

## Recent Dreissenid Mussel Colonization may Lead to More Toxic Cyanobacterial Blooms

Katelyn M. McKindles<sup>a</sup>, Paul V. Zimba<sup>b</sup>, Alexander S. Chiu<sup>b</sup>, Sue B. Watson<sup>c</sup>, Danielle B. Gutierrez<sup>d</sup>, Judy Westrick<sup>d</sup>, Timothy W. Davis<sup>a</sup>.

<sup>a</sup>Bowling Green State University, Bowling Green, Ohio <sup>b</sup>Center for Coastal Studies, Texas A&M University – Corpus Christi <sup>c</sup>Water Science and Technology, Environment Canada <sup>d</sup>Department of Chemistry, Wayne State University.

### Abstract

Lake Winnipeg (Manitoba, Canada), the world's 12<sup>th</sup> largest lake by area, is host to yearly cyanobacterial harmful algal blooms (cHABs) dominated by *Aphanizomenon* and *Dolichospermum*. cHABs in Lake Winnipeg are primarily a result of eutrophication but may be exacerbated by the recent introduction of dreissenid mussels. The invasion of dreissenids into Lake Erie has been hypothesized to be one factor promoting the toxic *Microcystis* blooms currently seen in the western basin. Prior to the invasion, Lake Erie cHABs were a mixed community of *Microcystis*, *Aphanizomenon* and *Dolichospermum*. Using methods to monitor the potential for toxin production in Lake Winnipeg in conjunction with environmental measures, this study defined the baseline composition of a Lake Winnipeg cHAB to measure potential changes due to dreissenid colonization. Surface water samples were collected in 2013 from 23 sites during summer and 18 sites in fall. Genetic and mass spectrometry cyanotoxin profiles identified microcystins (MC) as the most abundant cyanotoxin across all stations, with MC concentrations highest in the North Basin. In the fall, *mcyA* genes were sequenced to determine which species had the potential to produce MCs, and 12 of the 18 sites were a mix of both *Planktothrix* and *Microcystis*. Current blooms in Lake Winnipeg produce low levels of MCs, but the capacity to produce cyanotoxins is widespread across both basins. If dreissenid mussels continue to colonize Lake Winnipeg, a shift in physicochemical properties of the lake due to faster water column clearance rates may yield more toxic blooms potentially dominated by microcystin producers.

### Introduction

Lake Winnipeg in Manitoba, Canada, is subject to excess nutrient run-off (1, 2) (highly eutrophic), has distinct geographical lake features (Figure 1), has seasonal cyanobacterial harmful algal blooms (cHABs) (3, 4), and now has populations of dreissenid mussels. Lake Winnipeg cHABs are currently dominated by the less harmful cyanobacteria species *Aphanizomenon* and *Dolichospermum* (5) and the first recording of the mussels in Lake Winnipeg occurred in 2013 (6). If dreissenid mussels are successful in colonizing Lake Winnipeg, bloom dominance may shift towards more toxic cyanobacteria species such as *Microcystis*, potentially leading to toxic blooms and shifts in lower trophic level dynamics which could significantly impact the ecology of Lake Winnipeg and socioeconomics of the region. Because of this potential shift, **the work here outlines the toxin production of cHABs in Lake Winnipeg prior to extensive colonization by the dreissenid mussels.**

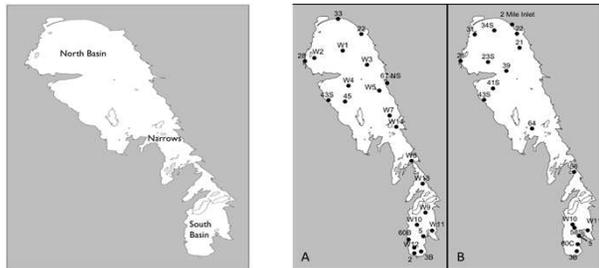


Figure 1. Distinct Geographical Features of Lake Winnipeg

Figure 2. Sampling sites on Lake Winnipeg. A. Summer 2013 B. Fall 2013

### Methods and Materials

Water samples were collected from 23 sites in June 2013 (summer) and 18 sites in September 2013 (fall) (Figure 2). Two filters were used for molecular analysis and one was used for instrumental toxin analysis as described below. Additional samples were collected and fixed at a 5% final concentration Lugol's solution for microscopy analysis of biomass and species composition.

CyanoDTeC™ cyanotoxin detection kit (Diagnostic TECHNOLOGY, Sydney, Australia) with primers for the detection of *cyrA*, *sxtA*, and *mcyE* genes, ELISA (total microcystins when chl-a exceeded 5 µg/L), and mass spectrometry multiple reaction monitoring (MS-MRM) was used to analyze samples from all collection sites for multiple MC congeners as well as SXT and CYN. PCR amplifications were then performed using *mcyA* primers that detect potential microcystin producing genotypes in *Microcystis*, *Planktothrix* and *Anabaena* (7) and have been used in previous Great Lakes cHAB phylogenetic studies (8, 9, 10, 11).

### Results

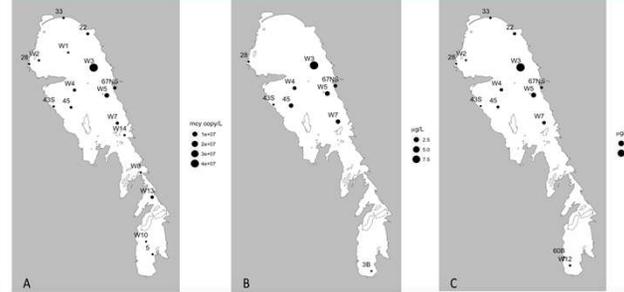


Figure 3. Summer 2013 - Multiplex q-PCR analyses of the 23 sites sampled during summer were positive for *cyr*, *sxt*, and *mcy* gene at six, two and 17 sites, respectively (Figure 3a, data not shown). Nine of the 23 sites were positive for MC using ELISA (Figure 3b), and MCs were detected using MS-MRM at 14 sites located predominantly on the eastern side of the NB (Figure 3c).

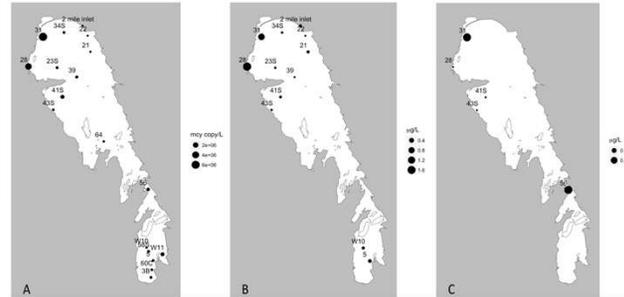


Figure 4. Fall 2013 - Multiplex q-PCR analysis of the 18 sites sampled during the fall were positive for *cyr* and *mcy* genes at three and 18 sites, respectively, and negative for *sxt* (Figure 4a, data not shown). 12 of the 18 sites were positive for MC using ELISA (Figure 4b), and MCs were detected using MS-MRM at only five sites (Figure 4c). Toxin levels were lower in the fall and were often close to detection limits.

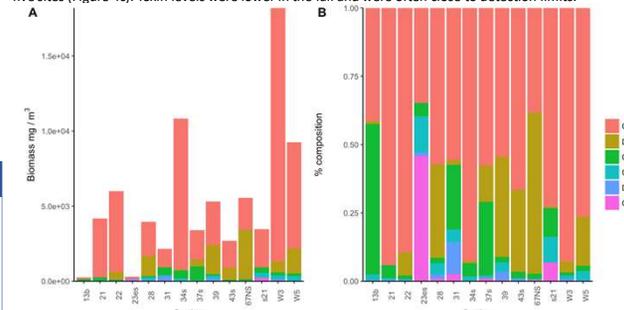


Figure 5. Total biomass and % phylum composition for 8 summer sites and 6 fall sites in Lake Winnipeg. The dominant taxa in 11 of the 14 sites was cyanobacteria, consisting primarily of *Aphanizomenon flos aquae* complex and *Dolichospermum* spp.

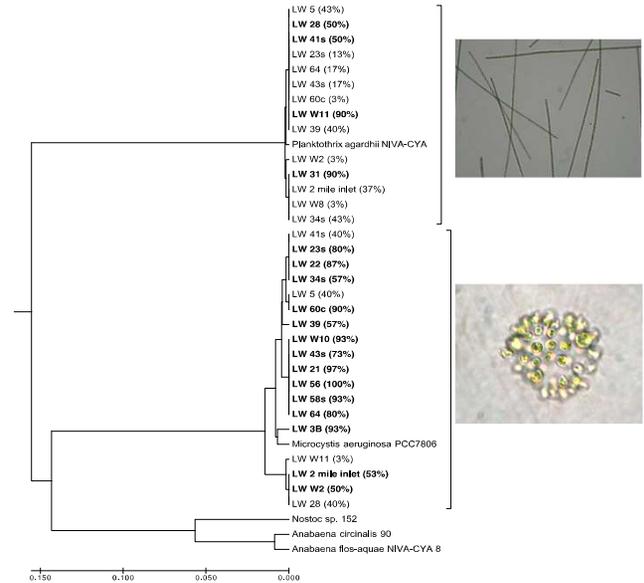


Figure 6. *mcyA* phylogenetic tree of MC producers. A majority of the sites (12 of 20) contained a mixture of both *Planktothrix* and *Microcystis* sequences. Four of these mixed sites were dominated by *Planktothrix* (NB sites 28 and 41a, SB sites W11 and 5) and 8 were dominated by *Microcystis* (NB sites 2 mile inlet, 34a, W2, 23a, 39, 43a, the Narrows site 64, and the SB site 60C) (bolded sites). Of the single species sites, 6 were *Microcystis* only (NB sites 22, 21, the Narrows site 56, and the SB sites W10, 58Sn and 3B) and two were *Planktothrix* only (NB site 31 and the Narrows site W8).

### Conclusions

- Current blooms produce low levels of MC, largely during the summer in the Northern Basin
  - May be due to less turbidity (higher light penetration)
- Potential MC producers are widespread in the Lake, demonstrating a potential for more toxic blooms in the future
- Of note, site 31 was visually (data not shown) and genetically dominated by *Planktothrix* and it has the highest fall hit for *mcy* genes and MS-MRM MCs
- Strong correlation between the three analysis measures suggests consistent analysis results
  - Summer qPCR analysis may be used in the future to predict toxic bloom formation

**This work establishes the baseline composition of Lake Winnipeg in two seasons in which cHABs are present, and before the dreissenid mussels have completely taken over. Future work will continue to monitor the lake under these conditions, hoping to relate the evolution of Lake Winnipeg cHABs to those of Lake Erie**

### Contact

Katelyn McKindles  
Bowling Green State University  
217 Life Sciences Building  
Bowling Green, OH 43403  
kmckind@bgsu.edu

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