Global Transcriptional Profiles Associated with Increasing Severity and Duration of a *Microcystis* Bloom

Lead: Lauren Krausfeldt, Nova Southeastern University

USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection, and management technologies through collaboration, partnership, and cutting-edge science.

Problem

Cyanobacterial harmful algal blooms (cyanoHABs) are a major concern in freshwater ecosystems around the world. CyanoHABs have significant negative consequences on the health of humans, animals, ecosystems, and the economy. There remains a need to develop novel approaches to predict, prevent, and disrupt the formation of cyanoHABs. Towards fulfilling this need, an understanding of the key physiological changes in cyanobacteria that lead to the formation and persistence of cyanoHABs is required.

Approach

To address the mechanistic understanding of HABs caused by *Microcystis*, we performed in situ mesocosms on the Caloosahatchee River in south Florida during ongoing Microcystis bloom. We added daily additions of N in the forms of NH_4 and urea, a commonly used N fertilizer in agriculture. Measurements for temperature, phycocyanin (PC), chlorophyll a (chl a), conductivity, pH, fluorescent dissolved organic matter (fDOM), turbidity, and dissolved oxygen (DO) were collected daily at time point (T) T0, T24, T48, and T72, and additionally at T264, a time point chosen to represent a longer term impact. These measurements were done prior to daily nutrient addition using a YSI EXO multiparameter water quality sonde. Surface water samples at 0.05 m depth were collected monthly by the South Florida Water Management District (SFWMD) from Lake Okeechobee for DNA sequencing and subsequent comparison. We characterized the global transcriptional response of the Microcystis bloomto different nutrient additions that yielded differential bloom outcomes using a metatranscriptomic approach.

Results

By performing in situ mesocosms on the Caloosahatchee River in southwest Florida, a *Microcystis* bloom was successfully intensified with the addition of nitrogen. Metagenomics identified that *Microcystis panniformis* caused the bloom and was highly identical to *M. panniformis* identified in Lake Okeechobee, a major source of freshwater to the Caloosahatchee River. Metatranscriptomes characterized the global transcriptional response associated with increased bloom intensity upon treatment with ammonium and urea for 72 h, which primarily included upreg-

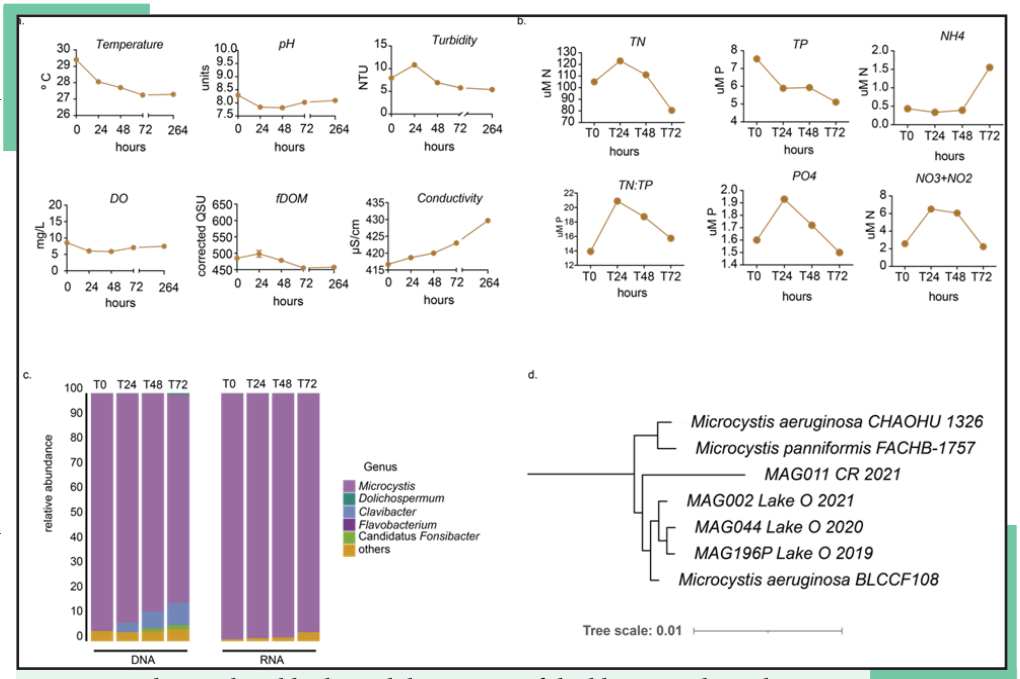

Figure 1. *Chemical and biological description of the bloom on the Caloosahatchee River (CR) in May 2021: (a) chemical data collected from the CR during the bloom on the CR using YSI; (b) nutrient chemistry in the CR during the bloom; (c) present and active microbial community composition from the bloom on the CR based on metagenomic (DNA) and metatranscriptome (RNA) reads, respectively; (d) phylogenetic tree showing relationships between Lake Okeechobee (O) and CR metagenome assembled genomes recovered from 2019-2021. MAGs on tree are labeled where and in what year they were recovered.*

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ulation photosynthesis machinery, phosphate transport, and carbon transport and metabolism. However, urea additions also caused upregulation of carbon storage, amino acid metabolism, microcystin biosynthesis, and several other processes associated with cell growth, and the bloom persisted several days longer in this treatment. Type II toxin-antitoxin systems, CRISPR-cas genes, and over 100 transposases were also upregulated indicating that phage defense and genome rearrangement were important in bloom proliferation. Importantly, these results show the stepwise response by Microcystis that leads to increased bloom intensity and duration.

Value to USACE Mission

This study, to our knowledge, is the first to successfully induce a Microcystis bloom using in situ mesocosms and characterize the global transcriptional response over several days. In doing this, the functions that are important in increasing severity and duration of a bloom experiencing N-limitation have been elucidated. This study also confirmed that phage defense and genome rearrangement are key mechanisms for the proliferation of Microcystis in nature. Finally, we demonstrate the potential importance of urea as N source in the Caloosahatchee River and elsewhere, as urea alone was able to support these outcomes. Since the use of urea as a N fertilizer is increasing globally, this demonstrates run-off containing urea could have severe implications on freshwater ecosystems.

Project Team

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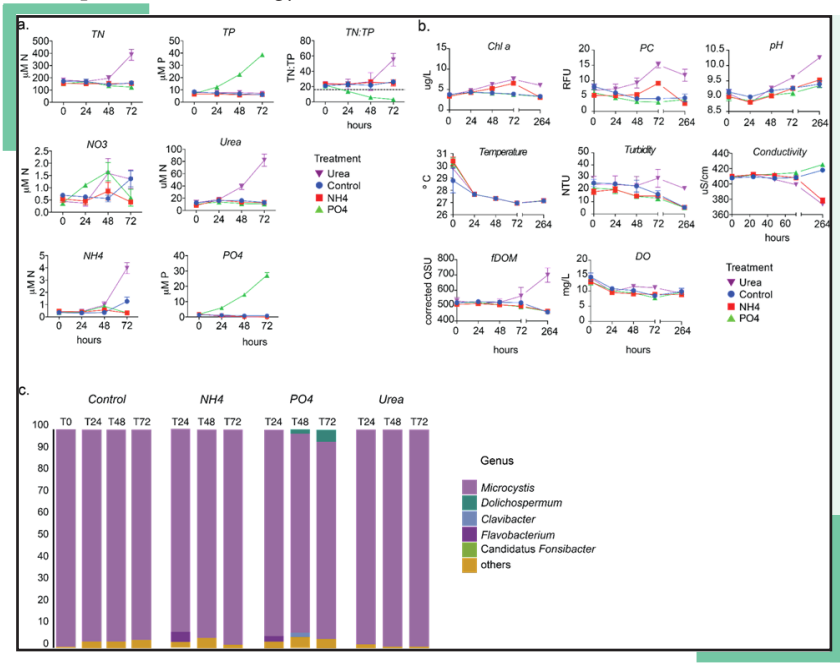

Figure 2. *Chemical and active microbial community in the mesocosms on the Caloosahatchee River: (a) nutrient concentrations in the mesocosm chambers at the surface at the start of the experiments (T0), after 24 h (T24), after 48 h (T48), and after 72 h (T72). Nutrients were added at increasing concentrations at T0, T24, and T48 and samples for nutrient concentrations were collected before nutrient dosing. Samples for dissolved nutrients were also collected after nutrient dosing and can be found in Figure S2; (b) chemical profiles in the mesocosm chambers at the surface at T0, T24, T48, T72, and one week later at T264. Chemical profiles were taken before nutrient dosing that occurred at T0, T24, and T48; (c) top 95% of the composition of the active microbial community in the control chambers at T0.*