmichael, W.W. and G.L. Boyer (2016) Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes, *Harmful Algae*, 54:194-212
  125. Watson S.B., C. Miller, G. Arhonditsis, G.L. Boyer, W. Carmichael, M. Charlton, R. Confesor, D. C. Depew, T.O. Höök, S. Ludsin, G. Matisoff, S.P. McElmurry, M.W. Murray, P. Richards, Y. R. Rao, M. Steffen, and S. Wilhelm (2016) The re-eutrophication of Lake Erie: Harmful algal blooms and hypoxia. *Harmful Algae*, 56 (2016) 44–66.
  126. Belisle, B.S, M.M. Steffen, L. L. Pound, J. M DeBeBruyn, S.B. Watson, R.A. Bourbonniere, G.L. Boyer and S.W. Wilhelm (2016) Urea in Lake Erie: Organic nutrient sources as potentially important drivers of phytoplankton biomass? *J. Great Lake Res.*, 42 (2016) 599–607.
  127. Watson, S. B., A. Zastepa, G.L. Boyer, R. Yang, E. Matthews, and B. Newbold (2016) Algal Bloom Response and risk management: on-site response tools. *submitted to Toxicon*
  128. Krausfeldt, L.E., X. Tang, J. van de Kamp, G. Gao, L. Bodrossy, G.L. Boyer, and S.W. Wilhelm (2016) Spatial and temporal variability in the phylogeny and function of nitrogen cyclers in Lake Taihu. *for submission to: FEMS Microbiology Ecology*.
  129. Jones D. N., M. M. Woller-Skar, G. L. Boyer, J. Lankton, and A.L. Russell (20--) Trophic Transfer of Microcystin from a Freshwater Lake to Little Brown Bats (*Myotis lucifugus*). *for submission to: PLOS one*.

### **TECHNICAL REPORTS:**

1. Watson, S.B., G. Boyer, M. Charlton, J. Ridal (2007) Biological metabolite impairments in Great Lakes Areas of Concern, Technical Report Prepared for the Bay of Quinte, Area of Concern, 15p.
2. Watson, S.B. and G.L. Boyer (2011) Harmful Algal Blooms (HABS) in the Great Lakes: current status and concerns, Technical report prepared for the International Joint Commission.
3. Watson, S.B., Boyer, G.L., Newbold, B., Matthews, E., Yang, R., 2012. Algal Bloom Response and risk management: evaluation of on-site toxin kits in Hamilton Harbour. In: RAP Report, Hamilton Harbour. NWRI Report. Canadian Centre for Inland Waters, Burlington, ON, pp. 24.
4. Watson, S. and G. Boyer (2014) *Indicator 3.4.5 Harmful and Nuisance Algae* pp 46-48 *In: Great Lakes Ecosystem Indicator Project Report, International Joint Commission, available online at <http://www.ijc.org/files/publications/Ecosystem%20Indicators%20-Final.pdf> .*
5. Watson, S.B. and G.L. Boyer (2011) Harmful Algal Blooms (HABS). pp 296-305, *In: State of the Great Lakes, 2011, Indicators to assess the status and trends of the Great Lakes Ecosystem, Environment Canada and the U.S. Environmental Protection Agency, 2014), available online at <http://binational.net/wp-content/uploads/2014/11/sogl-2011-technical-report-en.pdf>*
6. International Joint Commission, Science Advisory Board (2013) Taking Action on Lake Erie, A Science Summary Report prepared by the TAcLE Work Group as part of the Lake Erie Ecosystem Priority (LEEP). S.B. Watson and D Carpenter, Co-chairs, Available online at: <http://www.ijc.org/files/tinymce/uploaded/TAcLE%20Summary%20Report%20FINAL.pdf>
7. Watson, S.B. and G.L. Boyer (2016) Harmful and Nuisance Algal Blooms (HABS). *In: State of the Great Lakes, 2016, Indicators to assess the status and trends of the Great Lakes Ecosystem, Environment Canada and the U.S. Environmental Protection Agency, 2017), available online at <http://binational.net/>.*
8. Watson, S. and G. Boyer (2016) *Indicator Harmful and Nuisance Algae Blooms (HABS) In: Great Lakes Ecosystem Indicator Project Report, International Joint Commission, accepted.*

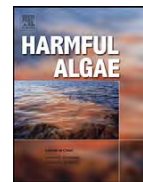
## **Exhibit B**





Contents lists available at ScienceDirect

## Harmful Algae

journal homepage: [www.elsevier.com/locate/hal](http://www.elsevier.com/locate/hal)

## The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms

Timothy W. Davis<sup>a</sup>, Dianna L. Berry<sup>a</sup>, Gregory L. Boyer<sup>b</sup>, Christopher J. Gobler<sup>a,\*</sup>

<sup>a</sup> School of Marine and Atmospheric Sciences, Stony Brook University, Southampton, NY, United States

<sup>b</sup> Chemistry Department, State University of New York, College of Environmental Science and Forestry, Syracuse, NY, United States

## ARTICLE INFO

## Article history:

Received 26 November 2008

Received in revised form 13 February 2009

Accepted 14 February 2009

## Keywords:

Microcystis

Microcystin

Eutrophication

Climate change

Global warming

Nutrient loading

Mcy

Microcystin synthetase

Harmful algal bloom

## ABSTRACT

In temperate latitudes, toxic cyanobacteria blooms often occur in eutrophied ecosystems during warm months. Many common bloom-forming cyanobacteria have toxic and non-toxic strains which co-occur and are visually indistinguishable but can be quantified molecularly. Toxic *Microcystis* cells possess a suite of microcystin synthesis genes (*mcyA–mcyJ*), while non-toxic strains do not. For this study, we assessed the temporal dynamics of toxic and non-toxic strains of *Microcystis* by quantifying the microcystin synthetase gene (*mcyD*) and the small subunit ribosomal RNA gene, 16S (an indicator of total *Microcystis*), from samples collected from four lakes across the Northeast US over a two-year period. Nutrient concentrations and water quality were measured and experiments were conducted which examined the effects of elevated levels of temperatures (+4 °C), nitrogen, and phosphorus on the growth rates of toxic and non-toxic strains of *Microcystis*. During the study, toxic *Microcystis* cells comprised between 12% and 100% of the total *Microcystis* population in Lake Ronkonkoma, NY, and between 0.01% and 6% in three other systems. In all lakes, molecular quantification of toxic (*mcyD*-possessing) *Microcystis* was a better predictor of *in situ* microcystin levels than total cyanobacteria, total *Microcystis*, chlorophyll *a*, or other factors, being significantly correlated with the toxin in every lake studied. Experimentally enhanced temperatures yielded significantly increased growth rates of toxic *Microcystis* in 83% of experiments conducted, but did so for non-toxic *Microcystis* in only 33% of experiments, suggesting that elevated temperatures yield more toxic *Microcystis* cells and/or cells with more *mcyD* copies per cell, with either scenario potentially yielding more toxic blooms. Furthermore, concurrent increases in temperature and P concentrations yielded the highest growth rates of toxic *Microcystis* cells in most experiments suggesting that future eutrophication and climatic warming may additively promote the growth of toxic, rather than non-toxic, populations of *Microcystis*, leading to blooms with higher microcystin content.

© 2009 Published by Elsevier B.V.

### 1. Introduction

Blooms of toxic cyanobacteria are a global public health and environmental concern. Toxic blooms are most commonly formed by *Microcystis*, a well-known producer of the hepatotoxin, microcystin (Carmichael, 1992, 1994; Fleming et al., 2002; Chorus and Bartram, 1999; Pearl, 2008). Long term exposure to microcystin has been associated with severe human health effects, including liver and colorectal cancers (Falconer et al., 1988; Carmichael and Falconer, 1993; Bell and Codd, 1994; Carmichael, 1994; Ito et al., 1997; Chorus and Bartram, 1999; Zegura and Sedmak, 2003). One complexity in field studies of harmful cyanobacteria blooms has been the existence and often co-existence of toxic and non-toxic strains of the same species within

a genus that are morphologically and taxonomically indistinguishable (Otsuka et al., 1999; Fastner et al., 2001; Kurmayer et al., 2002). While it is clear that the occurrence of toxic cyanobacteria blooms around the world have increased during recent decades (Chorus and Bartram, 1999; Hudnell and Dortch, 2008), the underlying causes of such blooms and the factors influencing the dynamics of toxic and non-toxic strains within them are poorly understood.

Cyanobacteria blooms are typically associated with eutrophic and poorly flushed waters (Paerl, 1988; Paerl et al., 2001; Philipp et al., 1991; Carmichael, 1994; Rapala et al., 1997; Oliver and Ganf, 2000). Due to increases in human population density, agriculture, and industrial activities, nutrient loading rates into many freshwater ecosystems has increased (Carpenter et al., 1998). As surface waters become enriched in nutrients, particularly phosphorus (P), there is often a shift in the phytoplankton community towards dominance by cyanobacteria (Smith, 1986; Trimbee and Prepas, 1987; Watson et al., 1997; Paerl and Huisman, 2008). Higher

\* Corresponding author. Tel.: +1 631 632 5043.

E-mail address: [christopher.gobler@stonybrook.edu](mailto:christopher.gobler@stonybrook.edu) (C.J. Gobler).

phosphorus levels have been shown to yield higher microcystin content per cell in some cyanobacteria (Utkilen and Gjølme, 1995; Rapala et al., 1997). However, nitrogen (N) may be equally important in the occurrence of toxic, non-N fixing cyanobacteria blooms, such as *Microcystis* sp. Laboratory studies have indicated that increasing N loads increases the growth and toxicity of this species (Watanabe and Oishi, 1985; Orr and Jones, 1998; Codd and Poon, 1988). Moreover, previous laboratory research suggests toxic strains of *Microcystis* are able to outgrow non-toxic strains at high nitrogen levels (Vézie et al., 2002). However, no field study has examined how nutrients directly affect the growth of toxic and non-toxic strains of cyanobacteria within wild populations.

The burning of fossil fuels and subsequent rise in atmospheric carbon dioxide has caused the earth's surface temperature to increase by approximately 1 °C during the 20th century, with most of the increase having occurred during the last 40 years (IPCC, 2001). In the current century, global temperatures are expected to increase an additional 2–5 °C (Houghton et al., 2001). Frequently, cyanobacteria dominate phytoplankton assemblages in temperate freshwater environments during the warmest periods of the year, particularly in eutrophic systems (Paerl, 1988; Paerl et al., 2001; Paerl and Huisman, 2008; Paul, 2008). Harmful cyanobacteria such as *Microcystis* have been found to have an optimal temperature for growth and photosynthesis at, or above, 25 °C (Konopka and Brock, 1978; Takamura et al., 1985; Robarts and Zohary, 1987; Reynolds, 2006; Jöhnk et al., 2008; Paerl and Huisman, 2008). Furthermore, the cellular toxin content of multiple genera of cyanobacteria increases with increasing temperature to a maximum above 25 °C (Van der Westhuizen and Eloff, 1985; Codd and Poon, 1988; Sivonen, 1990; Rapala et al., 1997). However, the growth response of toxic and non-toxic strains of cyanobacteria to increasing water temperature in an ecosystem setting has yet to be explored.

The purpose of this study was to elucidate the conditions that favor the growth and proliferation of the toxic and non-toxic strains of *Microcystis*. A two-year field study was established in four diverse lake systems across the Northeast US to determine the dynamics of toxic and non-toxic strains of *Microcystis* using molecular quantification of total *Microcystis* cells and *Microcystis* cells possessing the microcystin synthetase gene. Levels of microcystin, nutrients, and other environmental parameters were assessed concurrently. Experiments were conducted to examine the impacts of elevated nutrient concentrations (N and P) and increased temperature on the growth rates of toxic and non-toxic *Microcystis* populations.

## 2. Materials and methods

### 2.1. Study sites

During this study, four sites within the Northeast US were investigated. Lake Champlain lies between New York and Vermont, and is connected to the Richelieu River to the north and the Hudson River to the south. It is the largest lake in the Northeast United States other than the Great Lakes. Lake Champlain serves as a drinking water supply to millions of individuals, has been subjected to eutrophication since the 1970s, and is dominated by cyanobacteria in some regions during warm months (Myer and Gruending, 1979; Shambaugh et al., 1999; Boyer et al., 2004). Our sampling site was Missisquoi Bay (Latitude: 44.62°N; Longitude: 73.37°W), a basin in the northeast extent of Lake Champlain that experiences annual toxic cyanobacteria blooms (Boyer et al., 2004).

Our other sampling sites were located on Long Island, NY, USA, which has recently seen its population expand beyond seven million people. Lake Agawam (Latitude: 40.88°N; Longitude: 72.39°W) is a small (0.5 km<sup>2</sup>), shallow (4 m maximum depth) system which experiences annual toxic cyanobacteria blooms dominated by

*Microcystis* (Gobler et al., 2007). Mill Pond (Latitude: 40.91°N; Longitude: 72.36°W) is a deeper (8m maximum depth), hypereutrophic system (mean chlorophyll *a* = 200 µg L<sup>-1</sup>) which also experiences dense cyanobacteria blooms dominated by *Microcystis* cells during summer months. Finally, Lake Ronkonkoma (Latitude: 40.83°N; Lon: 73.12°W), is the largest body of freshwater on Long Island (area = 1.5 km<sup>2</sup>, maximum depth = 27 m), and experiences summer cyanobacteria blooms comprised of *Microcystis*. In all systems sampled on Long Island, lake transects indicated that blooms were spatially similar with regard to chlorophyll *a*, phycocyanin, and toxin concentrations (data not shown). As such, our sampling sites were representative of each system.

### 2.2. Water quality sampling

Field sampling was conducted bi-weekly before, during, and after cyanobacteria blooms (May–November). In 2005, Lake Ronkonkoma and Lake Agawam were sampled whereas in 2006, Lake Champlain, Lake Agawam, and Mill Pond were studied. At each site, general water quality was evaluated using a handheld YSI 556 sonde to determine surface and bottom temperatures and dissolved oxygen. Twenty liters of surface water was collected and taken to the lab where triplicate chlorophyll *a* and *in vivo* phycocyanin (as a proxy for total cyanobacteria) were measured with Turner Designs fluorometers using standard techniques (Parsons et al., 1984; Watras and Baker, 1988; Lee et al., 1994). For microcystin analysis, whole water was filtered onto triplicate 47 mm GFF glass fiber filters and placed in 5 mL cryovials which were stored at –80 °C until analysis. Water samples were filtered through 0.2 µm capsule filters to obtain samples for dissolved nutrient analysis. Nitrate was analyzed by reducing the nitrate to nitrite using spongy cadmium as per Jones (1984). Nitrite, ammonium, phosphate, and silicate were analyzed using techniques modified from Parsons et al. (1984). Duplicate whole water samples were preserved with Lugol's iodine solution (5% final concentration) to quantify phytoplankton assemblages. For molecular analysis of cyanobacteria, bloom water was filtered onto triplicate 0.22 µm polycarbonate filters, immediately placed in CTAB lysis buffer, and stored at –80 °C.

### 2.3. Impacts of nutrients and temperature on toxic and non-toxic *Microcystis*

On selected dates (Table 3), experiments were conducted to assess the impact of increased nutrient concentrations and temperature on toxic and non-toxic *Microcystis* populations. For each of the six experiments, two sets of 12 triplicate, 1 L bottles (*n* = 24) were filled with surface water from each experimental site and were either left unamended to serve as a control, or amended with nitrogen (20 µM NO<sub>3</sub><sup>-</sup>), phosphorus (1.25 µM orthophosphate), or both N and P in order to determine which nutrient may favor the proliferation of toxic and non-toxic *Microcystis*. One set of bottles (*n* = 12) was placed in an outdoor incubator receiving ambient light and circulating *in situ* water. In order to maintain *in situ* water temperatures, ambient lake water was continuously pumped through the incubation chamber throughout the duration of the experiment (48–72 h). The other set of bottles (*n* = 12) was incubated at natural light levels and circulating water maintained at elevated temperatures (+4 °C above ambient water temperature) achieved with a coupled heater/chiller design using commercially available heaters and chillers (Aquatic Eco-systems, Inc., FL, USA), approximating levels projected for the coming century (+4 °C; Houghton et al., 2001). Exact water temperatures administered during experiments were monitored every minute with *in situ* loggers (Onset Computer Corporation, MA, USA). At the end of the incubation period, aliquots from experimental bottles were filtered for levels of chlorophyll *a* and analyzed for *in vivo*

**Table 1**

A list of primers (Integrated DNA Technologies, IA, USA) and probes (Applied Biosystems, Foster City, CA, USA) used in the qPCR analysis.

DNA target	Primer	Sequence (5'–3')	Reference
pGEM plasmid DNA	M13F	CCCAGTCACGACGTTGTA AAAACG	Coyne et al. (2005)
	pGEMR	TGTGTGGAATTGTGAGCGGA	Coyne et al. (2005)
	pGEM probe	(Taq) FAM <sup>a</sup> -CACTATAGAATCACTCAAGCTTGCATGCCTGCA-BHQ-1 <sup>b</sup>	Coyne et al. (2005)
Microcystis 16S rDNA	184F	GCCGCRAGGTGAAAMCTAA	Neilan et al. (1997)
	431R	AATCCAAARACCTTCCTCC	Neilan et al. (1997)
	Probe	(Taq) FAM-AAGAGCTTGGCTCTGATTAGCTAGT-BHQ-1 <sup>b</sup>	Rinta-Kanto et al. (2005)
Microcystis mcyD	F2	GGTTCGCCTGGTCAAAGTAA	Kaebnick et al. (2000)
	R2	CCTCGCTAAAGAAGGGTTGA	Kaebnick et al. (2000)
	Probe	(Taq) FAM-ATGCTCTAATGCAGCAACGGCAAA-BHQ-1 <sup>b</sup>	Rinta-Kanto et al. (2005)

F: forward primer R: reverse primer.

<sup>a</sup> 6-Carboxyfluorescein.<sup>b</sup> Black Hole Quencher-1 (quenching range 480–580 nm).

phycocyanin. Samples were also filtered at the end of the incubation period as described above to preserve samples for determination of densities of toxic and non-toxic *Microcystis* using molecular methods. Net growth rates of each population were determined as follows:  $\mu = \ln[N_t/N_0]/t$  where  $\mu$  is the rate of population growth ( $d^{-1}$ ),  $N_0$  and  $N_t$  are initial and final cell densities, and  $t$  is the duration of incubation in days.

#### 2.4. Sample analysis

##### 2.4.1. Microscopic analysis

Densities of *Microcystis* and other co-occurring cyanobacteria were quantified using gridded Sedgewick-Rafter and Utermohl counting chambers. Utermohl chambers were used to quantify populations with cell densities that were low. For dense algal populations, a gridded Sedgewick-Rafter chamber allowed for accurate assessment of cell densities without the layering of cells which can occur when high biomass samples are concentrated within an Utermohl chamber. For all samples, at least 200 cells were enumerated. To quantify *Microcystis* as well as *Anabaena*, the number of colonies per chamber, as well as the number of cells in 20 colonies, was determined. Such a counting approach provided good reproducibility (<15% relative standard deviation) on live and preserved samples, as well as precise comparability between live and preserved samples. However, as individual trichomes of *Aphanizomenon* colonies are difficult to visually resolve, only colonies per chamber were counted.

##### 2.4.2. Microcystin analysis

Filters for microcystin analyses were extracted in 50% methanol containing 1% acetic acid using ultrasound (four, 20 second bursts with a 20 second pause between bursts). Previous work has shown that this extraction protocol gives 90% recovery of microcystin-LR (Boyer et al., 2004). Following extraction, the methanolic extract was stored at  $-80^\circ\text{C}$  until analysis. Microcystin concentration was measured using the protein phosphatase inhibition assay (PPIA; Carmichael and An, 1999). This analytical protocol does not allow for specific congeners of microcystin to be distinguished but rather provides an indication of the potential biological impact of the microcystins, specifically their ability to inhibit protein phosphatases (Carmichael and An, 1999). A certified microcystin-LR standard (Alexis Biochemicals, San Diego, USA) was used to create the standards for this analysis. This assay yielded a  $99.5 \pm 8.2\%$  recovery of samples spiked with known amounts of microcystin, a methodological relative standard deviation of 9.4%, and a detection limit between 0.05 and 0.50  $\mu\text{g L}^{-1}$ .

##### 2.4.3. Molecular analyses

Total cellular nucleic acids were extracted from field and experimental samples using methods described in Coyne and Cary

(2005). Filtered environmental or experimental samples were submersed in CTAB buffer (Dempster et al., 1999), supplemented with 20  $\mu\text{g L}^{-1}$  pGEM-3z(f+) plasmid (Promega; Table 1) which served as an internal control for extraction efficiency and PCR inhibition (Coyne et al., 2005). The filters were then flash frozen and stored at  $-80^\circ\text{C}$  until extraction. Nucleic acids were extracted after an initial heating step at  $65^\circ\text{C}$ , followed by a double chloroform extraction, and an isopropanol precipitation. Extracted nucleic acids were resuspended in 20  $\mu\text{L}$  of LoTE buffer. The quantity and quality of nucleic acids was assessed with a NanoDrop 1000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Two *Microcystis*-specific genetic targets were used during this study, the 16S rRNA gene (16S rDNA) and *mcyD* gene. The *Microcystis* 16S rRNA gene is specific to the *Microcystis* genus which allowed us to quantify the abundance of the total *Microcystis* population. The *mcyD* gene is found within the microcystin synthetase gene operon which is responsible for the production of microcystin and is only found in toxic strains of *Microcystis* (Tillett et al., 2000) allowing for the quantification of the toxic *Microcystis* population (Rinta-Kanto et al., 2005). qPCR was carried out using an ABI 7300 Real Time PCR instrument using TaqMan<sup>®</sup> labeled probes (Applied Biosystems) and *Microcystis*-specific *mcyD* and 16S rDNA primers (Table 1). Each 10  $\mu\text{L}$  reaction included 5  $\mu\text{L}$  of  $2\times$  TaqMan<sup>®</sup> Master Mix (Applied Biosystems), 10  $\mu\text{M}$  each primer (Integrated DNA Technologies), 10  $\mu\text{M}$  Taqman<sup>®</sup> probe (Table 1) and 1  $\mu\text{L}$  of a 1:25 dilution of the unknown DNA or standard. For amplification of the pGEM and 16S targets, the cycling conditions were  $95^\circ\text{C}$  for 10 min, followed by 55 cycles of  $95^\circ\text{C}$  for 15 s and  $60^\circ\text{C}$  for 1 min. In order to amplify the *mcyD* gene, the cycling conditions were  $95^\circ\text{C}$  for 10 min, followed by 55 cycles of  $95^\circ\text{C}$  for 15 s, followed by  $50^\circ\text{C}$  for 1 min, then  $60^\circ\text{C}$  for 1 min. To prepare standard samples, cultured *Microcystis aeruginosa*, clone LE-3 (Rinta-Kanto et al., 2005), was enumerated by standard microscopy and collected on polycarbonate filters which were prepared and extracted as outlined above. A standard curve of dilutions of the extracted LE-3 genomic DNA was run with each analytical run to serve as a reference for numbers of toxic *Microcystis* cells. Since some *Microcystis* cells may carry multiple copies of the 16S rDNA gene and *mcyD* gene, data was generally expressed as "cell equivalents" (Rinta-Kanto et al., 2005). The numbers of toxic and total *Microcystis* cells were determined using the  $\Delta\Delta\text{CT}$  method (Livak and Schmittgen, 2001; Coyne et al., 2005). The difference between the number of *mcyD* cell equivalents (toxic cells) and 16S rDNA cell equivalents (total cells) indicated the number of non-toxic cell equivalents (Rinta-Kanto et al., 2005). Toxic *Microcystis* cell abundances were determined for all locations and on all dates. Total *Microcystis* cells (16S rDNA) were quantified for all systems except Lake Agawam 2006 and Mill Pond 2006 when total *Microcystis* cells equivalents were only quantified on the days of experiments.

## 2.5. Statistical analysis

All time series data sets from each location (chlorophyll *a*, phycocyanin, toxic *Microcystis* cell densities, non-toxic *Microcystis* densities, total *Microcystis* densities, total cyanobacteria cells, microcystin concentration, dissolved inorganic phosphorus (DIP), dissolved inorganic nitrogen (nitrate + ammonium = DIN), and temperature) were statistically analyzed using a Pearson's correlation matrix to establish the degree to which individual variables were correlated. Student's *t*-tests were used to determine if the means of different populations (toxic and non-toxic) were significantly different from each other. The effects of nitrogen, phosphorus, and temperature on the net growth rate of toxic and non-toxic *Microcystis* populations were analyzed with three-way ANOVAs with nitrogen, phosphorus, and temperature considered treatment effects ( $\alpha = 0.05$ ). Post-hoc comparisons of significant impacts were elucidated with Tukey's multiple comparison tests. For all results the standard variance presented is  $\pm$  one standard deviation (SD).

## 3. Results

### 3.1. Dynamics of toxic *Microcystis* blooms

#### 3.1.1. Lake Agawam, Long Island, NY

During 2005, Lake Agawam hosted mixed cyanobacteria blooms numerically dominated by *Microcystis* from June through September (Table 2). Blooms contained mean chlorophyll *a* levels of  $97 \pm 28 \mu\text{g L}^{-1}$  (mean  $\pm$  SD) and coincided with peaks of *in vivo* phycocyanin (Fig. 1). Densities of non-toxic *Microcystis* ranged from  $3.9 \times 10^6$  to  $1.0 \times 10^8$  cell equivalents  $\text{L}^{-1}$  during blooms with peak densities achieved on 20 October (Fig. 1). Concurrently, toxic *Microcystis* cell densities (possessing the *Microcystis*-specific *mcyD* gene) ranged from  $5.0 \times 10^2$  to  $5.0 \times 10^5$  cell equivalents  $\text{L}^{-1}$  (Fig. 1), peaking on 21 June and representing between 0.01% and 1.56% of total *Microcystis* cells. Microcystin was detectable in the water column from May through October ranging from  $0.86$  to  $11.8 \mu\text{g L}^{-1}$ , with the highest levels occurring at the peak in toxic *Microcystis* cell equivalents (Fig. 1). Concentrations of microcystin were significantly correlated with levels of toxic *Microcystis* cell equivalents ( $p < 0.05$ ). DIN levels during the summer months (July–August) ranged from  $2.67$  to  $6.52 \mu\text{M}$ , while DIP concentrations never exceeded  $0.38 \pm 0.08 \mu\text{M}$  (mean =  $0.25 \pm 0.06 \mu\text{M}$ ; Fig. 1). During September when algal biomass levels declined, DIN and DIP levels increased (Fig. 1). Temperatures in Lake Agawam during 2005 rose from  $13^\circ\text{C}$  in May to  $29^\circ\text{C}$  in August, and then declined to  $15^\circ\text{C}$  in late October.

During 2006, the temporal dynamics of cyanobacterial blooms in Lake Agawam differed from 2005. Lake Agawam hosted cyanobacteria blooms dominated by *Anabaena* during June and early July with maximal concentrations reaching  $2.79 \times 10^8$  cells  $\text{L}^{-1}$  (Table 2). This bloom was succeeded by a bloom of *Microcystis* during mid-July which remained dominant through October (Table 2). *Aphanizomenon* was present throughout the sampling period but was never dominant (Table 2). Cyanobacteria blooms contained mean chlorophyll *a* levels of  $209 \pm 17.0 \mu\text{g L}^{-1}$  and coincided with peaks of *in vivo* phycocyanin (Fig. 2). Densities of toxic *Microcystis* ranged from  $5.3 \times 10^3$  to  $3.5 \times 10^6$  cell equivalents  $\text{L}^{-1}$ , peaking on 10 October (Fig. 2). Particulate microcystin was detectable throughout the study period ranging from  $0.69$  to  $81.1 \mu\text{g L}^{-1}$ , with the highest levels occurring concurrently with the peak in toxic *Microcystis* cell equivalents (Fig. 2). In a manner similar to 2005, microcystin concentrations were significantly correlated with toxic *Microcystis* cell equivalents ( $p < 0.001$ ). DIN levels during the bloom months (June–October) ranged from  $3.03$  to  $48.81 \mu\text{M}$  and were inversely related to phycocyanin concentrations, being higher before and after bloom

events (Fig. 2). During June and early July DIP concentrations were  $1.54 \pm 0.44 \mu\text{M}$  but rose later in the sampling season (Fig. 2). Surface temperatures in 2006 ranged from  $22^\circ\text{C}$  in June to  $29^\circ\text{C}$  in July, declining to  $13^\circ\text{C}$  by October.

#### 3.1.2. Lake Ronkonkoma, Long Island, NY

Compared to the other systems examined, Lake Ronkonkoma was the only system which hosted cyanobacteria blooms that were first dominated by *Microcystis* and were succeeded by *Anabaena* (Table 2). *Aphanizomenon* was either absent, or at low densities in Lake Ronkonkoma (i.e.  $< 50,000$  colonies  $\text{L}^{-1}$ ; Table 2). Blooms (June–August) contained mean chlorophyll *a* levels of  $20.2 \pm 8.0 \mu\text{g L}^{-1}$  and coincided with peaks of *in vivo* phycocyanin (Fig. 3). May through June *Microcystis*-dominated blooms (Table 2) were comprised almost exclusively of toxic cells ( $1.98 \times 10^8$  cell equivalents  $\text{L}^{-1}$  maximum on 27 May; Fig. 3), until late June bloom composition shifted toward dominance by non-toxic cells (peak densities  $1.20 \times 10^6$  cell equivalents  $\text{L}^{-1}$  on 25 July) before declining during the late summer (Fig. 3). Through the sampling period, toxic *Microcystis* cell equivalents comprised between 12% and 100% of the total *Microcystis* population and microcystin levels ranged from  $1.28$  to  $78.8 \mu\text{g L}^{-1}$  (Fig. 3). In a manner similar to Lake Agawam, there was a significant correlation between microcystin concentrations and toxic *Microcystis* cell equivalents ( $p < 0.001$ ). Nutrient concentrations during the bloom months (June–September) ranged from  $4.17$  to  $11.1 \mu\text{M}$  DIN and  $0.19$  to  $0.71 \mu\text{M}$  DIP (Fig. 3). Finally, surface temperatures ranged from  $16^\circ\text{C}$  in May to  $30^\circ\text{C}$  in August and decreased to  $25^\circ\text{C}$  by September.

#### 3.1.3. Mill Pond, Long Island, NY

Cyanobacterial bloom dynamics within Mill Pond during the summer of 2006 were similar to that of Lake Agawam during the same year. Mill Pond hosted cyanobacteria blooms dominated by *Anabaena* and *Aphanizomenon* from June through early July (Table 2). This bloom subsided and a bloom of *Microcystis* occurred and remained dominant throughout the summer and early fall (Table 2). Blooms (June–October) contained mean chlorophyll *a* levels of  $96.9 \pm 57.3 \mu\text{g L}^{-1}$  and coincided with peaks of *in vivo* phycocyanin (Fig. 4). Densities of toxic *Microcystis* ranged from  $3.3 \times 10^3$  to  $4.1 \times 10^6$  cell equivalents  $\text{L}^{-1}$ , achieving the highest concentration on 12 October coinciding with the highest levels of microcystin (Fig. 4). Microcystin levels ranged from  $5.50$  to  $154 \mu\text{g L}^{-1}$ , and were significantly correlated with toxic *Microcystis* cell equivalents during the sampling period ( $p < 0.001$ ). DIN concentrations varied widely ( $6.82$ – $80.9 \mu\text{M}$ ), with the highest concentrations present during late spring and fall (Fig. 4). DIP concentrations in Mill Pond were also elevated with a mean concentration of  $4.93 \pm 0.06 \mu\text{M}$  (Fig. 4). Surface temperatures in this system ranged from  $22^\circ\text{C}$  in June to  $28^\circ\text{C}$  in July declining to  $12^\circ\text{C}$  by October.

#### 3.1.4. Lake Champlain

Lake Champlain's Missisquoi Bay was devoid of cyanobacteria until July and was never numerically dominated by *Microcystis* in 2006 (Table 2). A cyanobacteria bloom which occurred during late summer contained mean chlorophyll *a* levels of  $52.0 \pm 27.5 \mu\text{g L}^{-1}$ , coincided with peaks of *in vivo* phycocyanin (mid-July through mid-October; Fig. 5), and was dominated by *Anabaena* at a density of  $4.91 \pm 0.21 \times 10^7$  cells  $\text{L}^{-1}$  on 1 August (Table 2). *Microcystis* was present in the water column from July through October (Table 2) with densities of non-toxic *Microcystis* ranging from  $1.4 \times 10^4$  to  $2.1 \times 10^7$  cell equivalents  $\text{L}^{-1}$  during blooms (peak densities on 1 August; Table 2). Toxic *Microcystis* cells were detectable from August through September ( $0.24$ – $9.79 \times 10^5$  cell equivalents  $\text{L}^{-1}$ ; Fig. 5) representing approximately 6% of total *Microcystis* cells. Microcystin was present in the water column from May through October at levels that were lower than other systems ( $0.10$ – $1.95 \mu\text{g L}^{-1}$ , Fig. 5) and

**Table 2**

Mean cyanobacterial densities (cells<sup>+</sup> or colonies<sup>\*\*</sup> L<sup>-1</sup>) (SD in parentheses) for all systems sampled. Counts were made using light microscopy. Dashed lines indicate samples were not available.

	Microcystis <sup>+</sup>	Anabaena <sup>+</sup>	Aphanizomenon <sup>**</sup>
<b>Lake Agawam 2005</b>			
26-May-05	68,666,000 (3,433,000)	1,100,000 (141,000)	0 (0)
7-Jun-05	–	14,667,000 (1,604,000)	0 (0)
21-Jun-05	28,300,000 (1,415,000)	700,000 (141,000)	0 (0)
5-Jul-05	53,300,000 (2,665,000)	8,733,000 (2,810,000)	30 (0)
18-Jul-05	58,700,000 (2,935,000)	17,400,000 (3,504,000)	0 (0)
1-Aug-05	60,600,000 (3,030,000)	0 (0)	0 (0)
12-Aug-05	201,300,000 (10,065,000)	3,467,000 (611,000)	140,000 (14,000)
23-Aug-05	52,000,000 (2,600,000)	3,067,000 (1,007,000)	260,000 (30,000)
20-Sep-05	2,000,000 (100,000)	1,700,000 (141,000)	0 (0)
13-Oct-05	–	–	–
20-Oct-05	–	–	–
8-Nov-05	–	–	–
<b>Lake Agawam 2006</b>			
2-Jun-06	76,945,000 (3,847,000)	279,000,000 (13,950,000)	520,000 (26,000)
16-Jun-06	1,200,000 (60,000)	45,500,000 (2,275,000)	30,000 (2,000)
22-Jun-06	55,491,000 (2,775,000)	201,500,000 (10,075,000)	0 (0)
27-Jun-06	3,755,000 (188,000)	265,000,000 (13,250,000)	370,000 (19,000)
6-Jul-06	14,850,000 (743,000)	60,000,000 (3,000,000)	30,000 (2,000)
11-Jul-06	122,960,000 (6,148,000)	28,500,000 (1,425,000)	90,000 (5,000)
19-Jul-06	693,000,000 (34,650,000)	50,000,000 (2,500,000)	390,000 (20,000)
25-Jul-06	429,418,000 (21,471,000)	8,500,000 (425,000)	340,000 (17,000)
10-Aug-06	1,483,227,000 (74,161,000)	15,000,000 (750,000)	570,000 (29,000)
12-Sep-06	76,691,000 (3,835,000)	35,000,000 (1,750,000)	140,000 (7,000)
28-Sep-06	625,527,000 (31,276,000)	12,500,000 (625,000)	150,000 (8,000)
10-Oct-06	436,582,000 (21,829,000)	15,000,000 (750,000)	0 (0)
24-Oct-06	332,000,000 (16,600,000)	16,500,000 (825,000)	40,000 (2,000)
<b>Lake Ronkonkoma 2005</b>			
27-May-05	1,000,000 (0)	0 (0)	0 (0)
8-Jun-05	113,000,000 (6,062,000)	0 (0)	0 (0)
23-Jun-05	–	–	–
5-Jul-05	67,667,000 (6,506,000)	8,000,000 (1,000,000)	0 (0)
25-Jul-05	7,000,000 (2,000,000)	9,500,000 (1,000,000)	25,000 (7,000)
5-Aug-05	2,000,000 (0)	2,500,000 (0)	50,000 (10,000)
18-Aug-05	4,000,000 (0)	5,167,000 (764,000)	20,000 (10,000)
15-Sep-05	–	–	–
<b>Lake Champlain 2006</b>			
22-May-06	0 (0)	0 (0)	0 (0)
19-Jun-06	293,000 (15,000)	0 (0)	0 (0)
3-Jul-06	0 (0)	0 (0)	0 (0)
17-Jul-06	953,000 (48,000)	1,067,000 (53,000)	0 (0)
1-Aug-06	11,480,000 (574,000)	41,867,000 (2,093,000)	213,000 (11,000)
19-Sep-06	1,027,000 (51,000)	2,400,000 (120,000)	5,893,000 (295,000)
3-Oct-06	200,000 (10,000)	0 (0)	80,000 (4,000)
17-Oct-06	60,000 (3,000)	0 (0)	0 (0)
<b>Mill Pond 2006</b>			
2-Jun-06	9,082,000 (454,000)	27,000,000 (1,350,000)	1,050,000 (53,000)
16-Jun-06	18,600,000 (930,000)	78,500,000 (3,925,000)	1,970,000 (99,000)
22-Jun-06	22,636,000 (1,132,000)	46,000,000 (2,300,000)	620,000 (31,000)
27-Jun-06	5,155,000 (258,000)	0 (0)	840,000 (42,000)
6-Jul-06	61,600,000 (3,080,000)	3,000,000 (150,000)	13,970,000 (699,000)
11-Jul-06	244,873,000 (12,244,000)	0 (0)	490,000 (25,000)
19-Jul-06	268,736,000 (13,437,000)	0 (0)	2,370,000 (119,000)
25-Jul-06	181,909,000 (9,095,000)	1500,000 (75,000)	4,410,000 (221,000)
10-Aug-06	264,955,000 (13,248,000)	5,000,000 (250,000)	60,000 (3,000)
12-Sep-06	158,782,000 (7,939,000)	69,000,000 (3,450,000)	1,630,000 (82,000)
28-Sep-06	75,164,000 (3,758,000)	2,000,000 (100,000)	2,020,000 (101,000)
10-Oct-06	529,200,000 (26,460,000)	0 (0)	390,000 (20,000)
24-Oct-06	17,338,000 (867,000)	0 (0)	690,000 (35,000)

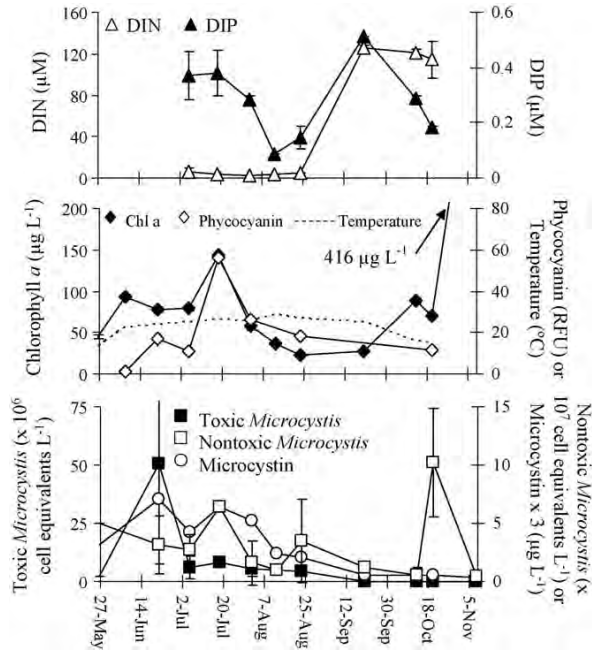
peaked on 1 August during the maximum densities of toxic *Microcystis* cells (Fig. 5). As was found in other systems, microcystin concentrations in Lake Champlain were significantly correlated with toxic *Microcystis* cell equivalents L<sup>-1</sup> ( $p < 0.05$ ). Nutrient levels were elevated before the onset of *Microcystis* blooms (May early July DIN =  $11.95 \pm 4.97 \mu\text{M}$ ; DIP =  $0.32 \pm 0.08 \mu\text{M}$ ), but were lower during the peak of the bloom (mid July–September DIN =  $8.12 \pm 3.91 \mu\text{M}$ ; DIP =  $0.26 \pm 0.19 \mu\text{M}$ ; Fig. 5). Nutrient levels increased again in October. The bloom peak also corresponded with

the highest temperatures of the sampling campaign (26 °C), with initial and final sampling temperatures close to 10 °C.

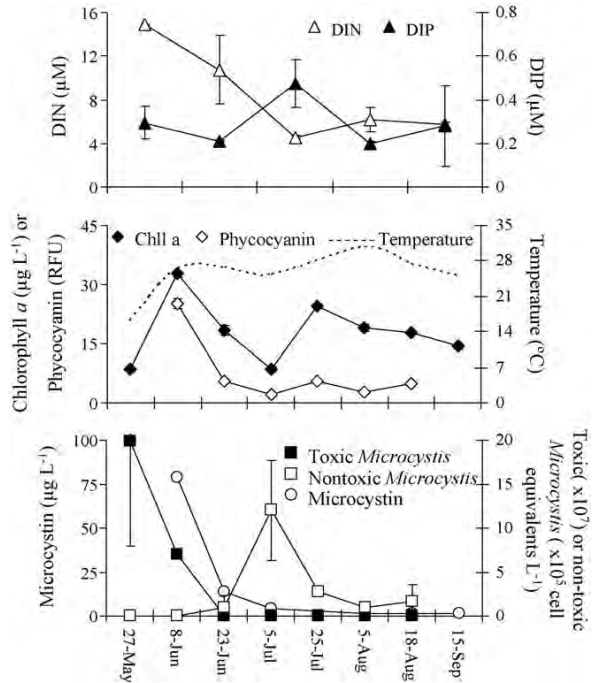
### 3.2. Effects of increased temperature and nutrients on toxic and non-toxic strains of *Microcystis*

#### 3.2.1. Lake Agawam

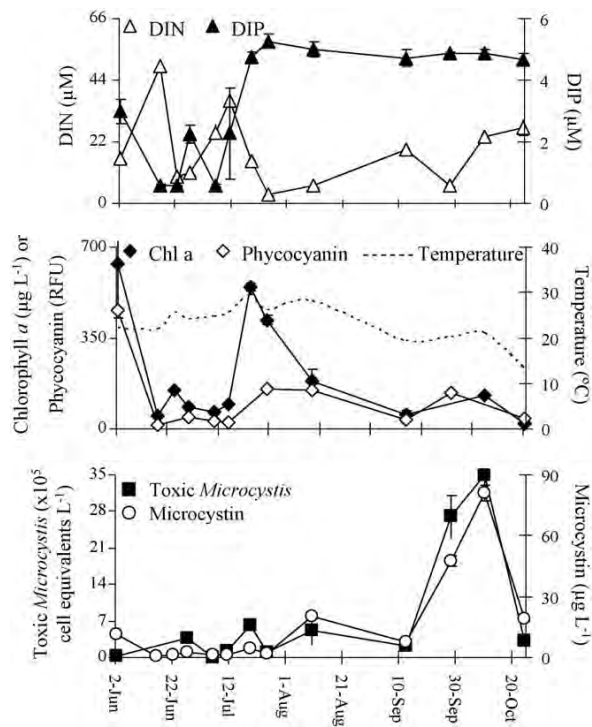
During the July 2005 experiment in Lake Agawam, ambient water temperature was  $26.9 \pm 1.7 \text{ °C}$  while the elevated temperature



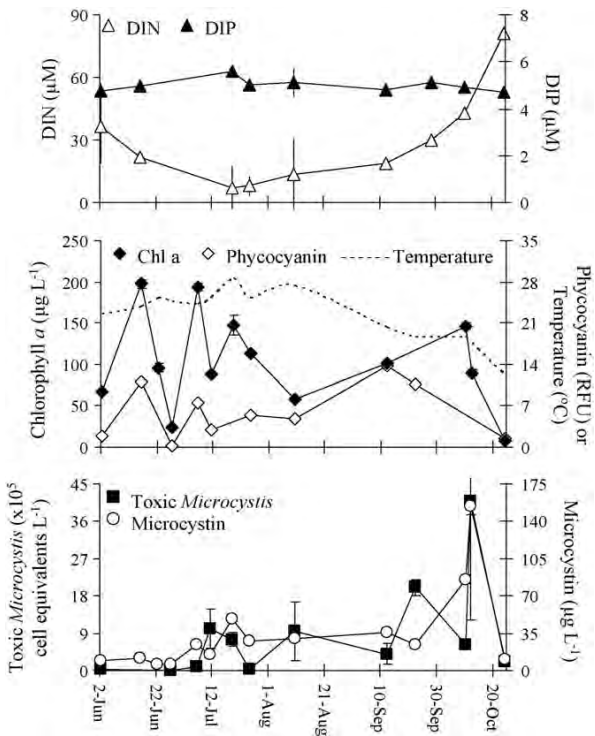
**Fig. 1.** Time series of parameters measured in the Lake Agawam, 2005. (A) Concentrations of dissolved inorganic nitrogen (DIN  $\Delta$ ) and orthophosphate (DIP  $\blacktriangle$ ). (B) Levels of total chl a  $\blacklozenge$ , phycoerythrin  $\diamond$ , and temperature (---). (C) Densities of toxic  $\blacksquare$  and non-toxic *Microcystis*  $\square$  as well as concentrations of microcystin  $\circ$ . Error bars represent  $\pm 1$ SD of replicated samples.



**Fig. 3.** Time series of parameters measured in Lake Ronkonkoma, 2005. Further details as in Fig. 1.



**Fig. 2.** Time series of parameters measured in Lake Agawam, 2006. Further details as in Fig. 1.



**Fig. 4.** Time series of parameters measured in Mill Pond, 2006. Further details as in Fig. 1.

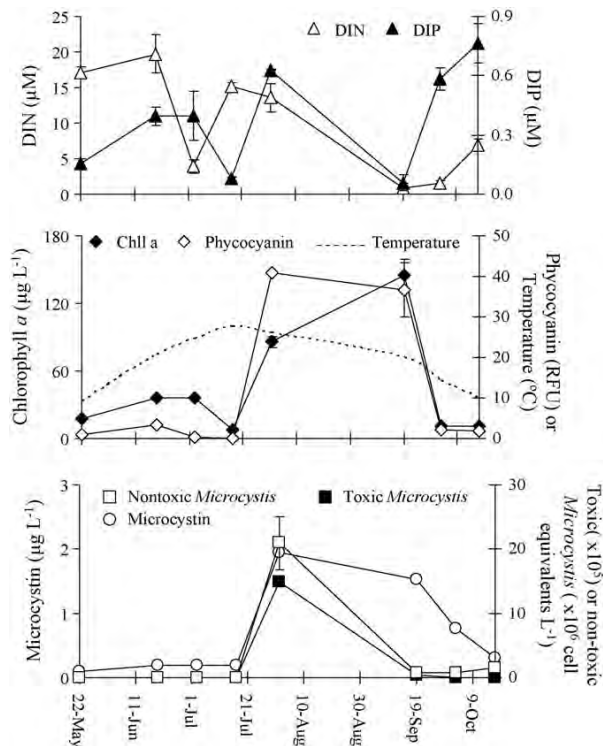


Fig. 5. Time series of parameters measured in Lake Champlain, 2006. Further details as in Fig. 1.

treatment was  $30.6 \pm 1.4$  °C. Elevated temperature was a significant treatment effect which enhanced both toxic and non-toxic *Microcystis* growth rates by 2–3 fold ( $p < 0.01$ ; Table 3; Fig. 6). Nutrients did not significantly alter growth rates and there were no significant interactions between treatments for either sub-population of *Microcystis* (Table 3; Fig. 6).

During the October 2006 experiment in Lake Agawam, temperature was a significant treatment effect which doubled toxic *Microcystis* growth rates compared to the control treatment ( $p < 0.05$ ; Table 3; Fig. 6). Furthermore, although the interaction between temperature and phosphorus was not significant, this dual treatment yielded a growth rate that was 125% greater than the control and was the highest growth rate of either population in any treatment ( $1.87 \pm 0.13$  d<sup>-1</sup>; Fig. 6). Interestingly, increased temperatures decreased the growth rate of the non-toxic population

relative to the control ( $p < 0.05$ ; Table 3; Fig. 6). Similarly, the interaction of temperature and phosphorus yielded a growth rate for non-toxic *Microcystis* that was significantly lower than the control ( $p < 0.05$ ; Table 3; Fig. 6). Ambient and elevated temperatures were  $10.6 \pm 0.98$  and  $14.9 \pm 0.81$  °C, respectively for this experiment.

### 3.2.2. Lake Ronkonkoma

During the Lake Ronkonkoma experiment, ambient water temperature was  $20.9 \pm 0.97$  °C. Experimentally enhanced temperatures ( $26.7 \pm 0.7$  °C) significantly increased growth rates of toxic *Microcystis* strains by 89% ( $p < 0.05$ ; Table 3; Fig. 6). N enrichment lead to a significant decrease in the growth rate of the non-toxic subpopulation of *Microcystis* ( $p < 0.05$ ; Table 3; Fig. 6), but did not alter the growth rate of the toxic strains (Table 3; Fig. 6). In addition, P enrichment yielded a significant, ~2-fold increase in the growth rates of both toxic and non-toxic strains of *Microcystis* ( $p < 0.001$ ; Table 3; Fig. 6). There was a significant interaction between N and P on toxic *Microcystis* growth rates ( $p < 0.01$ ; Table 3; Fig. 6), likely due to the slightly lower growth response in the N and P treatments compared to P only. There was a significant interaction between temperature and P on the non-toxic strains of *Microcystis* ( $p < 0.01$ ; Table 3; Fig. 6). There was also a significant interaction between N, P, and temperature for the non-toxic population of *Microcystis* ( $p < 0.05$ ) perhaps due to the growth rate being lower than that of the temperature/P enrichment (Fig. 6).

### 3.2.3. Mill Pond

During the June experiment in Mill Pond, ambient water temperature was  $23.6 \pm 2.7$  °C and elevated temperature was  $26.4 \pm 1.9$  °C. Temperature was a significant treatment effect which increased the growth rate of toxic *Microcystis* by 22% relative to the control ( $p < 0.05$ ; Table 3; Fig. 6). Higher phosphorus concentrations also increased the growth rate of toxic *Microcystis* (by 33% relative to the control), but not significantly ( $p > 0.05$ ; Fig. 6). However, the additive interaction between temperature and phosphorus yielded a growth rate for toxic *Microcystis* that was 63% higher than the control and was the highest growth rate of either population in any treatment ( $1.55 \pm 0.36$  d<sup>-1</sup>,  $p < 0.05$  for P/temperature interaction; Table 3; Fig. 6). The growth rate of the non-toxic *Microcystis* population was not significantly altered by any treatment or interaction (Fig. 6). Moreover, growth rates of this population were lower than the growth rates for toxic *Microcystis* in all treatments (Fig. 6).

During the July experiment, water temperatures were similar to that of the June experiment ( $23.8 \pm 3.1$  and  $28.9 \pm 1.6$  °C for ambient and elevated, respectively). Although none of the individual treatments yielded a significantly increased growth rate for either population, the interaction between temperature and phosphorus was significant for toxic *Microcystis* cells as this dual treatment

Table 3

Experimentally significant treatment effects and interactions on the growth rates of toxic and non-toxic *Microcystis* as determined by a 3-way ANOVA.

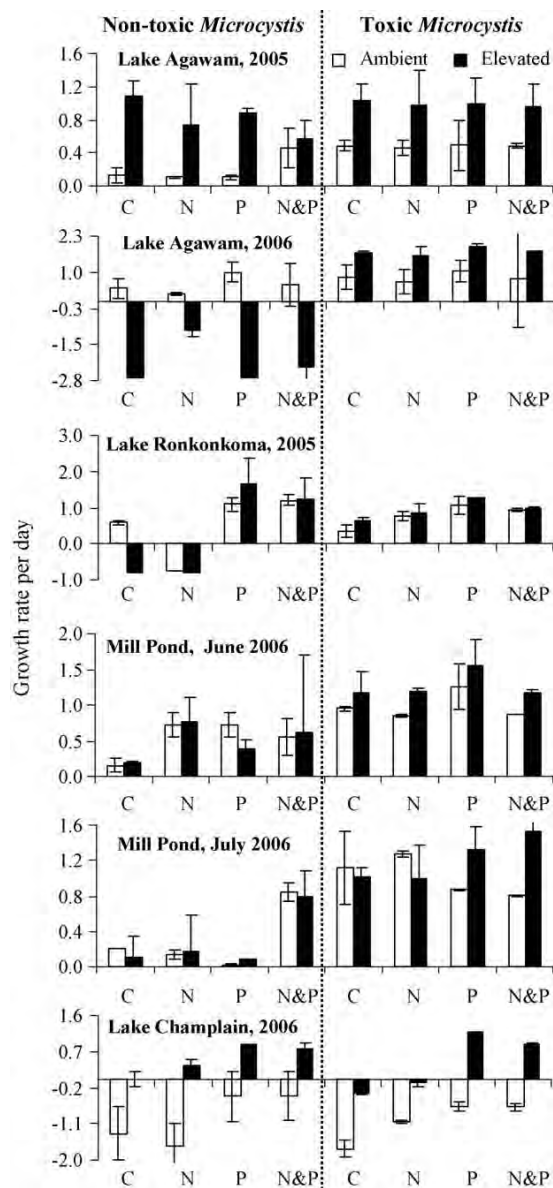
	Treatment effects		Interactions	
	Toxic	Non-toxic	Toxic	Non-toxic
Lake Agawam				
18-Jul-05	T**	T**	–	–
24-Oct-06	T*	–T***	–T and N**, –T and P**	–
Lake Ronkonkoma				
5-Jul-05	T*, P***	P***	N and P**	T and P**, T and N and P*
Lake Champlain				
1-Aug-06	T***, N**, P***	T***, P***	T and N**, T and P***, N and P***	–
Mill Pond				
27-Jun-06	T*	–	–	–
11-Jul-06	–	–	T and P**	N and P**

A negative sign before a nutrient indicates that the treatment yielded a significant decrease in growth rates, whereas all others yielded significantly higher growth rates.

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

\*\*\*  $p < 0.001$ .



**Fig. 6.** Net growth rates of toxic *Microcystis* (right half of figure) and nontoxic *Microcystis* (left half of figure) during nutrient amendment experiments ( $t = 72$  h) conducted in various systems during the 2005 and 2006 field seasons at ambient (white bars) and elevated (black bars) temperatures. C: control; N: nitrate; P: orthophosphate. Error bars represent  $\pm 1$  SD of triplicate experimental bottles.

yielded toxic population growth rates that were 20% higher than the control ( $p < 0.01$ ; Table 3; Fig. 6). Furthermore, although not statistically significant, concurrent enrichment of temperature, N and P yielded a growth rate for toxic *Microcystis* that was 40% greater than the control and was the highest growth rate for either population among all the treatments ( $1.54 \pm 0.11 \text{ d}^{-1}$ ; Fig. 6). On the other hand, the interaction between N and P was significant for the non-toxic population yielding growth rates which were enhanced 3-fold over unamended controls ( $p < 0.01$ ; Table 3; Fig. 6).

### 3.2.4. Lake Champlain

During the Lake Champlain experiment, ambient water temperatures were  $24.9 \pm 2.0$  °C. Experimentally enhanced tem-

peratures ( $29.1 \pm 1.3$  °C) significantly increased the growth rates of both toxic and non-toxic *Microcystis* populations by 80% and 101% respectively ( $p < 0.001$ ; Table 3; Fig. 6). Increased nitrogen concentrations stimulated growth rates of the toxic *Microcystis* population, increasing them 40% over the unamended controls ( $p < 0.01$ ; Table 3; Fig. 6). Furthermore, phosphorus enrichment significantly increased the growth rates of both toxic and non-toxic populations by 60% and 68%, respectively ( $p < 0.001$ ; Table 3; Fig. 6). Finally, nutrients (N or P) interacted with temperature, to enhance toxic *Microcystis* growth rates ( $p < 0.01$ ; Table 3; Fig. 6) with the enhanced P and temperature treatment yielding the highest growth rates of any population among all treatments ( $1.17 \pm 0.03 \text{ d}^{-1}$ ).

## 4. Discussion

Harmful cyanobacterial blooms have increased globally in frequency and intensity in recent decades. Eutrophication and warmer temperatures are often cited as key factors which promote these events (Paerl, 1988; Chorus and Bartram, 1999; Hudnell and Dortch, 2008; Paerl and Huisman, 2008). Previous studies have investigated the effects of singular environmental factors on the growth and/or abundance and/or photosynthesis of total *Microcystis* populations such as light (Codd and Poon, 1988; Wiedner et al., 2003; Kim et al., 2005), nutrient enrichment (Watanabe and Oishi, 1985; Codd and Poon, 1988; Fujimoto et al., 1997; Orr and Jones, 1998; Lee et al., 2000; Oh et al., 2000; Paerl et al., 2001; Vézic et al., 2002; Downing et al., 2005; Gobler et al., 2007), salinity (Tonk et al., 2007) and temperature (Konopka and Brock, 1978; Takamura et al., 1985; Robarts and Zohary, 1987). Other studies have found that *Microcystis* can out-compete other species of phytoplankton at high temperatures ( $\geq 30$  °C; Fujimoto et al., 1997). However, to our knowledge, this is the first field study to investigate the effects of increased temperature and nutrient concentrations on the growth rates of toxic and non-toxic subpopulations of *Microcystis*. Our ability to examine this phenomenon along with the *in situ* dynamics of these two populations within four distinct ecosystems during two years has generated data set which provides new insight regarding the ecology of toxic *Microcystis* blooms.

### 4.1. Seasonal dynamics of toxic and non-toxic *Microcystis*

Microcystin was detected in all four ecosystems studied on every date analyzed (Figs. 1–5). Toxic *Microcystis* cells comprised between 0.01% and 100% of the total *Microcystis* population among the four systems, a range larger than those found in prior studies (Kurmayer and Kutzenberger, 2003; Rinta-Kanto et al., 2005; Rinta-Kanto and Wilhelm, 2006; Yoshida et al., 2006; Hotto et al., 2008). However, there were notable differences, even among the systems presented here. In Lake Ronkonkoma, toxic *Microcystis* comprised between 12% and 100% of total cells, whereas the range was between 0.01% and 6% in other systems. The seasonal dynamics of toxic and non-toxic *Microcystis* observed in Lake Ronkonkoma, the only stratified lake studied, were consistent with the findings of Kardinaal et al. (2007) who also found toxic strains of *Microcystis* were succeeded by non-toxic strains in a deep stratified lake. However, in the well-mixed systems we studied (Lake Agawam, northeastern Lake Champlain, Mill Pond), toxic strains comprised a small portion of total cells where as Kardinaal et al. (2007) found the toxic strains dominated *Microcystis* populations in the two unstratified lakes. The seasonal dynamics of cyanobacterial blooms in general, and toxic and non-toxic strains of *Microcystis* in particular, likely vary based on system-specific physical and/or environmental conditions.

The World Health Organization (WHO) currently recommends monitoring chlorophyll *a* concentrations and total cyanobacterial



cell counts to protect against human exposure to high levels of microcystin (Chorus and Bartram, 1999). However, of the five field data sets generated by this study (Figs. 1–5), only two displayed mildly significant correlations between chlorophyll *a* concentrations and concentrations of microcystin ( $p < 0.05$ ) while only three showed significant correlations between total cyanobacterial cell counts and microcystin concentrations ( $p < 0.05$ ). This is not surprising as all phytoplankton contain chlorophyll *a* and nearly every major species of cyanobacteria has both toxic and non-toxic strains (Chorus and Bartram, 1999). However, in all five of the time series data sets generated by this study, densities of toxic *Microcystis* cells were significantly correlated with microcystin levels ( $p < 0.05$  for all; Figs. 1–5), often at a very high level of significance (Lake Ronkonkoma, Lake Agawam, Mill Pond;  $p < 0.001$ ). Obviously, the predominance of other microcystin producing genera will influence the relationship between toxic *Microcystis* cells and microcystin (Rantala et al., 2006; Rinta-Kanto and Wilhelm, 2006) and the presence of the *mcyD* gene does not necessarily translate into the synthesis of microcystin *in situ* (Gobler et al., 2007). Despite this, our data demonstrates that *mcyD*-containing *Microcystis* cells were a better predictor of pelagic microcystin concentrations than the total cell counts of cyanobacteria, total *Microcystis*, or chlorophyll *a* and thus may be a better predictor of microcystin in aquatic ecosystems than parameters currently recommended to be monitored by the WHO.

#### 4.2. Effects of temperature on the growth rates of toxic and non-toxic strains of *Microcystis*

As seasonal temperatures increase from 10 to 30 °C in freshwater ecosystems, the phytoplankton group with the highest growth rates generally shifts from diatoms to green algae to cyanobacteria (Canale and Vogel, 1974; Reynolds, 1997). Furthermore, it has generally been accepted that cyanobacteria dominate phytoplankton assemblages in temperate freshwater environments during the warmest periods of the year, particularly in eutrophic systems (Canale and Vogel, 1974; Reynolds and Walsby, 1975; Konopka and Brock, 1978; Tilman and Kiesling, 1984; Paerl, 1988, 2008; Paerl and Huisman, 2008). Consistent with this idea, cyanobacteria in general, and *Microcystis* in particular, dominated all of our study sites as temperatures reached their annual pinnacle (Figs. 1–5). While toxic *Microcystis* cell densities also peaked during annual maximum temperatures in two of our study sites (Lake Agawam, Lake Champlain; Figs. 1 and 5) they were slightly off-set in two other sites (Mill Pond, Lake Ronkonkoma; Figs. 3 and 4) likely reflecting the additive role other factors, such as nutrients, can play in bloom dynamics (see Section 4.3). More importantly, during five of the six (83%) experiments conducted, a ~4 °C increase in experimental temperatures yielded significantly higher (22–115%) growth rates for toxic (*mcyD*-containing) *Microcystis* cells (Fig. 6). In contrast, the growth rates of non-toxic *Microcystis* were significantly increased by higher temperature in only a third of experiments conducted (Fig. 6). Furthermore, in five of six experiments, the growth rate of toxic *Microcystis* cells exceeded those of non-toxic cells within the enhanced temperature only treatment (Fig. 6). All experimentally elevated temperatures were between 15 and 30 °C the optimal range for *Microcystis* (Chorus and Bartram, 1999), and in all but one experiment (Lake Agawam, 2006; temperature = 15 °C), the elevated temperatures fell between 25 and 30 °C, a range which yields maximal cellular toxin content in *Microcystis* (Van der Westhuizen and Eloff, 1985; Codd and Poon, 1988). Kim et al. (2005) found that toxic *Microcystis* strains cultured at 25 °C had more *mcyB* transcripts than cultures reared at 20 or 30 °C. Our observed increase in the abundance of 'toxic' *Microcystis* during higher temperatures could represent more gene copies per cell, more toxic cells, or both. However, any of these scenarios could lead to higher levels of microcystin in aquatic ecosystems (Tillett

et al., 2000; Rinta-Kanto and Wilhelm, 2006). Hence, as surface water temperatures continue to rise (Houghton et al., 2001), toxic *Microcystis* may out-grow non-toxic *Microcystis* or may synthesize more microcystin synthetase, yielding blooms that are comprised of a larger proportion of toxic cells and/or have higher microcystin concentrations. Moreover, as predicted by Paerl and Huisman (2008), our data also suggests future warming of temperate aquatic systems could lead to toxic *Microcystis* dominating for longer time periods than they presently do.

#### 4.3. The effects of nutrient enrichment on the growth rates of toxic and non-toxic *Microcystis*

During two of the six experiments conducted (Lake Ronkonkoma, Lake Champlain), phosphorus loading yielded significantly higher growth rates for both toxic and non-toxic populations of *Microcystis* relative to control treatments, although growth rates were not significantly different between these populations (Table 3; Fig. 6). These findings are contrary to those of Vézic et al. (2002) who found that at higher P concentrations the growth rates of toxic *Microcystis* exceeded non-toxic strains. However, our results were reasonable in light of the ambient nutrient levels as DIP was low (0.2 and 0.6 μM, respectively) and DIN concentrations were greater than 11 μM during these experiments (Figs. 3 and 5). DIP levels were substantially higher within the two shallower and more eutrophic systems we studied (Mill Pond, Lake Agawam; Figs. 1, 2 and 4) and P never affected *Microcystis* growth in these systems. Nitrogen loading significantly increased the growth rate of toxic *Microcystis* in Lake Champlain (Table 3; Fig. 6), a result consistent with Vézic et al. (2002) who found that increasing N concentrations in N-limited cultures significantly increased the growth rates of both toxic and non-toxic subpopulations of *Microcystis*. This result is also consistent with Gobler et al. (2007) who found that nitrogen can promote the growth and microcystin production of *Microcystis* during bloom events.

During many experiments we conducted, nutrients (N or P) and temperature interacted to promote the growth of toxic *Microcystis*. In Lake Champlain, Mill Pond (June 2006), and Lake Agawam (2006), the concurrent enhancement of temperature and P yielded growth rates for toxic *Microcystis* that not only increased 170%, 125%, and 20%, respectively, relative to the controls but also yielded the highest growth rate of either population in any treatment (Fig. 6). Similarly, during the Mill Pond experiment conducted during July 2006, the enrichment of N, P, and temperature yielded a growth rate of toxic *Microcystis* that was 40% greater than the control and was the highest growth rate of either population in all treatments (Fig. 6). These results are somewhat consistent with the findings of Jiang et al. (2008), who documented that higher temperature and P yielded greater abundance of total *Microcystis* cells. More importantly, these results suggest that future nutrient loading coupled with climatic warming may promote toxic, rather than non-toxic, populations of *Microcystis* and thus may lead to more toxic blooms.

This study has demonstrated the manner in which temperature and nutrients can interact to strongly influence the abundance and relative dominance of toxic and non-toxic strains of *Microcystis* within bloom events across the Northeast US. However, our previous research has demonstrated that even toxic populations of *Microcystis* show seasonal changes in expression of the microcystin synthetase gene (Gobler et al., 2007). As such, an important open question is the degree to which environmental factors such as nutrient loading and temperature changes influence microcystin synthetase gene expression and cellular microcystin synthesis within an ecosystem setting. Moreover, whether other microcystin synthesizing strains of other toxic cyanobacteria genera are promoted by warming and eutrophication remains unknown.

In conclusion, we found that the portion of wild *Microcystis* populations that were comprised of toxic cells varied seasonally and by location from 0.01% to 100%. Molecularly quantifying toxic (*mcyD*-containing) *Microcystis* was a better predictor of *in situ* microcystin levels than proxies currently recommended by the WHO to protect against human exposure to microcystin (total cyanobacteria cell densities or chlorophyll *a*; Chorus and Bartram, 1999). Warmer temperatures frequently (83% of experiments) shifted *Microcystis* toward populations comprised of a larger percentage of toxic *Microcystis* and/or cells with more *mcyD* copies per cell, scenarios which could yield more toxic blooms in an ecosystem setting. Finally, this study demonstrated that higher temperatures coupled with elevated P concentrations frequently yielded growth rates of toxic *Microcystis* cells which exceeded all other treatments and populations. Therefore, continued climatic warming and eutrophication could lead to a shift in cyanobacterial dominance toward blooms that contain a greater percentage of toxic *Microcystis* cells and/or more microcystin synthetase gene copies and hence greater concentrations of microcystin.

### Acknowledgements

We thank EPA-ECO HAB, New York Sea Grant, and the New Tamarind Foundation for financial support. We thank Charles Wall, Jen Goelski, Alison Citro, Michelle Weiss, Patrick Curran, Amanda Burson, Maria Alejandra Marcoval, Michael Satchwell, Matthew Harke, Colleen Norman, Justin Fischdicke and Lindsay Koza Moore for field and laboratory assistance. We thank Tim Mihuc and the Lake Champlain Research Institute for logistical support in Lake Champlain and the Stony Brook–Southampton Marine Science Center for logistical support in Lake Agawam.[SS]

### References

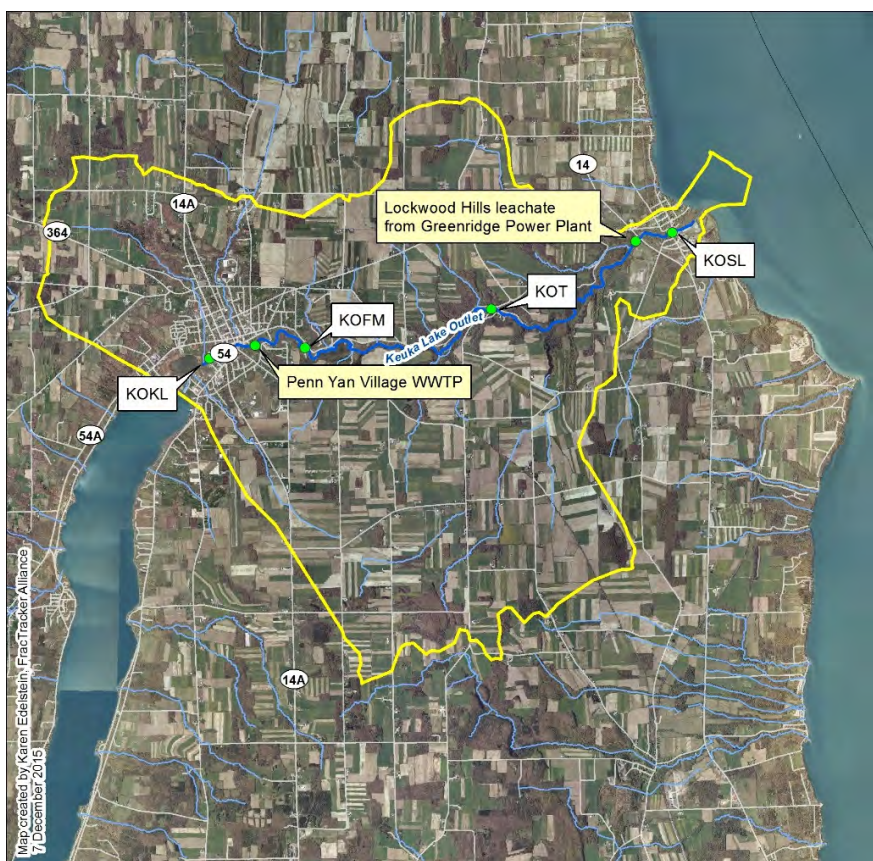
- Bell, S.G., Codd, G.A., 1994. Cyanobacteria toxins and human health. *Rev. Med. Microbiol.* 5, 256–264.
- Boyer, G.L., Matzin, M.C., Shambaugh, A.D., Satchwell, M.F., Rosen, B.H., Mihuc, T., 2004. The occurrence of cyanobacterial toxins in Lake Champlain. In: Manley, T. (Ed.), *Partnerships Research in the New Millennium*. Kluwer Academic/Plenum Publishers, pp. 241–257.
- Canale, R.P., Vogel, A.H., 1974. Effects of temperature on phytoplankton growth. *J. Environ. Eng. (American Society of Civil Engineers)* 100, 229–241.
- Carmichael, W.W., 1992. A status report on planktonic Cyanobacteria (Blue-Green Algae) and their Toxins. EPA/600/R-92/079, United States Environmental Protection Agency, Cincinnati, OH.
- Carmichael, W.W., Falconer, I.R., 1993. Diseases related to freshwater blue green algal toxins, and control measures. In: Falconer, I.R. (Ed.), *Algal Toxins in Seafood and Drinking Water*. Academic Press, pp. 187–209.
- Carmichael, W.W., 1994. The toxins of cyanobacteria. *Sci. Am.* 270, 78–86.
- Carmichael, W.W., An, J.S., 1999. Using an enzyme linked immunosorbent assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystins and nodularins. *Nat. Toxins* 7, 377–385.
- Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* 8 (3), 559–568.
- Chorus, I., Bartram, J., 1999. *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management*. World Health Organization/E&FN Spon/Routledge, London.
- Codd, G.A., Poon, G.K., 1988. Cyanobacterial toxins. In: Rogers, L.J., Gallon, J.R. (Eds.), *Biochemistry of the Algae and Cyanobacteria*. Clarendon Press, Oxford, England, pp. 283–296.
- Coyne, K.J., Handy, S.M., Demir, E., Whereat, E.B., Hutchins, D.A., Portune, K.J., Doblin, M.A., Cary, S.C., 2005. Improved quantitative real-time PCR assays for enumeration of harmful algal species in field samples using an exogenous DNA reference standard. *Limnol. Oceanogr.: Methods* 3, 381–391.
- Coyne, K.J., Cary, S.C., 2005. Molecular approaches to the investigation of viable dinoflagellate cysts in natural sediments from estuarine environments. *J. Euk. Microbiol.* 52, 90–94.
- Dempster, E.L., Pryor, K.V., Francis, D., Young, J.E., Rogers, H.J., 1999. Rapid DNA extraction from ferns for PCR-based analyses. *Biotechniques* 27, 66–68.
- Downing, T.G., Sember, C.S., Gehringer, M.M., Leukes, W., 2005. Medium N:P ratios and specific growth rate comodulate microcystin and protein content in *Microcystis aeruginosa* PCC7806 and *M. aeruginosa* UV027. *Microb. Ecol.* 49, 468–473.
- Falconer, I.R., Smith, J.V., Jackson, A.R.B., Jones, A., Runnegar, M.T.C., 1988. Oral toxicity of a bloom of the cyanobacterium *Microcystis aeruginosa* administered to mice over periods up to 1 year. *J. Toxicol. Environ. Health* 24, 291–305.
- Fastner, J., Erhard, M., von Döhren, H., 2001. Determination of oligopeptide diversity within a natural population of *Microcystis* spp. (Cyanobacteria) by typing single colonies by matrix-assisted laser desorption/ionization-time of flight mass spectrometry. *Appl. Environ. Microbiol.* 67, 5069–5076.
- Fleming, L.E., Rivero, C., Burns, J., Williams, C., Bean, J.A., Shea, K.A., Stinn, J., 2002. Blue green algal (cyanobacterial) toxins, surface drinking water, and liver cancer in Florida. *Harmful Algae* 1, 157–168.
- Fujimoto, N., Sudo, R., Sugiura, N., Inamori, Y., 1997. Nutrient-limited growth of *Microcystis aeruginosa* and *Phormidium tenue* and competition under various N:P supply ratios and temperature. *Limnol. Oceanogr.* 42, 250–256.
- Gobler, C.J., Davis, T.W., Coyne, K.J., Boyer, G.L., 2007. The interactive influences of nutrient loading and zooplankton grazing on the growth and toxicity of cyanobacteria blooms in a eutrophic lake. *Harmful Algae* 6, 119–133.
- Hotto, A.M., Satchwell, M.F., Berry, D.L., Gobler, C.J., Boyer, G.L., 2008. Spatial and temporal diversity of microcystins and microcystin-producing genotypes in Oneida Lake, NY. *Harmful Algae* 7, 671–681.
- Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., Van der Lin-den, P.J., Dai, X., Maskell, K., Johnson, C.A., 2001. *Climate Change 2001: The Scientific Basis*. Cambridge University Press, Cambridge, p. 881.
- Hudnell, K.H., Dortch, Q., 2008. A synopsis of research needs identified at the interagency, international symposium on cyanobacterial harmful algal blooms (ISOC-HAB). In: Hudnell, K.H. (Ed.), *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs Series: Advances in Experimental Medicine and Biology*, vol. 619, 2008, XXIV, 950 pp.
- IPCC, 2001. A report of working group I of the Intergovernmental Panel on Climate Change. Summary for Policymakers and Technical Summary.
- Ito, E., Kondo, F., Terao, K., Harada, K.-I., 1997. Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin-LR. *Toxicol.* 35, 1453–1457.
- Jiang, Y., Ji, B., Wong, R.N.S., Wong, M.H., 2008. Statistical study on the effects of environmental factors on the growth and microcystins production of bloom-forming cyanobacterium-*Microcystis aeruginosa*. *Harmful Algae* 7, 127–136.
- Jöhnk, K.D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P.M., Stroom, J.M., 2008. Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biol.* 14, 495–512.
- Jones, M.N., 1984. Nitrate reduction by shaking with cadmium: Alternative to cadmium columns. *Water Res.* 18, 643–646.
- Kaebnick, M., Neilan, B.A., Borner, T., Dittmann, E., 2000. Light and the transcriptional response of the microcystin biosynthesis gene cluster. *Appl. Environ. Microbiol.* 66, 3387–3392.
- Kardinaal, E.W., Tonk, L., Janse, I., Hol, S., Slot, P., Huisman, J., Visser, P.M., 2007. Competition for light between toxic and nontoxic strains of the harmful cyanobacterium *Microcystis*. *Appl. Environ. Microbiol.* 73, 2939–2946.
- Kim, H.R., Kim, C.K., Ahn, T.S., Yoo, S.A., Lee, D.H., 2005. Effects of temperature and light on microcystin synthetase gene transcription in *Microcystis aeruginosa*. *Key Eng. Mater.* 277–279, 606–611.
- Konopka, A., Brock, T.D., 1978. Effect of temperature on blue-green algae (cyanobacteria) in Lake Mendota. *Appl. Environ. Microbiol.* 36, 572–576.
- Kurmayer, R., Dittmann, E., Fastner, J., Chorus, I., 2002. Diversity of microcystin genes within a population of the toxic cyanobacterium *Microcystis* spp. in Lake Wannsee (Berlin, Germany). *Microb. Ecol.* 43, 107–118.
- Kurmayer, R., Kutzenberger, T., 2003. Application of real time PCR for quantification of microcystin genotypes in a population of the toxic cyanobacterium *Microcystis* sp. *Appl. Environ. Microbiol.* 69, 6723–6730.
- Lee, T., Tsuzuki, M., Takeuchi, T., Yokoyama, K., Karube, I., 1994. In vivo fluorometric method for early detection of cyanobacterial waterblooms. *J. Appl. Phycol.* 6, 489–495.
- Lee, S.J., Jang, M.-H., Kim, H.-S., Yoon, B.-D., Oh, H.M., 2000. Variation of microcystin content of *Microcystis aeruginosa* relative to medium N:P ratio and growth stage. *J. Appl. Microbiol.* 89, 323–329.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta Ct}$  method. *Methods* 25, 402–408.
- Myer, G.E., Gruendling, G.K., 1979. *Limnology of Lake Champlain Lake Champlain Basin Study*. New England River Basins Commission, Burlington, VT.
- Neilan, B.A., Jacobs, D., DelDot, T., Blackall, L.L., Hawkins, P.R., Cox, P.T., Goodman, A.E., 1997. rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. *Int. J. Syst. Bacteriol.* 47, 693–697.
- Oh, H.-M., Lee, S.J., Jang, M.-H., Yoon, B.-D., 2000. Microcystin production by *Microcystis aeruginosa* in a phosphorus-limited chemostat. *Appl. Environ. Microbiol.* 66, 176–179.
- Oliver, R.L., Ganf, G.G., 2000. In: Whitton, B.A., Potts, M. (Eds.), *Freshwater Blooms. The Ecology of Cyanobacteria. Their Diversity in Time and Space*. Kluwer Academic, Dordrecht, The Netherlands, pp. 149–194.
- Orr, P.T., Jones, G.J., 1998. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnol. Oceanogr.* 43, 1604–1614.
- Otsuka, S., Suda, S., Li, R., Watanabe, M., Oyaizu, H., Matsumoto, S., Watanabe, M.M., 1999. Phylogenetic relationships between toxic and non-toxic strains of the genus *Microcystis* based on 16S to 23S internal transcribed spacer sequence. *FEMS Microbiol. Lett.* 172, 15–21.
- Paeerl, H.W., 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnol. Oceanogr.* 33, 823–847.

- Paerl, H.W., Fulton, R.S., Moisaner, P.H., Dyble, J., 2001. Harmful freshwater algal blooms with an emphasis on cyanobacteria. *Sci. World* 1, 76–113.
- Paerl, H.W., Huisman, J., 2008. Blooms like it hot. *Science* 320, 57–58.
- Paerl, H.W., 2008. Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum. In: Hudnell, K.H. (Ed.), *Cyanobacterial Harmful Algal Blooms: State of the Science Research Needs Series: Advances in Experimental Medicine and Biology*, vol. 619. XXIV, 950 pp.
- Parsons, T.R., Maita, Y., Lalli, C.M., 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, Oxford.
- Paul, V.J., 2008. Global warming and cyanobacterial harmful algal blooms. In: Hudnell, K.H. (Ed.), *Cyanobacterial Harmful Algal Blooms: State of the Science Research Needs Series: Advances in Experimental Medicine and Biology*, vol. 619. XXIV, 950 pp.
- Philipp, R., Rowland, M.G.M., Baxter, P.J., McKenzie, C., Bell, R.H., 1991. Health risks from exposure to algae. *CDR (London: England Rev.)* 1, R67–R68.
- Rantala, A., Rajaniemi-Wacklin, P., Lyra, C., Lepisto, L., Rintala, J., Mankiewicz-Boczek, J., Sivonen, K., 2006. Detection of microcystin-producing cyanobacteria in Finnish lakes with genus-specific microcystin synthetase gene E (mcyE) PCR and associations with environmental factors. *Appl. Environ. Microbiol.* 72, 6101–6110.
- Rapala, J., Sivonen, K., Lyra, C., Niemela, S.I., 1997. Variation of microcystin, cyanobacterial hepatotoxins, in *Anabaena* spp as a function of growth stimulation. *Appl. Environ. Microbiol.* 63, 2206–2212.
- Reynolds, C.S., Walsby, A.E., 1975. Waterblooms. *Biol. Rev.* 50, 437–481.
- Reynolds, C.S., 1997. Successional development, energetics, and diversity in planktonic communities. In: Abe, T., Levin, S.A., Higashi, M. (Eds.), *Biodiversity: An Ecological Perspective*, Springer, 247 pp.
- Reynolds, C.S., 2006. *Ecology of Phytoplankton*. Cambridge University Press, Cambridge, 550 pp.
- Rinta-Kanto, J.M., Ouellette, A.J.A., Boyer, G.L., Twiss, M.R., Bridgeman, T.B., Wilhelm, S.W., 2005. Quantification of Toxic *Microcystis* spp. during the 2003 and 2004 Blooms in Western Lake Erie using Quantitative Real-Time PCR. *Environ. Sci. Technol.* 39, 4198–4205.
- Rinta-Kanto, J.M., Wilhelm, S.W., 2006. Diversity of microcystin-producing cyanobacteria in spatially isolated regions of Lake Erie. *Appl. Environ. Microbiol.* 72, 5083–5085.
- Robarts, R.D., Zohary, T., 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *N. Z. J. Mar. Freshwater Res.* 21, 391–399.
- Shambaugh, A., Duchovany, A., McIntosh, A., 1999. A survey of Lake Champlain's plankton. *Lake Champlain in Transition: From research toward restoration Water Science Application*, vol. 1. American Geophysical Union, Washington, pp. 323–340.
- Sivonen, K., 1990. Effects of light, temperature, nitrate, orthophosphate, and bacteria on growth of and hepatotoxin production by *Oscillatoria agardhii* strains. *Appl. Environ. Microbiol.* 56, 2658–2666.
- Smith, V.H., 1986. Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton. *Can. J. Fish. Aquat. Sci.* 43, 148–153.
- Takamura, N., Iwakuma, T., Yasuno, M., 1985. Photosynthesis and primary production of *Microcystis aeruginosa* Kütz. in Lake Kasumigaura. *J. Plankton Res.* 7, 303–312.
- Tillett, D., Dittmann, E., Erhard, M., von Dohren, H., Borner, T., Neilan, B.A., 2000. Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide–polyketide synthetase system. *Chem. Biol.* 7, 753–764.
- Tilman, D., Kiesling, R.L., 1984. Freshwater algal ecology: taxonomic trade-offs in the temperature dependence of nutrient competitive abilities. In: Klug, M.J., Reddy, C.A. (Eds.), *Current Perspectives in Microbial Ecology*. American Society for Microbiology, Washington, DC, pp. 314–319.
- Tonk, L., Boch, K., Visser, P., Huisman, J., 2007. Salt tolerance of the harmful cyanobacterium *Microcystis aeruginosa*. *Aquat. Microb. Ecol.* 46, 117–123.
- Trimbee, A.M., Prepas, E.E., 1987. Evaluation of total phosphorus as a predictor of relative biomass of blue-green algae with an emphasis on Alberta lakes. *Can. J. Fish. Aquat. Sci.* 44, 1337–1342.
- Utkilen, H., Gjølme, N., 1995. Iron-stimulated toxin production in *Microcystis aeruginosa*. *Appl. Environ. Microbiol.* 61, 797–800.
- Van der Westhuizen, A.J., Eloff, J.N., 1985. Effect of temperature and light on the toxicity and growth of the blue-green alga *Microcystis aeruginosa* (UV-006). *Planta* 163, 55–59.
- Vézic, C., Rapala, J., Vaitomaa, J., Seitsonen, J., Sivonen, K., 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis* strains and on intracellular microcystin concentrations. *Microb. Ecol.* 43, 443–454.
- Watanabe, M.F., Oishi, S., 1985. Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. *Appl. Environ. Microbiol.* 49, 1342–1344.
- Watras, C.J., Baker, A.L., 1988. Detection of planktonic cyanobacteria by tandem in vivo fluorometry. *Hydrobiologia* 169, 77–84.
- Watson, S.B., McCauley, E., Downing, J.A., 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. *Limnol. Oceanogr.* 42, 487–495.
- Wiedner, C., Visser, P.M., Fastner, J., Metcalf, J.S., Codd, G.A., Mur, L.R., 2003. Effects of light on the microcystin content of *Microcystis* strain PCC 7806. *Appl. Environ. Microbiol.* 69, 1475–1481.
- Yoshida, T., Takashima, Y., Tomaru, Y., Shirai, Y., Takao, Y., Hiroisha, S., Nagasaki, K., 2006. Isolation and characterization of a cyanophage infecting the toxic Cyanobacterium *Microcystis aeruginosa*. *Appl. Environ. Microbiol.* 72, 1239–1247.
- Zegura, B., Sedmak, B., Filipic, M., 2003. Microcystin-LR induces oxidative DNA damage in human hepatoma cell line HepG2. *Toxicol.* 41, 41–48.

## **Exhibit C**

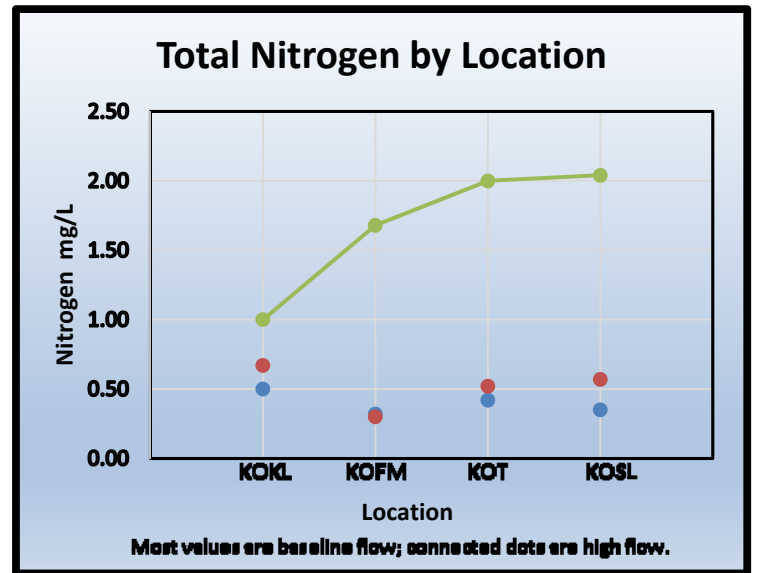
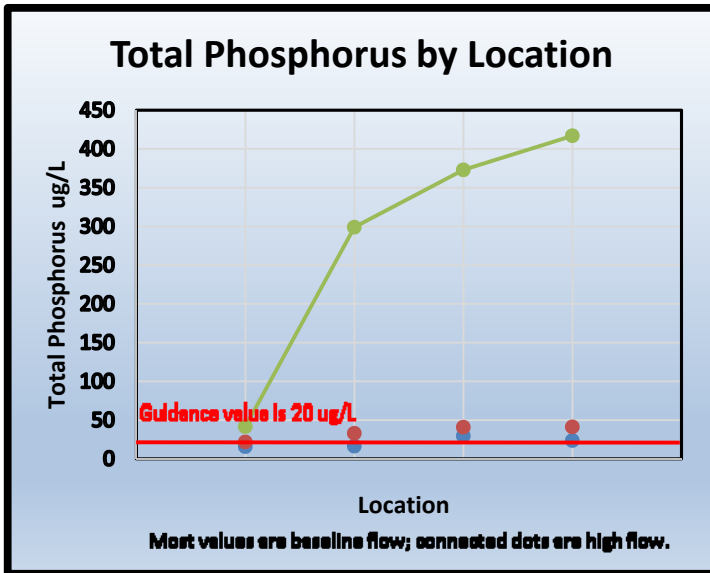
## Keuka Outlet Fact Sheet

Keuka Outlet is the largest tributary to Seneca Lake, its watershed area representing 35% of total. It is the sole outlet of Keuka Lake, and flows northeast from Penn Yan to Dresden, mid-way on the western shore of Seneca Lake. The flow from Keuka Lake is controlled by gates in a dam at Penn Yan. The drainage area is largely made up of agriculture and forested land, though the outlet does receive effluent from the Penn Yan WWTP. The stream has a long history of mills and industry on its shores, and historically provided water for a canal connecting the two lakes. The Keuka Outlet stream is a DEC Class C fishing stream. The maps shows the sampling locations on the outlet stream, though the watershed encompasses all that flows into Keuka Lake.



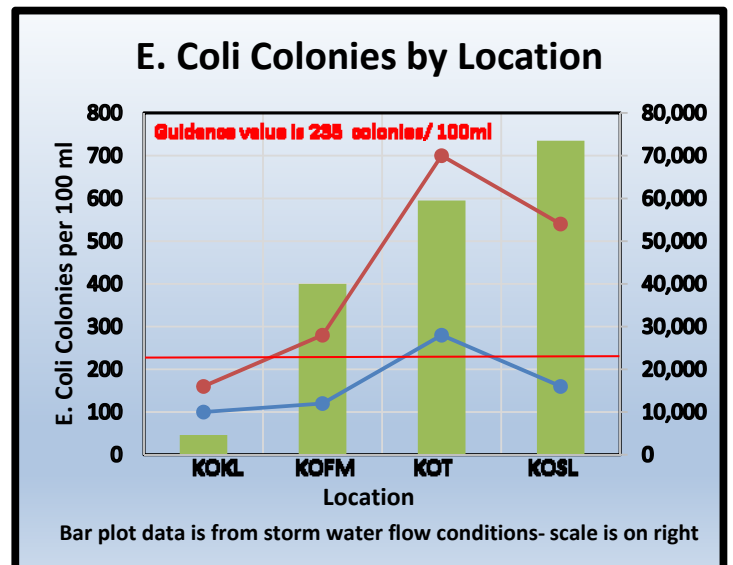
SLPWA (and its partner CSI) has sampled and tested the outlet throughout 2015 at the four locations shown on the map (KOKL, KOFM, KOT, KOSL). Results for phosphorus and nitrogen (nutrients that encourage plant and algae growth), and E. coli bacteria (indicator of human and animal wastes) are the most concerning of the attributes tested.

## Results



The graph showing phosphorus levels, at the locations sampled, shows that at normal stream flow conditions the levels are reasonable at the 1-2X the DEC guidance level of 20ug/l. During the one storm event tested the phosphorus levels increased dramatically to ~20X the guidance level. The graph for nitrogen is similar, with normal flow conditions showing levels at or below the guidance level. Again the high flow of the storm event showed nitrogen levels 4X the guidance level. High flow levels combined with high levels of nutrients results in much added nutrients flowing into Seneca Lake, adding to the issue of weed and algae growth. 2015 saw Seneca Lakes first confirmed toxic blue-green algae blooms.

The graph to the right shows the levels of E.coli during normal flow and the one high flow event captured. At normal flow, the E.coli levels increase as the stream flows from Keuka to Seneca Lake, starting below the guidance level and ending at or above that level, The real concern is the dramatically high levels seen during the high flow event, with levels increasing from 20X to 310X . E. coli is not regulated in Class C streams. The very high levels that are seen in the highest of flow conditions pollute Seneca Lake (a "Class A" drinking water supply) with this dangerous bacteria. Investigative sampling will be required to determine if the Penn Yan waste water treatment facility is a significant contributor to the high bacteria levels.



Actions to address these issues can occur at the community and state level. Upgrades or replacement of the WWTP can address the high phosphorus output. Improvement and controls on agricultural practices can have impact on the dramatic run-off effects seen for both phosphorus and E.coli levels

### Keuka Outlet Data

Location Code GPS Coordinates	Guidance Values	Site Name	Team Contact	Sampling Date	Type of Event	Water Temperature (degrees C)	pH (Classes A-C) 6.0-9.0 (Class D)	Dissolved Oxygen mg/L	Chloride mg/L	Soluble Reactive Phosphorus ug/L	Total Phosphorus (ug/L)	Nitrate-Nitrite Nitrogen (mg/L)	Kjeldahl Total Nitrogen (mg/L)	Specific Conduct (Ms/cm)	Coliform (Col/100 ml)	E.coli (Col/100 ml)	Turbidity (NTU)	Hardness Mg/L as CaCo3	Total Suspended Solids (ml/L)
KOKL 42.6574 -77.0589		Penn Yan Boat Launch	Fred Geyer	7/13/2015	BF	23.5	8.00	7.8	35.2	15.10	15.8	0.08	0.50	315.0	4000	100	1.71	117.0	0.63
				8/17/2015	BF	25.2	8.25	6.8	37.5	17.20	21.8	0.08	0.67	305.0	9000	60	3.89	110.0	0.63
				9/30/2015	SW	21.5	7.25	5.1	31.7	23.20	41.7	0.07	1.00	287.0	20000	4620	80.00	141.0	53.00
KOFM 42.6596 -77.0371		Fox Mills Rd.	Fred Geyer	7/13/2015	BF	23.5	8.25	7.6	35.9	9.14	16.3	0.09	0.32	323.0	7000	120	2.62	118.0	2.25
				8/17/2015	BF	24.8	8.25	7.4	38.6	20.00	32.9	0.05	0.30	331.0	5500	160	4.59	130.0	12.50
				9/30/2015	SW	-	8.00	7.7	33.0	175.00	299.0	2.16	1.68	384.0	100000	40000	80.00	141.0	53.00
KOT 42.6669 -76.9947		KO Tributary near Ridge Rd.	Larry Martin	7/13/2015	BF	23.5	8.25	7.9	37.3	16.40	29.6	0.30	0.42	333.0	5000	280	4.82	122.0	15.30
				8/17/2015	BF	23.2	8.25	---	42.6	32.50	40.8	0.72	0.52	366.0	12500	420	1.86	132.0	1.25
				9/30/2015	SW	17.0	8.25	8.0	38.6	204.00	373.0	4.80	2.00	454.0	100000	59500	95.00	177.0	65.00
KOSL 42.6805 -76.9538		Charles St. Bridge, Dresden	Larry Martin	7/13/2015	BF	23.2	8.00	7.8	39.3	12.60	23.5	0.27	0.35	339.0	20000	160	1.05	119.0	7.00
				8/17/2015	BF	22.7	8.25	7.4	47.1	30.10	41.3	1.04	0.57	402.0	11000	380	2.79	138.0	0.63
				9/30/2015	SW	16.8	8.00	8.0	31.3	215.00	417.0	4.25	2.04	401.0	100000	73500	150.00	161.0	102.00

BF= Base Flow; SW= Storm Water