Phosphorous Competition and pH Limited Growth in Grand Lake Saint Marys *Microcystis* Isolates

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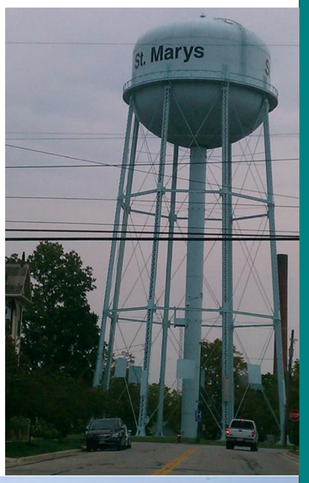
Introduction

Harmful Algal Blooms (HABs) are a seasonal event in many eutrophic lakes in temperate regions. Multiple factors are believed to contribute to these proliferations of photosynthetic microorganisms, including selective grazing, irradiance levels, both macronutrient and micronutrient loading, and possibly pH. As some of the organisms involved are nitrogen fixers and semi-independent of inputs, phosphorous is believed to be a primary driver.(4)

In addition to poor water quality conditions like odors and anoxia, many of the algal species involved produce hepatotoxins and neurotoxins. One of the bloom forming organisms of interest is *Microcystis*, a colonial cyanobacteria that poses identification, culturing, and quantification challenges, as well as producing microcystins – potent toxins that are under investigation as possible liver tumor promoters.



Grand Lake Saint Marys in midstate Ohio experiences season algal blooms and toxin levels in excess of ten times recommended levels for drinking water sources. Economic losses from lost tourism and recreational use have been estimated in the millions of dollars.

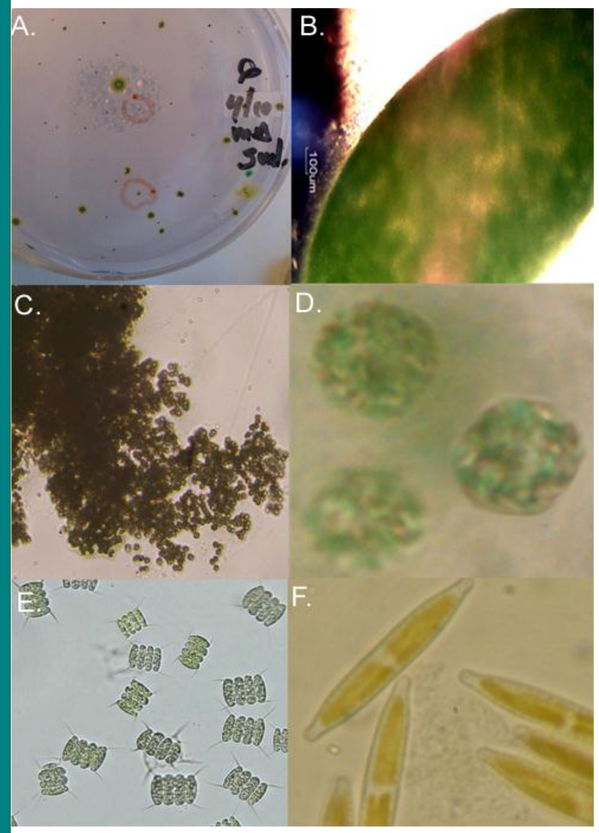
Methods and Materials

Algal isolations and identifications were performed from surface bloom samples from Grand Lake St. Mary's using a combination of streak and pour plating on cyanobacterial media/agarose plates.(3) Samples were visually evaluated against sample isolates provided by Japanese National Institute for Environmental Studies (MCC-NIES sequenced strain NIES-843) and University of Texas (unialgal UTEX 2385). Confirming taxonomic identifications were provided by Dr. Miriam Kannan at NKU.

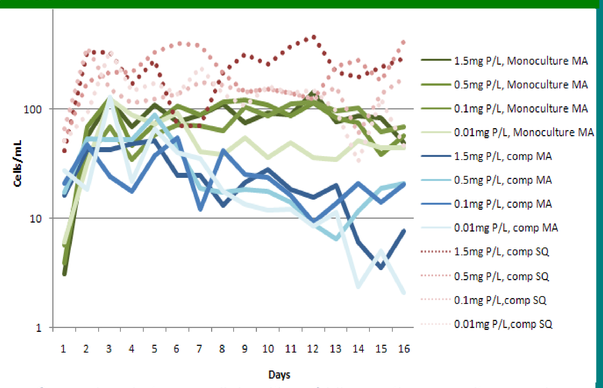
Phosphorous competition cultures were acclimatized to environmental chamber conditions reflective of summer bloom-forming conditions (30C, 16:8hr days and 300mol/m^{-2} s^{-1} photons) and grown in BG-11 nutrient media, in monoculture or in competition, with phosphorous limited to levels reflective of oligotrophic through hypereutrophic environmental Carlson trophic states.(1) Cell densities were evaluated over 14 day trials via brightfield microscopy on hemocytometers after boiling to separate *Microcystis* colonies.(2)

Preliminary Results

Five unialgal cultures of *Microcystis* were successfully isolated. Additional cultures of a chlorophyte (*Scenedesmus quadricauda*) and a diatom (*Nitzschia sp.*) were also isolated for nutrient competitions.



Plating isolation (A) and identification of *Microcystis* sp. Colonies are surrounded by a collective mucilage sheath(B), form large random-shaped, grazer-resistant colonies(C), and produce gas vacuoles that enable colonies to stay high in the water column (D). *S. quadricauda* (E) and *Nitszchia* sp. (F) were also isolated



Comparison between cell densities of *Microcystis* monocultures and Microcystis grown with *Scenedesmus quadricauda* at four phosphorous treatment levels

Microcystis growth was lowered in competition with *Scenedesmus* at all phosphorous levels, however St. Marys isolates showed less variation in cell densities between phosphorous treatments than preliminary testing using UTEX lab strains. pH levels were found to be raised appreciably from starting neutral media levels of 7.1-7.2 pH.

	1.5 mg P/L	0.5 mg P/L	0.1 mg P/L	.01 mg P/L
Microcystis trial 1	8.73	8.75	8.47	6.65
Microcystis trial 2	8.75	8.75	8.85	7.50
Microcystis trial 3	8.88	8.87	8.87	7.70
M.A. vs. S.Q	8.91	8.88	8.93	8.93

Final pH measurements in test cultures

Discussion

Preliminary isolations and quantification methods appear to be producing promising results that may yield information about nutrient competition in algal bloom communities.

Environmental isolates appear to be more sensitive to pH than long-term cultured lab organisms such as those maintained by UTEX. Published formulations of BG-11 media do not routinely include buffering compounds, but are mostly usually used to proliferate laboratory isolated acclimated to high pH cultures.

Future direction

Microcystis monocultures and nutrient competitions with both *Nitzschia* and *Scenedesmus* will be repeated and evaluated after addition of tricine as a buffering compound.

Microcystis blooms are often identified by purely visual characteristics; in addition to visual identification, the EPA has since confirmed production of microcystin toxins in all our isolates from GLSM via ELISA, supporting taxonomic assessments. Genetic analyses via intergenic repeat sequence PCR and 16s ribosomal sequencing comparisons are also planned.

The biological role of microcystins is not well understood, with allelopathy and herbivory studies producing conflicting results. As studies investigating microcystin allelopathy in *Nitzschia* are lacking, we will perform growth inhibition experiments using our *Nitzschia* isolates and exposing them to purified toxins. The results of this will clarify any possible allelopathy present in the nutrient competitions.

References & Citations

1) Carlson, R. E. (1977). A Trophic State Index for Lakes. *Limnology and Oceanography* , 22, 361-369.

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4) Smayda, T. (2008). Complexity in the Eutrophication–Harmful Algal Bloom Relationship, with Comment on the Importance of Grazing. *Harmful Algae 8* , 140-151.