Combining monitoring, advanced molecular techniques and near real-time instrumentation to investigate the response of cyanoHABs in Lake Erie and Lake St. Clair to different environmental conditions

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Co-authors & Funding Great La Restor

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Lake St. Clair bloom during late August, 2013



Spatial sampling of Lake St. Clair, 23 August, 2013

Samples collected (17 sites)

- Sterivex filter (DNA)
- Cell enumeration (Lugols)
- Dissolved and total nutrients

Dearborn

FI (Fighting Island)

• Total microcystins (ELISA)



Genetic analysis

- Targeted the *mcyA* region of the MC gene operon
- All samples yielded PCR products for mcyA
- Samples were sequenced at GENEWIZ, NJ

Microcystis spp. dominated the cyanobacterial community



Site



MC producers were homologous throughout LSC and similar to strains in Lake Erie and Lake Ontario

- Maximum-likelihood tree was generated using the Jones-Taylor-Thornton (JTT) algorithm
- Bootstrap values were obtained for 1,000 replicates
 - Bay of Quinte, Lake Ontario Hamilton Harbour, Lake Ontario Lake St. Clair
 - Northshore Lake Erie



- Planktothrix spp.

Davis et al., 2014; PLOS ONE



Using molecular tools to monitor CHABs:



<u>% toxic Microcystis</u> = proportion of Microcystis cells containing the genetics to produce microcystins (mcyD or E / 16S x 100)

Determined by (1) qPCR and (2) metagenomics

Shift in toxicity not due to changes in



<u>Community shifts correlate with (1) seasonal</u> <u>parameters (e.g., temp) (2) the bloom (pH)</u>



Berry, Davis et al., in press, Environmental Microbiology

<u>Microcystis has ~1900 core genes</u> and a diverse flexible genome



- Within an individual strain 40-50% are core genes
 - Prochlorococcus 40-67% (Kettler et al., 2007 PLoS Genetics)
 - Anabaena 40% (Simm et al., 2015 Frontiers in Microbiology)
- Of all genes identified
 - 11%-14% of identified genes are highly represented (core genome)
 - 62% of genes are rare (only found in 1-2 strains)

Meyer, Davis et al., subm., PLoS ONE

<u>Many of the strain specific genes found in new</u> <u>Microcystis</u> isolates involve genome flexibility

	Microcystis aeruginosa LE3	Microcystis cf aeruginosa/ botrys LSC13-02	<i>Microcystis wesenbergii</i> LE013-01
Unique genes	195	475	232
Transposases	5	13	5
Transferases	12	16	21
Endonucleases	6	20	1
CRISPR-Cas	5	1	0

- Most Great Lakes strain specific genes are currently hypothetical
 - LE3: 42%
 - LSC13-02: 43%
 - LE013-01: 43%

Meyer, Davis et al., subm., PLoS ONE

Toxic strains decline with lower nitrate concentrations



Microcystin synthesis genes up-regulated within 4 hours of exposure to increased N



Bars = Mean \pm SE; A and B = significantly up-regulated (p < 0.05)

Chaffin, Davis, et al, in prep.

Nitrogen constrains growth and toxicity of Planktothrix in Sandusky Bay

 $NH_4 + PO_4$ - $NO_3 + PO_4$ -+Urea only- $+NH_4 only$ - $+NH_3 only$ -+PO₄ only- $Urea + PO_4$ Control 10 16 After 48 hours of incubation



Davis et al., 2015 ES&T

Niche differentiation among cyanobacterial populations



Microcystis is an excellent P scavenger



Microcystins may protect cyanobacteria from oxidative damage....

The Cyanobacterial Hepatotoxin Microcystin Binds to Proteins and Increases the Fitness of *Microcystis* under Oxidative Stress Conditions

Yvonne Zilliges¹, Jan-Christoph Kehr², Sven Meissner², Keishi Ishida³, Stefan Mikkat⁴, Martin Hagemann⁵, Aaron Kaplan⁶, Thomas Börner⁷, Elke Dittmann²*



<u>Colony-associated catalase gene expression shows similar</u> trends to microcystins concentrations



Microcystis and many other cyanos lack catalase genes



n = number of genomes queried



48 hours @

ambient light and temperature

 $+H_2O_2$ treatments = 1081 ± 439 nM Control treatments = 364 ± 158 nM

(Average ± SD of 18 measurements)



Isolation of bloom-associated heterotrophic bacteria from Lake Erie



compare to LE 16S

Isolate	Class	Family	Genus	Boot-	Seq-	OTU ^c	OTU	Cata-
Code				strap	match		%ID	lase
LE-L5	Bacilli	Bacillaceae		100			96.1	
PE-	vor half c	fallicol	atos nro	C II		ata	lac	Δ ς
PE-5			accs pro	u u	0.988	ala	194.5	CS
	Alphaproteobacteria							

<u>Cultured bacteria are abundant during Lake Erie</u> <u>cyanoHABs, rapidly decompose H₂O₂</u>



<u>Heterotrophic bacteria protect</u> <u>Microcystis</u> from external H₂O₂

Dependence of the Cyanobacterium *Prochlorococcus* on Hydrogen Peroxide Scavenging Microbes for Growth at the Ocean's Surface

J. Jeffrey Morris¹, Zackary I. Johnson², Martin J. Szul¹, Martin Keller³, Erik R. Zinser¹*

. PLoS ONE | www.plosone.org

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Future directions

- How do the results from Lake Erie compare to Saginaw Bay and Green Bay?
- Investigate the ecological adaptations of Great Lakes CHAB species
 - Further understanding the interactive roles of light, nutrients, and temperature on toxin production and community composition
- Develop an ESP network for western Lake Erie
- Further develop the ESP capabilities
- Develop toxin prediction models
- Investigate changes in microcystin congeners over time
- Develop a genomic database for Great Lakes cyanobacteria



