

Assessing species-specific phytoplankton phosphorus limitation and competition for phosphorus uptake across the trophic gradient in Lake Erie.

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Executive Summary:

Using a combination of observational and manipulative lab experiments we propose to address the role of species-specific phosphorus limitation as a mechanism driving phytoplankton community dynamics in the western basin of Lake Erie. In particular, we are interested in understanding the environmental and physiological conditions that allow the toxic cyanobacterium *Microcystis aeruginosa* to achieve bloom proportions in the late-summer under low phosphorus loading conditions that were previously thought to limit *Microcystis* growth. Selective grazing by zebra mussels on other taxa besides *Microcystis* has been cited as one mechanism allowing *Microcystis* to flourish, however intense phosphorus recycling by zebra mussels could create spatially and temporally heterogeneous patches of elevated phosphorus that could also allow *Microcystis* to out-compete other taxa such as diatoms. In order to test the latter hypothesis, we will first assess the level of phosphorus limitation of natural phytoplankton communities along a phosphorus-loading gradient (east to west from Maumee Bay) using a new technique for assessing alkaline phosphatase activity (APA) at a species-specific level. In addition, we conduct bio-assays on collected phytoplankton communities to determine the species-specific relationship between nutrient-deficiency, alkaline phosphatase activity and nutrient uptake kinetics in order to determine the physiological conditions of competing phytoplankton taxa across and nutrient gradient.

Scientific Rationale

Project Description

It is reasonably well-established that zebra and quagga mussel (*Dreissena polymorpha* and *Dreissena burgensis*) invasion to the Great Lakes region is the cause of recent increases in biomass and blooms of the toxic cyanobacterium *Microcystis aeruginosa* in both inland lakes and shallow basins of the Great Lakes (Vanderploeg et al. 2001, 2002, Raikow et al. 2004, Sarnelle et al. 2005). A solid understanding of the mechanisms driving this interaction is necessary to design a management strategy that can mitigate the factors that lead to bloom conditions. One mechanism that has empirical support involves selective feeding by *Dreissena* on non-*Microcystis* taxa (Vanderploeg et al. 2001). However, survey work suggests an important and complex role of trophic state (nutrient loading) (Raikow et al. 2004), so an alternative, but not exclusive, mechanism may involve nutrient-mediated dominance by *Microcystis* over other phytoplankton taxa.

Nutrient-mediated mechanisms explaining the recent shift in dominance by *Microcystis* could involve environmental changes in Lake Erie, such as changes in nutrient ratios that would favor *Microcystis* over other taxa (Tilman et al. 1982) or a combination of temporal and/or spatial heterogeneity of available phosphorus combined with specialized physiology of *Microcystis* to allow the population to persist in an otherwise unfavorable phosphorus-limited environment (Sommer 1985). With respect to important nutrient ratios, the Si:P ratio appears to have remained the same (Makarewicz et al. 2000) or even increased (Holland et al. 1995), indicating that diatoms, which make up the bulk of the spring and summer phytoplankton biomass, are not silica-limited and therefore Si:P ratios would not favor *Microcystis*. In contrast, soluble reactive phosphorus (SRP) levels have increased in the western basin since the introduction of *Dreissena* (Makarewicz et al. 2000), likely as response to the more than 10-fold increase in the recycling rate of phosphorus attributable to *Dreissena* (Arnott and Vanni 1996) and lower overall biomass of phytoplankton (Makarewicz et al. 1999). Furthermore, *Dreissena* reefs in Lake Erie are not uniformly distributed and benthic boundary layer dynamics can result in concentrated recycled nutrient lenses near the reef surface (Ackerman et al. 2001) that are mixed with the rest of the water column intermittently according to meteorological and current conditions.

Given some evidence of an overall increase in SRP levels and locally-concentrated nutrient patches in the western basin as a possible indirect effect of *Dreissena*, it is therefore necessary to examine the role of phosphorus in mediating the competitive interaction of *Microcystis* and other non-toxic phytoplankton. Typical equilibrium-based models (Tilman et al. 1982) that predict competitive exclusion of *Microcystis* by most diatoms and even some chlorophytes may not adequately predict mixed-culture competition experiments if phosphorus is added in periodic pulses (Sommer 1985). Common taxa that are poor phosphorus competitors can persist under overall P-limited conditions, given that they can achieve temporarily rapid growth rates right after a pulse (velocity-specialists sensu Crowley 1975) or they are able to take up large-quantities of phosphorus during pulses to sustain growth after the ambient P-levels

decrease (storage specialists sensu Sommer 1985). *Microcystis* is not a good competitor for phosphorus compared to other taxa (i.e. external P-requirement for growth is high) and has a low maximum growth rate compared to other taxa, so it appears that *Microcystis* cannot persist in pulsed conditions as a velocity-specialist. However, *Microcystis* does have relatively high capacity for acquiring and storing phosphorus that could allow it to persist or even out-compete other taxa in pulsed conditions (Olsen 1989).

As an alternative to equilibrium competition models (Tilman et al. 1982) the variable internal stores model (VIS) accounts for the nutrient status of the cells (cell quota) in driving cell division rates and ultimately competitive ability (Droop 1973, 1974). Briefly, cell division can only occur at a minimum cell quota and growth rate increases as a function of the cell quota above this minimum. Modifications of the cell quota concept have also been applied to the uptake rates of the limiting nutrient, whereby uptake rates decline as the maximum cell quota is reached (Brown and Harris 1978). The result of these modifications provides a quantitative explanation for how taxa such as *Microcystis*, with comparatively low maximum growth rate (μ_{\max}) and high minimum phosphorus requirement for growth (Q_{\min}), could dominate if the species/taxa is a superior competitor for acquiring excess phosphorus during nutrient pulses (Grover 1991). Specifically, two physiological strategies could be utilized by *Microcystis* under pulsed conditions: one is to have a higher phosphorus uptake rate ($U_{\text{Microcystis}} > U_{\text{competitor}}$) under all phosphorus cell quotas; another strategy is to have a higher maximum cell quota such that competing taxa reach Q_{\max} earlier after a pulse and thus $U_{\text{competitor}}$ approaches 0, while $U_{\text{Microcystis}}$ remains high until the nutrient pulse is consumed. Under either strategy, the relative uptake rate (uptake P cell⁻¹ pulse⁻¹) needs to be higher for *Microcystis* in order to more biomass of *Microcystis* per pulse compared to the competing taxa.

Competition experiments have demonstrated that *Microcystis aeruginosa* can outcompete *Straurastrum luetkemulleri* (Chlorophyceae) under pulsed, but not continuous, phosphorus conditions despite having a higher minimum cell quota and lower maximum growth rate (Olsen et al. 1989). This shift in competitive advantage was attributed to the ability for *Microcystis* to acquire up to 10x more phosphorus than *Straurastrum* during pulses (1 to 60 ug P L⁻¹) under a range of background phosphorus levels similar to Lake Erie (0 to 30 ug P L⁻¹) (Olsen 1989). Depending on the phosphorus uptake physiology of competing taxa in Lake Erie, *Microcystis* may be able to utilize transient patches of high phosphorus in a similar fashion to alter the apparent competitive advantage held by fast-growing diatoms and low P-quota taxa based on equilibrium based models (e.g. Tilman et al. 1982).

Some of the parameters needed to verify this hypothesis would be the phosphorus status (Q_0 to Q_{\max}) of *Microcystis* and other taxa and the relative uptake rates of phosphorus as a function of the taxa's cell quota. Given these parameters, we can then establish whether *Microcystis* has the capacity to out-compete other taxa if there is a patchy phosphorus environment in the Lake Erie. A potential phosphorus-mediated explanation for recent blooms of *Microcystis* in Lake Erie would provide the foundation for additional research to determine the relative importance of direct effects like selective grazing by *Dreissena* (Vanderploeg et al. 2001) or indirect effects like nutrient-recycling as a cause and effect mechanism linking *Microcystis* blooms to the invasion of *Dreissena* to Lake Erie and inland lakes.

Project Objectives

- 1) Assess the phosphorus status (cell quota, Q_0 to Q_{\max}) of *Microcystis* vs. competing taxa across a phosphorus gradient in the western basin of Lake Erie leading eastward from Maumee Bay.
- 2) Determine whether taxon-specific alkaline phosphatase activity (APA) can be used as a proxy for P cell quota.
- 3) Determine phosphorus uptake kinetics of *Microcystis* and competing taxa as a function of cell quota in order to calculate the relative phosphorus uptake potential for the species/taxon during pulsed phosphorus conditions.
- 4) Conduct a competition experiment using the natural phytoplankton assemblage from Lake Erie in which the frequency and intensity of pulses of P input are manipulated to determine whether these variables contribute to the success of *Microcystis* in Lake Erie.

Project Approach/Methods

Taxon-specific assessment of phosphorus status across a phosphorus gradient

We will employ a suite of techniques, both established and newly-emerging, to determine the phosphorus status (cell quota) of the phytoplankton community at a taxon-specific level across the phosphorus gradient extending eastward from Maumee Bay in the western basin of Lake Erie. To enhance collaborative efforts to understand the biotic and abiotic factors leading to *Microcystis* blooms and reduce redundant sampling and analyses, we would like to pair our sampling with the research programs of Hank Vanderploeg and Gary Fahnenstiel of NOAA-GLERL who will be investigating the role of nutrient recycling by *Dreissena* and environmental factors leading to HABs in the western basin of Lake Erie during 2005. For our investigation, we will collect water samples from at least three locations extending from a high-phosphorus station near Maumee bay to a low-phosphorus station near the middle of the western-basin. If possible, we would like to take discrete water samples (with a van Dorn sampler) near and far from *Dreissena* reefs to identify vertical and horizontal heterogeneity in nutrient concentrations that could serve as a likely source of phosphorus pulses in Lake Erie. More importantly, we will take vertically-integrated water samples from each of the sampling stations to collect a representative assemblage of the phytoplankton community.

In conjunction with standard nutrient analyses (especially partitioning of SRP, dissolved and particulate phosphorus) and analyses of taxon-specific biomass of the phytoplankton community at each of the stations, we will also apply a relatively new technique for assessing alkaline phosphatase activity (APA) of phytoplankton at a taxon-specific level. Alkaline phosphatase is an enzyme produced by most taxa to cleave phosphate from organically-bound phosphorus and is widely accepted as an indicator of phosphorus deficiency in lake water (Pettersen 1980). Until recently, only whole water assays of APA were available, which though useful for assessing overall phytoplankton community P-deficiency, do not provide taxon-level information about P-status. A new assay that attaches an insoluble fluorescent label at the site of membrane-bound alkaline phosphatase of algal cells allows for qualitative and quantitative assessment of APA for individual species/taxon (Gonzales-Gil et al. 1998). This technique has been applied successfully to both marine and freshwater systems and APA-labelling has been

confirmed for many common freshwater taxa including *Microcystis aeruginosa* and many common species of diatoms, cryptophytes, dinoflagellates and chlorophytes (e.g. Rengefors et al. 2003).

We expect APA expression (as % of total cells expressing fluorescence) to increase across all taxa as a function of decreasing phosphorus levels along the phosphorus gradient from west to east in Lake Erie. This observation should also correspond to increased community expression of APA measured by traditional bulk APA assessment (Pettersson and Jansson 1978). However, we predict that APA will differ among the major taxa depending on relative P-deficiency. Therefore, species/taxa with low P-requirements (low Q_0) should be satiated with P at lower ambient phosphorus levels compared to taxa with high P-requirements such as *Microcystis*. Thus, we also predict that *Microcystis* will have higher cell-specific APA than competing phytoplankton taxa in the western basin in general. The latter lack of satiation in *Microcystis* provides the scope for responding to pulses of high P. Besides visual categorization of APA labeling using epi-fluorescence microscopy, we will also employ flow cytometry to provide a quantitative assessment APA fluorescent signatures of a large number of phytoplankton cells that can be categorized according to size and excitation of phycocyanin (corresponding to cyanobacteria). Similar studies (Dignum et al. 2004) were able to partition APA activity among cyanobacteria and other taxa using a flow cytometry arrangement similar to the one we will use on campus at Michigan State University. Conveniently, the fluorescence labeling process can be conducted immediately in the field and samples can be preserved for up to a month for later analysis in East Lansing. Fluorescence determination of taxon-specific APA will be paired with empirical measurements of P cell quota (particulate phosphorus per cell) after separating *Microcystis* from the remaining taxa through density fractionation. This will allow us to compare APA activity to P-cell quota of the fractionated seston.

Bioassay experiments to determine P uptake kinetics as a function of P cell quota for populations of Microcystis and competing taxa across the phosphorus gradient

Immediately upon collection of lake water, we will fractionate natural populations of *Microcystis* and competing taxa in order to create relatively mono-specific assemblages of *Microcystis* and mixed-species cultures of competing taxa to conduct phosphorus uptake assays on the ship. These assays will complement the taxon-specific measurements of APA and P cell quota across the natural phosphorus gradient in Lake Erie. Uptake rates for *Microcystis* and “competing taxa” assemblages at each of the sampling stations will be assessed by removal of supplemented SRP. We predict that P-uptake (SRP removed $\text{cell}^{-1} \text{minute}^{-1}$) will be highest for P-limited assemblages (taxon-specific Q_0) and lowest for P-replete assemblages (taxon-specific Q_{max}), which should vary across the natural phosphorus gradient.

Competition experiment between Microcystis and competing taxa under varying cell quotas and varying intensity and frequency of phosphorus pulses

Based on our assessment of the P-deficiency (cell quotas and APA expression) for *Microcystis* and competing taxa across the phosphorus gradient in Lake Erie and our analysis of quota-dependent phosphorus uptake rates in the bioassays, we will be able to estimate the relative uptake potential of *Microcystis* compared to competing taxa at each of the stations when we sampled. We expect that *Microcystis* will be relatively more P-deficient owing to a higher

Q_{\max} than other taxa, and therefore may maintain higher P-uptake rates than competing taxa that have reached Q_{\max} . If *Microcystis* has a higher relative uptake rate than other taxa, then *Microcystis* may be able to out-compete other taxa under pulsed phosphorus conditions as was observed for competition experiments between *Microcystis aeruginosa* and *Straurastrum luetkemulleri* (Olsen et al. 1989). To test this hypothesis, we will conduct a competition experiment in the laboratory using natural phytoplankton assemblages collected from Lake Erie during the cruise. Mixed assemblages (*Microcystis* and competing taxa together) will be cultured under pulsed or continuous P supply to maintain three levels of overall P concentrations corresponding to those found across the phosphorus gradient in the western basin of Lake Erie. We expect that competing taxa will dominate at very low overall P concentrations and under a continuous P supply regime while *Microcystis* may be able to dominate at intermediate P-supplies and under pulsed conditions if it is superior competitor for acquiring and storing phosphorus pulses.

Project Relevance

Our proposed research directly applies to the one of the primary goals of the IFYLE program to characterize and understand the physical and biological factors leading to harmful algal blooms. The main thrust of our research is to understand the role of phosphorus as a “habitat field” that can influence *Microcystis* formation. This information can be directly applied to forecasting models to predict phosphorus conditions that can lead to blooms. Secondly, our investigation utilizes a new technology to assess the physiological nutrient status of individual phytoplankton taxa. This rapid assay combined with our analyses of taxon-specific phosphorus uptake could be incorporated into future sampling efforts on Lake Erie to forecast blooms based on the physiological condition of the phytoplankton community.

Collaborations/Project Linkages

The investigation of *Microcystis* blooms, and HABs in general, is a focus of several researchers at NOAA-GLERL with our project having the most relevance to the research programs of Hank Vanderploeg and Gary Fahnenstiel. In discussions with Fahnenstiel, our proposed investigation of physiological and environmental conditions leading to blooms would dovetail nicely with his research plans to determine the environmental factors controlling microcystin toxin production and primary production in the western basin. Vanderploeg’s continuing research on the filtering and nutrient excretion of *Dreissena* will provide the basis for determining whether *Dreissena* reefs could be producing a heterogeneous phosphorus environment (pulses) that our research may determine favors *Microcystis* dominance.

Governmental/Societal Relevance

Microcystis blooms have developed into a major societal concern primarily due to its propensity to produce a potent liver toxin (Codd 1995) and that can pose a direct threat to public health (Carmichael and Falconer 1993, Chorus and Bertram 1999). To this extent, drinking water standards for *Microcystis* toxins were exceeded during a bloom in 1997 in Saginaw Bay (Vanderploeg 2001). In response, the importance of harmful algal blooms like *Microcystis* is reflected in the priority goals of several funding agencies of the national ECOHAB program (NOAA, EPA, NSF) and by the research goals of the Lake Erie Rapid-Response Funding Program (NOAA-GLERL) to “describe and forecast the development of harmful algal blooms in Lake Erie” and encourage “novel research concerning the formation of HABs in western Lake

Erie.” Our research will provide the scientific basis for understanding the role of the phosphorus environment in mediating the dominance of *Microcystis* over other less-harmful phytoplankton. Some aspects of the phosphorus environment, such as total phosphorus loading, can be controlled by policy measures and therefore our research could serve an insightful role in policy decisions.

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