

Final Report, Ohio Water Resources Center Project

“Source Tracking of *Microcystis* Blooms in Lake Erie and its Tributaries,” George S. Bullerjahn, PI

Problem and Research Objectives, Methodology, and Principal Findings and Significance:

The objective of this study is to determine the source of the diverse *Microcystis* that comprise the annual Lake Erie cyanobacterial blooms. Whereas some toxic and nontoxic forms may arise within Lake Erie sediments, the diversity of *Microcystis* detected in blooms may reveal genotypes that originate in sites physically removed from the lake. Understanding the origins of toxic and nontoxic forms can inform management strategies in the Great Lakes that may limit the expansion of *Microcystis* blooms in Lake Erie. Our methodology involves sequencing target genes from *Microcystis* populations to determine the toxic genotypes present, and the sites upstream from Lake Erie in which they may originate. Phylogenetic analysis of ribosomal 16S genes, toxin (*mcy*) genes and the N-responsive *ntcA* gene has provided fine-structure analysis of cyanobacterial populations that occur in the Lake Erie drainage basin. One result of our work described below is that while Lake Erie *Microcystis* genotypes are found in abundance upstream in Lake St. Clair, nearshore sites in Lake Erie (Sandusky Bay and Maumee River) are dominated by microcystin-producing *Planktothrix*. This latter finding has led to a future study on *Planktothrix* ecophysiology that has recently been funded by Ohio Sea Grant.

We proposed to monitor *Microcystis* genotypes from the Laurentian Great Lake Basin using archives of DNA from across the Great Lakes and tributaries/connecting channels, together with parallel measurements of nutrients, chlorophyll and physico-chemical parameters. The results will establish the diversity and geographic origins of the bloom-forming genotypes. Analysis of focal cyanobacterial harmful algal bloom (cHAB) species diversity from physically remote sites and Erie blooms has been conducted for two full bloom seasons, 2013 and 2014.

During the summer of 2013 we conducted a spatial and temporal survey of Lake St. Clair (LSC). Three sites around the southeast corner of LSC where the Thames River enters

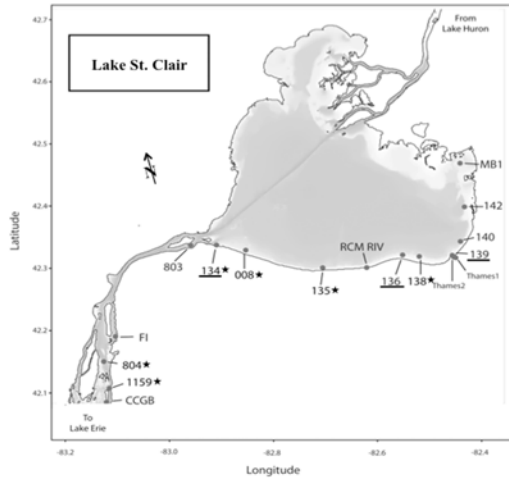


Figure 1: Map of Lake St. Clair indicating sampling sites. The underlined sites were the seasonal monitoring sites; starred sites where DNA was extracted and sequenced for genetic diversity.

LSC were monitored from June through September for CHAB genetic and phytoplankton community composition. In late August, a high biomass event occurred. During this event a spatial survey was conducted from Mitchel's Bay to the mouth of the Detroit River (17 sites; see Figure 1). We collected samples for DNA, dissolved and total nutrients, phytoplankton community composition and toxicity. We targeted the *mcyA* gene, part of the microcystin synthetase gene operon, to investigate the phylogenetics of potential microcystin producers in LSC and the Detroit River. The *mcyA* primers we used amplify the gene segment from *Microcystis*,

Planktothrix and *Anabaena* provided a robust picture of the potential microcystin producers in LSC and helped determine whether those strains were similar to strains found in the lower Great Lakes. We found that *Microcystis* was the predominant microcystin producer and that all toxic *Microcystis* strains found in Lake St. Clair clustered with toxic strains found in samples previously collected from Lakes Erie and Ontario, demonstrating extensive genetic connectivity between the three systems and establishing Lake St. Clair as an important immediate and historical source of toxic *Microcystis* to lakes Erie and Ontario. Also, we found that *Microcystis* blooms in Lake St. Clair can produce microcystin at levels that could negatively affect human health. These data will be presented at the 2014 International Association for Great Lakes Research and have been incorporated into a manuscript submitted to the international peer-reviewed journal PLOS ONE.

Bullerjahn's work is also informed by metagenomic and metatranscriptomic analyses provided through an approved project DOE-Joint Genome Institute ("Metagenomics and metatranscriptomics of the Lake Erie 'dead zone': a seasonal source of greenhouse gases," PIs McKay, Bullerjahn and Bourbonniere). Sampling pelagic Lake Erie and nearshore sites, we currently have metagenomes from Sandusky Bay and metagenomes and metatranscriptome data from the Erie central basin. The N-responsive regulator gene *ntcA* is unique to cyanobacteria, and we have used *ntcA* sequences as a measure of cyanobacterial diversity and relative abundance at these sites. The expression of *ntcA* in the metatranscriptome is also a measure of nitrogen bioavailability, as high levels of *ntcA* mRNA is diagnostic for N-limited cyanobacteria. For example, the recent

availability of the Sandusky Bay metagenome has allowed us to examine total cyanobacterial diversity and diversity of microcystin producers through phylogenetics of *ntcA* and *mcyA*. Regarding total cyanobacterial diversity, toxic *Planktothrix*, not *Microcystis*, dominates in nearshore environments such as Sandusky Bay, with heterocystous cyanobacteria present as minor members of the community. Importantly, the metagenome indicates that the number of *Planktothrix* genotypes is very low, allowing the design of some fairly straightforward experiments to test mechanisms of bloom formation and toxigenicity. Given that Sandusky Bay often experiences N limitation (see below), and that *Planktothrix* is not an N fixer, we speculate that *Planktothrix* is particularly successful at scavenging nitrogen, likely provided from nitrogen fixing taxa present. We hypothesize that N scavenging by *Planktothrix* is responsible for this genus outcompeting *Microcystis* in nearshore waters. Examining the diversity of N fixers by binning all *nifH* reads from the metagenome, only about one-third of the nitrogen fixers are cyanobacteria, the remainder being heterotrophic proteobacteria.

Collectively, these data sets on Erie and Sandusky Bay have allowed the development of RT-PCR primers specific for endemic *Microcystis* and *Planktothrix ntcA*, *mcyA* and cyanobacterial/bacterial *nifH*. qRT-PCR will be employed in field samples and laboratory experiments to assess the nutrient regimes (N speciation/SRP/organic P) that promote *Microcystis* and *Planktothrix* growth and toxigenicity. Additionally, in work stemming from genomic analysis of the nitrogen fixation genes, we are currently measuring nitrogen fixation rates from sediment and planktonic microbes to determine inputs of new N into the bloom sites.

In summary, the funded work targeted sampling at Sandusky Bay, the Maumee River and Lake St. Clair cyanobacteria, comparing the bloom-forming genera at each site using high-throughput DNA sequencing of diagnostic target genes. The results clearly indicated that all toxic *Microcystis* strains found in Lake St. Clair clustered with toxic strains found in samples previously collected from Lakes Erie and Ontario, demonstrating extensive genetic connectivity between the three systems and establishing Lake St. Clair as an important immediate and historical source of toxic *Microcystis* to lakes Erie and Ontario (Figure 1). Furthermore, while Lake Erie *Microcystis* genotypes are found in abundance upstream in Lake St. Clair, nearshore sites in Lake Erie (Sandusky Bay and Maumee River) are dominated by microcystin-producing *Planktothrix*.

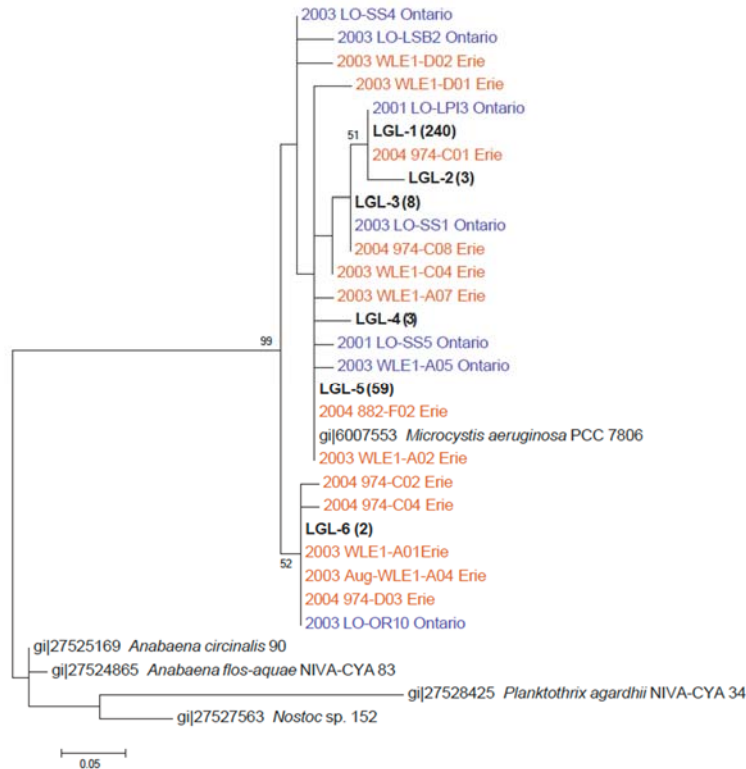


Figure 2. Phylogenetic tree of microcystin synthetase toxin (*mcyA*) gene sequences from Lake St. Clair, compared with environmental sequences from Lakes Erie and Ontario. The Lake St. Clair sequences (LGL) exist as six genotypes that are detected as abundant bloom formers downstream in Lakes Erie (orange) and Ontario (blue).

For further information see the below publications:

Davis, T., S.B. Watson, M.J. Rozmarynowycz, J.J.H. Ciborowski, R.M. McKay and G.S. Bullerjahn. 2014. Phylogenies of microcystin-producing cyanobacteria in the lower Laurentian Great Lakes suggest extensive genetic connectivity. PLOS ONE 9(9): e106093.

Davis, T.W., G.S. Bullerjahn, T. Tuttle, M. Rozmarynowycz, R.M. McKay and S.B. Watson. 2015. Evaluation of increasing nitrogen and phosphorus concentrations on the toxicity and chlorophyll content during cyanobacterial blooms in Sandusky Bay, Lake Erie. Environ. Sci. Technol., in revision.