Project Title: Lake Erie Center for the Great Lakes and Human Health

Funding Period: Sept. 1 2018-Aug. 30, 2023

The Lake Erie Center Project Description:

The National Institutes of Health (NIH) and the National Science Foundatio (NSF)-supported Lake Erie Center for Fresh Waters and Human Health is supporting policy relevant research examining the influence of climate change on the proliferation and toxicity of cyanobacterial Harmful Algal Blooms (cHABs) and their impact on human health (Figs. 1 and 2).

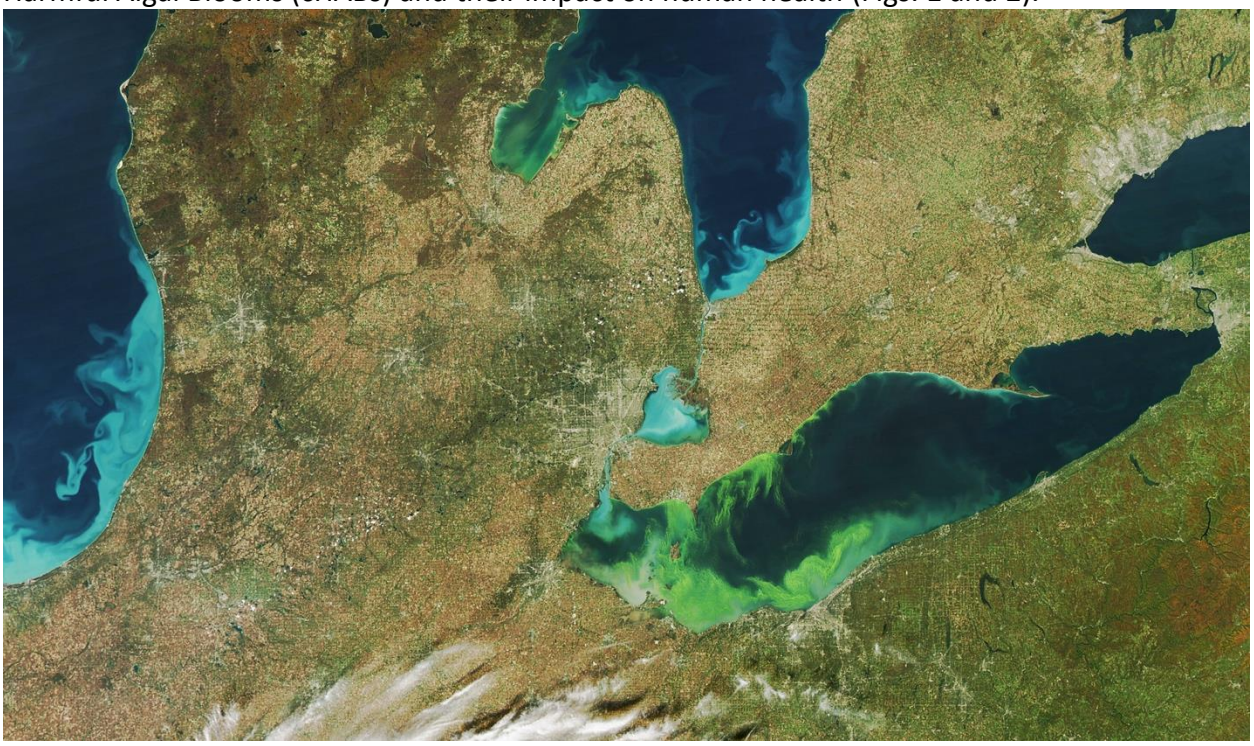

Figure 1: Remote sensing (NASA-MODIS) image of cyanobacterial blooms on Lake Erie, USA-Canada, that triggered the Toledo drinking water crisis in 2014.

Figure 2: Aerial photograph of the cyanobacterial bloom in Western Lake Erie. Photograph courtesy of T. Essick, National Geographic Magazine.

The Center at Bowling Green State University (BGSU) has received funding for five years (2018-2023) from the Centers for Oceans and Human Health 3: Impacts of Climate Change on Oceans and Great Lakes program (COHH3). Support is for research and facilities operations and community engagement activities that build on robust academic and agency partnerships that have developed in the wake of the 2014 Toledo Water Crisis (Fig. 3).

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Toledo-area water advisory expected to continue through Sunday as leaders await tests; water stations to remain open

Microcystin found in samples; boiling not recommended

Locating this COHH3 at BGSU reflects a leading role assumed by BGSU personnel studying the chronic cyanobacterial Harmful Algal Blooms of Lake Erie, and complements significant research investments made by BGSU. A COHH3 Lake Erie Center for Fresh Waters and Human Health will promote interinstitution collaboration to generate cutting-edge research on identifying the environmental drivers of cHABs and the effect of a changing climate on the proliferation and toxicity of bloom events and their impact on human health. This Center will shape the national research agenda; promote community engagement; and leverage strong institutional and agency support. BGSU personnel were pressed into action to help lead the scientific response to the August 2014 Toledo Water Crisis following contamination of Toledo's water supply by cHAB toxins. With the crisis still unfolding, BGSU coordinated with Center affiliates (Senior Personnel below) to study the bloom event and communicate issues related to the crisis to a concerned public. With the water crisis still receiving national attention, BGSU assumed the lead in organizing an international symposium held at BGSU in April 2015 that provided a forum to share ideas related to cHABs and their mitigation. BGSU also worked closely with federal legislators in the aftermath of the crisis to address issues of water quality in Lake Erie leading to the passing of H.R. 212, The Drinking Water Protection Act. The proposed research activities of Center affiliates are consistent with the goals and structure outlined for COHH3 with projects addressing cHABs in the framework of climate change, toxin discovery and toxicity, enhanced monitoring/forecasting and community engagement. This application pursues four overarching goals: 1) Heighten the scientific influence, innovation, and productivity of Center affiliates; 2)

Increase Center affiliate competitiveness for extramural funding; 3) Conduct three research projects whose collective goal is to understand drivers of cHAB growth, decline and toxicity 4) Provide information to community stakeholders and the scientific community through shared data and multiple outreach activities. These goals will be accomplished through integration of projects and by supporting four complementary cores: Administrative, Research, Community Engagement, and Facilities. Center affiliates are already providing national leadership, and our vision is to continue to foster an environment of innovation and collaboration that yields high impact research on cutting-edge issues related to climate change and its effect on cHABs and their impact on human health.

While BGSU will house the Center by providing the Administrative Core (see below) and lead one of the three research projects (Project 1), the Center is a collaborative entity that involves university and agency partners from several institutions that include the following Senior Personnel:

Greg Boyer, SUNY Environmental Sciences and Forestry (Facilities Core) Justin Chaffin, Ohio State University Stone Laboratory (Facilities and Community Engagement Cores) Heather Triezenberg, Michigan State University Michigan Sea Grant (Community Engagement Core) Hans Paerl, University of North Carolina (Research Project 1) Steven Wilhelm, University of Tennessee (Research Project 1) Greg Dick, University of Michigan (Research Project 2) David Sherman, University of Michigan (Research Project 2) Tom Bridgeman, University of Toledo (Research Project 3) Greg Doucette, NOAA (Research Project 3)

Research projects described in this request address overarching thematic priorities:

How do environmental cues (climate change, nutrient availability) promote or constrain the proliferation of cHAB species in mixed populations?

How do these environmental cues promote or constrain toxin production of cHAB species? How do other members of the microbial community promote or constrain growth and toxin production by cHAB species?

The UNC-CH Component Study:

Research Project 1: The synergistic effects of climate and anthropogenic drivers on toxic cyanobacterial blooms. George S. Bullerjahn and Robert M. McKay (BGSU), with Hans Paerl (University of North Carolina) and Steven W. Wilhelm (University of Tennessee).

Specific Aims

Competition between co-occurring microbes in nature is fierce and shapes not only environmental health, but often the interaction(s) of human populations with essential natural resources. Nowhere is this more apparent than in fresh waters, which globally are threatened by harmful cyanobacterial algal blooms (cHABs) (Harke et al., 2016). Indeed, the bloom of

Microcystis in Lake Erie in August 2014 (Bullerjahn et al., 2016) created a water crisis that forced the shutdown of the public water supply to over 400,000 people in Toledo, OH. Blooms in Lake Erie have been occurring sporadically for decades, and have now become a regular occurrence (Steffen et al., 2014), and climate change increases the likelihood for more expansive blooms, exposing larger populations to water-borne toxins (Paerl and Huisman 2009). While the growth and toxicity of cyanobacteria in Lake Erie has been studied for years, it is only recently that the tools of systems biology (combining ecology, molecular biology and environmental chemistry with limnetic techniques) have been brought to bear on this issue. The objective of this project is to determine the factors that contribute to biomass and toxin production by the cyanobacteria dominated microbial communities of the eutrophic waters of the Great Lakes. The physiology and success of the cyanotoxin producers *Microcystis* and *Planktothrix* will be tested to ascertain the contributions of climate and anthropogenic drivers to their success. The goals of this study are to develop the information necessary to determine the environmental cues promote or constrain the proliferation (i.e., growth and biomass accumulation) of cHAB species in mixed populations.

Our overarching aim in this project is to address the extent to which N availability and temperature play in the development, toxigenicity and persistence of cHAB taxa in Lake Erie and other bloom-affected freshwaters.

We hypothesize that temperature and N bioavailability are important drivers in yielding the specific composition of cHABs, and thus the effects of variations in N loadings and cycling rates can be differentiated from the effects of climate change. We will evaluate this hypothesis guided by a multidisciplinary approach that integrates environmental metagenomics, microcosm work and chemostat studies under controlled regimes of N availability and temperature. This study will be conducted in two eutrophic but contrasting systems, the western basin of Lake Erie (including Maumee Bay) and Sandusky Bay, in which cHABs routinely occur and act as trigger points for more widespread progression of cHABs.

Another and more general aim of this project is to serve as a data resource for the other projects integrated into the Center proposal. This research project provides the common source for transcriptomics and metabolomics for the Center.

To address the role of N availability and temperature in structuring HABs, we will test hypotheses using

4 systems biology approaches in molecular biology (RNA-sequencing analyses coupled to highly resolved bioinformatics), metabolomics and lipidomics (to examine cellular metabolism), assessments of toxin (concentration and chemistry) all coupled to a broad suite of environmental metrics. We will employ uni- and multivariate statistical approaches to resolve the major drivers of bloom formation and toxin production, which will subsequently be tested in microcosm assays in lab-based assays with chemostats to validate physiological mechanisms. Whereas all collaborating laboratories are involved in addressing all hypotheses, a separate institution (BGSU, UNC or UT) is designated to lead particular elements. Additionally, this project will be generating all the ‘omics data to be shared among the other projects working within the Center.

Hypothesis 1. Nitrogen availability controls cHAB community structure.

Hypothesis 2. Microcystin production is dependent on temperature and N speciation (nitrate vs. urea) Hypothesis 3. The success of cHAB species is promoted by the activity of the limnetic microbiome Our overarching goal is to these test hypotheses on samples collected from Lake Erie during the initiation, peak and demise of the annual cHAB events that have been occurring since 1995 (Brittain et al., 2000). To do this, weekly surveys (commencing in mid-May of Years 1-4 and continuing through to early October – date to be determined by the fall mixing event), will provide samples for biochemical, molecular and geochemical characterization. In parallel, we will collect other samples from other stations that will be reserved for targeted "event response" metrics: our goal here is to ensure we sample bloom conditions and have pre- and post-bloom samples. These samples would be critical in the instance that bloom events, which are notoriously patchy, do not exist at our specific sample locations. Finally, other event response samples from non-standard locations will be collected by the Center in response to bloom events: these may include samples collected upon notification of bloom initiation, or samples collected from locations where bloom taxa are particularly abundant. The Charter Boat Captain program (see Community Engagement Core) will likely provide these samples.

Hypotheses 1-3 above are reliant on the availability of multiple seasonal metatranscriptomes, metabolomes and lipidomes ('omics) obtained from the routine weekly sampling trips. Hypotheses 1 and 2 also will involve experimental manipulations of microcosms and chemostats to understand responses of cHAB communities to nutrients (specifically N) and temperature.

Testing Hypothesis 1: Nitrogen availability controls cHAB community structure (*Microcystis* vs. *Planktothrix*). This component of the project will focus on how *Planktothrix* and *Microcystis* compete in mixed microcosms of bloom biomass. Given our prior studies, we hypothesize that *Planktothrix* will dominate over *Microcystis* when N is limiting, and *Microcystis* will proliferate in N-replete conditions. However, prior to conducting these studies, we need to gain a broad perspective on the functioning of the individual bloom communities, as mentioned above we will collect water samples weekly from ten stations (May – Oct) in Years 1-4. This will provide a valuable dataset from a broad spectrum of stations on a regular basis. For detailed molecular analyses, we obtain water column metatranscriptomes and metabolomes from Maumee Bay (NOAA station WE02), Sandusky Bay (EC 1163) for Years 1-3. Two stations each week for up to 20 weeks each year will yield 40 metatranscriptomes for the first three years of the project. NOAA weekly surveys at WE02 and Ohio Department of Natural Resources Watercraft Division at EC 1163 will assist in water sampling throughout the study. Whereas NOAA site WE02 regularly is dominated by a *Microcystis* bloom, EC 1163 (in Sandusky Bay) is commonly dominated by *Planktothrix*. From four seasons, we will have a thorough picture of community gene expression during bloom development, maintenance and decline at both sites: this will inform us not only abut community function, but also provide insight into which members of the community are completing which tasks.

5 This work will be complemented by incubations of water samples with 15 N nitrate to obtain Michaelis Menten kinetic assessment of uptake rates (Dugdale and Wilkerson, 1986). These data will test our earlier hypothesis that *Planktothrix* is a more efficient scavenger for N species (Davis et al., 2015). Moreover, metabolomic analyses of these samples will allow for an

identification of processes associated with specific members of the community in a complement to sequencing information: while many metabolites/biochemicals are universal (e.g., ATP) others are often specific to a subgroup (e.g., photosynthetic pigments) and time course resolution of these incubations will resolve nutrient fluxes. And while competition for N may be a major factor by which *Planktothrix* dominates *Microcystis* in the nearshore, we cannot discount possible allelopathic effects, temperature or predation (e.g., viruses). However, each of these will have indicators within the community metatranscriptomes (Harke and Gobler, 2015, Steffen et al., 2015, Harke et al., 2017).

Metatranscriptomes obtained during the bloom seasons at all sites will identify active N cycling genes and their taxonomic affiliation, identifying microbes responsible for N sources (fixation) and sinks. From the *Planktothrix*- and *Microcystis*-dominated communities, genes expressed involved in N assimilation (e.g., glnA and nirA; Luque and Forchhammer 2008) and degradation of phycobilosomes and cyanophycin (nblA, cphAB; Collier and Grossman, 1994, Hai et al. 2002, Tonk et al. 2008; Van de Waal et al. 2010) will reveal how assimilation, luxury uptake and N utilization from internal stores are regulated during periods of high and low N availability. Microcystin synthetase (mcy) gene transcripts as a consequence of nutrient status will reveal whether N availability or speciation can be linked to toxigenicity or to dominance of toxin-producing genotypes: while we have shown these are decoupled in laboratory studies, behavior in the environment may be different. Metabolomic analyses provided within the center framework will detect depletions and accumulations of metabolites that may yield changes in microcystin congener synthesis (e.g., incorporated amino acids) indicative of subtle community shifts in the cHAB populations. With respect to Sandusky Bay and its active N cycling, phylogenetic analysis and quantification of transcripts ascribed to denitrification/nitrification and its transcriptional regulation (e.g. amoA, dnr, narXL, nirS/K, nosZ; see Arat et al. 2015) and N fixation (nifHDK) will reveal the activity and shifts in the microbial community with respect to the N cycle over the course of a bloom event. Genes for specific pathways can be used to demonstrate both production and consumption of specific chemical forms of N (e.g., urea transport genes urtA-E and urea utilization genes ureA-G).

Informed by the ‘omics data obtained above, microcosm studies will be performed in triplicate with mixed *Microcystis* and *Planktothrix* bloom biomass grown under varying N:P ratio, N concentration and N speciation (Hypothesis 1), along with chemostat studies of individual *Microcystis* and *Planktothrix* cultures under varying regimes of N availability and temperature (Hypothesis 2). These studies will assess the degree to which N concentration and temperature can promote the success of *Planktothrix* over *Microcystis* (and vice versa) as well as shape the toxigenicity of cHAB communities.

Testing Hypothesis 2. Microcystin production is dependent on temperature and N speciation (nitrate vs. urea). There is a delicate balance between growth and physiology occurring in most microorganisms. Our preliminary data clearly demonstrate that during batch culture growth there are trade-offs in microcystin production and gene regulation as temperature increases. As an offshoot of this hypothesis, we predict that early in the season we will see fewer, more toxic *Microcystis* cells that are then replaced by a larger populations of less toxic cells as temperatures increase. How this will manifest under future climate scenarios is unclear, as expanded summer seasons, warmer surface temperatures and altered nutrient flow patterns (due to altered weather

conditions) are all in play. To test our hypothesis and establish the baseline of information needed to make predictions, core information from field sampling efforts will provide quantitative metrics on total toxin, microbial community composition and activity with respect to environmental conditions (nutrient concentrations, temperature, etc). *Microcystis* and *Planktothrix* activity will be determined by the presence of specific housekeeping transcripts (e.g., rpoB; Peng et al., 2018) and cell numbers from direct counts of microscopic samples. There is also sufficient divergence in several of the microcystin biosynthesis genes to assign transcripts to specific genera (Rinta-Kanto and Wilhelm, 2006): we will be able to ascribe cellular efforts with respect to toxin production from this information. Lipidomics will identify taxon-specific lipids in the community, plus reveal changes in fatty acid saturation due to temperature effects on community members. Similar to the work in described above for Hypothesis 1, laboratory manipulation of chemostats containing individual *Planktothrix* and *Microcystis* cultures at two different temperatures (25 and 30 o C) will reveal the role to which

temperature affects growth and toxin production per cell, as prior work had indicated that increasing concentrations of urea affect microcystin production (Han et al., 2014, Belisle et al., 2016). Since these initial experiments have only been performed on *Microcystis*, responses of *Planktothrix* to nitrate and urea may be different, thereby requiring different lake management strategies to blooms of each type.

Testing Hypothesis 3. The proliferation of cHAB species is promoted by the activity of the limnetic microbiome. This element of the proposal provides a meta-analysis of the environmental metatranscriptomes, documenting the activities of the entire microbial consortium. Microbial communities are complex ecosystems where materials are assimilated, transformed and then rapidly transferred to partner members. With the advent of high-throughput sequencing technologies it became clear that in many ecosystems microbial processes ("what is occurring") were conserved but the organisms undertaking these processes ("who is doing it") were different. For example, while historically limnologists have focused on heterocyst-forming cyanobacteria as the major nitrogen fixers in natural systems, both genomic (Steffen et al., 2012, Davis et al. 2015) and transcriptional profiles (Krausfeldt et al., 2017) have demonstrated that in many instances the heterotrophic members of the microbial community are they major N 2 - fixers. To this end, just as the microbiome an organism can shape the hosts health and response to disease states (Stough et al., 2016, Villarino et al., 2016), the idea that there is a "core microbiome" associated with toxic cyanobacterial blooms (Wilhelm et al., 2011) (Tang et al., 2010) is one that demands further investigation. Given that we are examining the microbial community associated with two distinctly different microcystin-producing cyanobacteria (*Microcystis* vs. *Planktothrix*), this affords an excellent opportunity to determine whether phylogenetic and/or physiological differences exist that may support each genus in bloom development and maintenance.

To accomplish this survey, environmental metatranscriptomic data will be initially characterized by both community function ("what organisms are trying to do") and community phylogenetics ("who is doing it"). Seasonal variations will then be statistically resolved with respect to environmental conditions collected as part of metadata analyses. Once within this framework, network analyses will then be used to address questions on when toxin biosynthesis pathways are active and when microcystin (or other emerging cHAB toxins) are present. The goal here will be

to use information from the 2 master stations (EC 1163 in Sandusky Bay, WE02 in Maumee Bay) to generate hypotheses concerning microbiome drivers of associated with blooms. We will then test these phylogenetic and functional hypotheses against ‘omics datasets from “samples of opportunity” collected from other stations in Lake Erie. These samples will be obtained from targeted bloom events as they develop and persist in western Lake Erie, and will be obtained through research vessel access through the Facilities Core at the University of Toledo and the Community Engagement Core through the Charter Boat Captains.