

INTEGRATED FIELD AND LABORATORY INVESTIGATION OF LINKS BETWEEN NUTRIENTS, DISSOLVED ORGANIC CARBON, REACTIVE OXYGEN SPECIES, AND TOXICITY OF HARMFUL ALGAL BLOOM

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Overview and Objectives:

This project aims to uncover the environmental and ecological factors that shape the toxicity of cyanobacterial harmful algal blooms (CHABs). These CHABs are a threat to freshwater ecosystems and human health worldwide. Despite decades of study and advances in understanding the primary causes of CHABs, the drivers of toxicity are still poorly understood. Specific objectives are as follows:

1. Sequence the genomes of a collection of Great Lakes cyanobacterial isolates to provide a genetic framework for interpreting laboratory and field experiments.
2. Investigate the physiological and genetic response of toxic and non-toxic Great Lakes *Microcystis* strains, individually and in competition with each other, to elevated ROS (H₂O₂) concentrations under nitrogen (N) or phosphorus (P) replete and deplete conditions.
3. Conduct field experiments spiking natural bloom populations with increased ROS concentrations to understand how the natural assemblage of phytoplankton in general and specifically the *Microcystis* cells are responding at a physiological and genetic level.

Accomplishments:

Although we were unable to find a PhD student to start in Fall of 2014 as hoped, the Long Term CILER Fellowship allowed us to recruit a graduate student, Derek Smith, who will start the Department of Earth and Environmental Sciences PhD program in Fall of 2015. As of June 1 Mr. Smith is working with PIs Dick, Johengen, and Davis as a temporary employee. He is participating in weekly sampling of Lake Erie, enabling our team to continue a molecular biology sampling program in 2014 that was funded by the U-M Water Center.

In the meantime, we have completed the first objective of the project, genome sequencing and annotation of 13 strains of Great Lakes cyanobacteria. These include major HABs species (*Microcystis*, *Planktothrix*, *Dolichospermum/Anabaena* and

Synechococcus). Interestingly, genes for catalase, the main enzyme for decomposing and thus tolerating H₂O₂, is absent from both of the Lake Erie *Synechococcus* isolates. This gene is also absent from *Prochlorococcus* genomes, accounting for their sensitivity to H₂O₂. Searching publicly available genomes on Integrated Microbial Genomes, we found that of the 30 *Synechococcus* genomes sequenced, 16 have genes for catalase, showing that its presence is variable in this clade. Curiously, only one of the 15 available *Microcystis aeruginosa* genomes has catalase, indicating that its high tolerance of oxidative stress must be due to other pathways or traits, such as microcystins.

To explore this hypothesis, initial field experiments were conducted on Lake Erie HAB communities. Whole lake water was incubated in 2L microcosms in triplicate and either left alone to serve as controls or supplemented with increased H₂O₂ (ROS), nitrogen and phosphorus or both. At the end of the experiment (48 hours) we sampled for microbial community composition, toxic and non-toxic strains of *Microcystis*, gene expression (RNA), total, dissolved and conjugated microcystins and pigments (chlorophyll *a* and phycocyanin). Our initial results show that ROS alone did not affect pigment or microcystin concentrations. However, increased nutrients elevated all pigments and total particulate microcystins. When ROS and nutrients were combined the results were similar to the nutrients only for pigment response but particulate microcystins were lower and no change in % conjugated microcystins was observed. In none of the treatments did the dissolved microcystins significantly differ from the control. These results are intriguing and follow up experiments are planned for this summer on-board the Canadian research vessel *Limnos*. Further the long-term fellow (Smith) is currently setting up laboratory experiments to investigate the response of microcystin-producing Lake Erie strains of *Microcystis* to varying N, P and H₂O₂ conditions.

Publications:

Four associated publications are currently in preparation. First, a review article focused on methods of molecular studies of CHABs for a special issue of Harmful Algae. Second, a study of the concentrations and sources of H₂O₂ in Lake Erie. Third, a study of microbial communities, toxins, ROS, and nutrients in Lake Erie CHABs throughout the 2014 season. Fourth, a study of the effect of experimentally added H₂O₂ to natural CHABs communities. We plan to submit the first three this summer while the fourth will likely be submitted this fall.

Presentations:

Davis, T.W. et al., Using an integrated approach to better understand the environmental drivers of toxic cyanobacterial harmful algal blooms in Lake Erie, USA, International

HABs symposium, Korean Institute of Science and Technology, Seoul, South Korea, May 2015

Davis, T.W., et al., *Why are our lakes the same color as our lawns? Understanding the role of eutrophication and climate change in promoting Cyanobacterial Harmful Algal Blooms (CHABs)*, University of Illinois HABs workshop, Champagne-Urbana, IL, May, 2015

Dick, G.J. et al. *Omic approaches to freshwater microbial communities and CHABs*. Workshop on Global Solutions to Regional Problems: Collecting Global Expertise to Address the Problem of Harmful Algal Blooms, Bowling Green State University, April 2015. [oral presentation]

Meyer, K.A., T. Davis, S. Jain, S.B. Watson, G.S. Bullerjahn, R.M.L. McKay, and G.J. Dick. *Genome sequencing of thirteen Great Lakes cyanobacteria*. Workshop on Global Solutions to Regional Problems: Collecting Global Expertise to Address the Problem of Harmful Algal Blooms, Bowling Green State University, April 2015. [poster presentation]

Jain, S. and G.J. Dick. *SuperMoM: A graph-based database to store and analyze community omics and environmental data*. Workshop on Global Solutions to Regional Problems: Collecting Global Expertise to Address the Problem of Harmful Algal Blooms, Bowling Green State University, April 2015. [poster presentation]

Berry, M.A., P. Den Uyl, R.M. Cory, T. Davis, M. Duhaime, G.W. Kling, T.H. Johengen, G.J. Dick, and V.J. Denef. *Lake Erie bacterial community dynamics during a toxic algal bloom*. Workshop on Global Solutions to Regional Problems: Collecting Global Expertise to Address the Problem of Harmful Algal Blooms, Bowling Green State University, April 2015. [poster presentation]

Outreach Activities:

We helped to co-organize a symposium on CHABs held at BGSU April 13-15. This included two sessions for which we were the primary organizers: a poster session on CHABs April 13th (> 100 attendees) and a workshop and oral session on “omic approaches to CHABs on April 15th (~40 attendees).

Relevant Web content:

We are developing a public web portal for a database that will store the genome sequences and offer tools for analysis of omics data. In the meantime, we have constructed a github website to disseminate our code and tools:

<https://github.com/sunitj/SuperMoM>

Supplemental Material:

None to date.