Laboratory Studies to Assess Toxic Microcystis Growth and Toxicity under Varying Temperatures in Lake Pontchartrain, Louisiana

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Problem Statement and Proposed Research

Estuaries in the northern Gulf of Mexico are undergoing profound alterations due to changes in climate and implementation of coastal restoration activities and these may lead to increasing frequency of harmful cyanobacteria blooms (CyanoHABs). The introduction of large volumes of nutrient-rich, fresh Mississippi River water into nutrient-poor estuarine Lake Pontchartrain is known to substantially change the chemistry and ecology of the lake, and warm temperatures paired with the persistent nutrient loading into the lake supports the proliferation of CyanoHABs. Different environmental factors can control cyanobacteria community structure and their toxin production. With climate change drivers increasing the temperature regime of these freshwater ecosystems and selecting for more cyanobacteria, these CyanoHABs are posing a serious threat to the use and sustainability of existing freshwater resources. Therefore, management strategies targeting the specific environmental conditions that lead to cyanobacteria bloom development and the associated toxin production are critically needed. In this proposed project, we used existing field data to characterize estuarine spring conditions and conduct series of laboratory experiments to understand the effect of temperature variability on locally isolated *Microcystis* growth and toxin production.

Introduction

Cyanobacteria (blue-green algae) blooms (CyanoHABs) are a global phenomenon that are currently on the rise. The success of these blooms is caused by an assortment of factors: including augmented nutrient concentration, increased temperatures, and low turbidity (1, 2). Several cyanobacteria species, such as *Microcystis* and *Dolichospermum* (formally known as *Anabaena*), can produce chemical compounds (e.g., microcystin and anatoxin) that are toxic to humans and other animals and lowering overall water quality (3, 4).

Cyanobacteria thrive in calm, nutrient-rich, warm waters, and some species, including *Microcystis* and *Dolichospermum*, can survive unfavorable growth conditions at cold temperatures by overwintering. During the overwintering period, cyanobacteria cells react to stressful environmental factors by settling on the sediment surface and entering a dormant state that requires little less energy until the environment is optimum for activity (5). As climate change causes temperatures to continue to rise, CyanoHABs are occurring earlier and more frequently each year, and they are posing a severe and increasing threat to the usability and sustainability of freshwater resources (6).

Louisiana has an abundance of warm, nutrient-rich freshwater/estuarine systems, where CyanoHABs have been commonly observed in recent years (7, 8). Lake Pontchartrain, located just north of New Orleans, Louisiana, is a shallow, brackish estuary hydrologically connected to the Mississippi River by the flood control structure the Bonnet Carré Spillway (BCS). While Lake Pontchartrain is normally a nutrient limited system, seasonal flooding events introduce large volumes of nutrient-rich freshwater from the BCS and other tributaries into the lake. When the estuary becomes enriched with this influx of nutrient-rich freshwater, the chemistry and ecology of the lake substantially changes, resulting in the proliferation of CyanoHABs in warm months (7, 9).

The timing and duration of the nutrient loading from the BCS and Lake Pontchartrain's tributaries are critical criteria to characterize and predict the succession of dominant phytoplankton communities in the lake (10). Nutrient loading in early spring supports the increase of diatoms and chlorophytes followed by *Microcystis* and other potentially toxic cyanobacteria common to this estuary (7, 9). This was seen in 2019, when the Bonnet Carré

Spillway was opened twice, on February 27 and again on May 10, where large and long-standing cyanobacteria blooms were identified in summer.

The production of cyanobacteria toxins is dependent on several environmental conditions like temperature variance which is known to impact growth. However, it is uncertain at what temperatures cellular toxins are produced at a higher rate. Bargu Lab asks what water temperature variants seen in early- to mid-spring in the Lake Pontchartrain estuary better influence *Microcystis* growth and toxin production using controlled laboratory experiments. The null hypothesis states that *Microcystis* growth and toxin production will be unaffected by temperature variance and the alternative hypothesis states that temperature variance will affect *Microcystis* growth and toxin production, with higher growth at higher temperatures.

Materials and Methods

Microcystis Isolation from Lake Pontchartrain and Laboratory Acclimation

Stock cultures of *Microcystis* were successfully produced by isolating cells from Lake Pontchartrain. Water samples were collected from surface water along a historical transect stretching 30km from the BCS to the Lake Pontchartrain Causeway (Fig. 1). Isolated cells were maintained in Carolina Freshwater Media at 22°C in six well plastic cell culturing plates. Cultures of *Microcystis* cells were then further isolated and reinoculated every week until a pure culture was acquired. Three milliliters of each pure culture were then transferred to 50mL culture tubes with around 40mL of Carolina Freshwater Media. Weekly *Microcystis* re-inoculations proceeded in order to conserve and promote optimum, continuous growth in cultures.

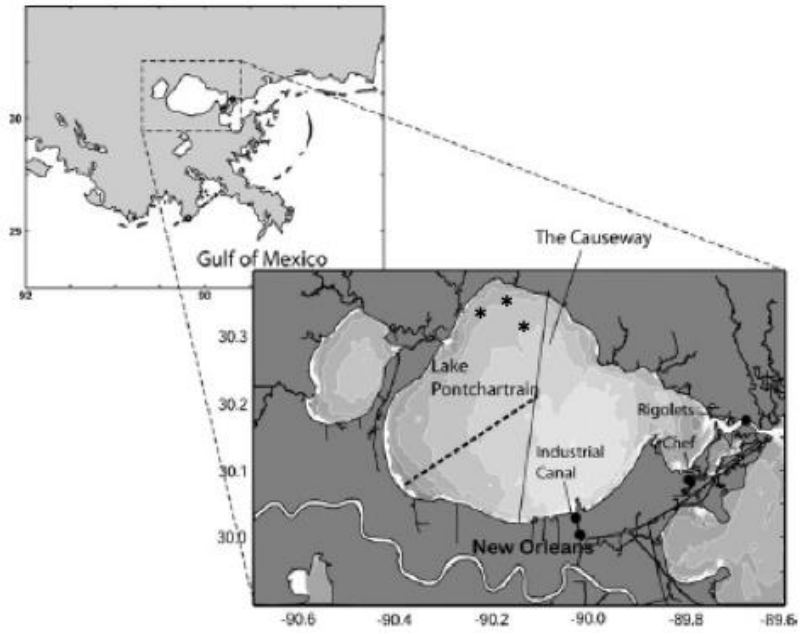


Figure 1: Lake Pontchartrain transect stretching from the Bonnet Carré Spillway to the Lake Pontchartrain Causeway and additional three northern stations (taken from Bargu 2011).

Experimental Settings

To assess the spring growth of *Microcystis* that are potentially resuspended into the surface water after overwintering period, two different temperatures (16°C and 20°C), based on the average monthly water temperatures for March and April by USGS' Environmental Atlas of the Lake Pontchartrain Basin, were picked. Fifty milliliter stock cultures of *Microcystis* sourced from Lake Pontchartrain Estuary were acclimated to 16°C and 20°C as the first step.

Microcystis stock cultures originally maintained at 22°C were transferred to 100mL culture flasks and acclimated to each temperature variant by lowering the incubator temperature by 1°C every three days until the desired temperature was reached. All cultures were grown in Carolina Freshwater Media and acclimated to the desired experimental temperature to ensure cells not get stressed or harmed.

After *Microsystis* stock cultures acclimated to desired temperature, five milliliters of it were added to 95ml of Carolina freshwater media and grown until reaching an exponential phase. This phase was determined by measuring the chlorophyll *a* on a fluorometer daily. Once the growth of *Microcystis* stock reached its maximum, five milliliters from that culture and 595ml of Carolina freshwater media were added into one-liter culture flasks (n = 6) and placed in an incubator set to 16°C or 20°C with 12-hour dark/light intervals for three weeks. At regular intervals, samples were taken for phycocyanin and the toxin microcystin measurements.

Microcystis Growth

Phycocyanin (PC) measurements were taken for a three-week growth period to determine changes in *Microcystis* biomass. Ten milliliters from each 600mL culture were taken to measure phycocyanin raw fluorescence units (RFUs) using the Turner field-portable fluorometer, CyanoFluor™. RFU values were converted to phycocyanin concentrations ($\mu g\ L^{-1}$) using a standard curve [concentrations ($\mu g\ L^{-1}$): 0, 10, 50, 100, 500, 1000 and 2000] created with phycocyanin (Sigma-Aldrich #P217210MG) dissolved in a phosphate buffer.

Microcystis Toxin Production

Water samples were analyzed for the particulate form of the cyanobacterial toxin microcystin. Fifty milliliters from each sample were filtered through GF/F filters (Whatman Inc., Clifton, NJ, USA) and then were kept at -20°C until processed. Filters were extracted for microcystin using methanol:water:acetic acid (50:49:1), method previously described by Boyer (2008) (11) with modifications described by Garcia et al (2010) (12). Toxins in extracted water samples were quantified using commercially available, highly sensitive enzyme-linked

immunosorbent assay (ELISA) kits (Abraxis LLC). Samples were analyzed following the protocol included in the kits, with each sample run in duplicate and at several dilutions to reduce interference from matrix effects. Absorbances were read using a microplate ELISA photometer.

Results

Phycocyanin Results

Phycocyanin production represents the changes in biomass of a given cyanobacteria species. *Microcystis* increased in biomass at both 16°C and 20°C until the end of experiment period (19 days) (Fig. 2). However, there were large differences in phycocyanin production rates between 16°C and 20°C throughout the experiment, with much faster biomass increase (reaching to 15,241 ± 1,707 µg PC/L) in *Microcystis* cultures grown at 20°C compared to *Microcystis* cultures grown at 16°C (reaching to 5,359 ± 2,602 µg PC/L) (Fig. 2).

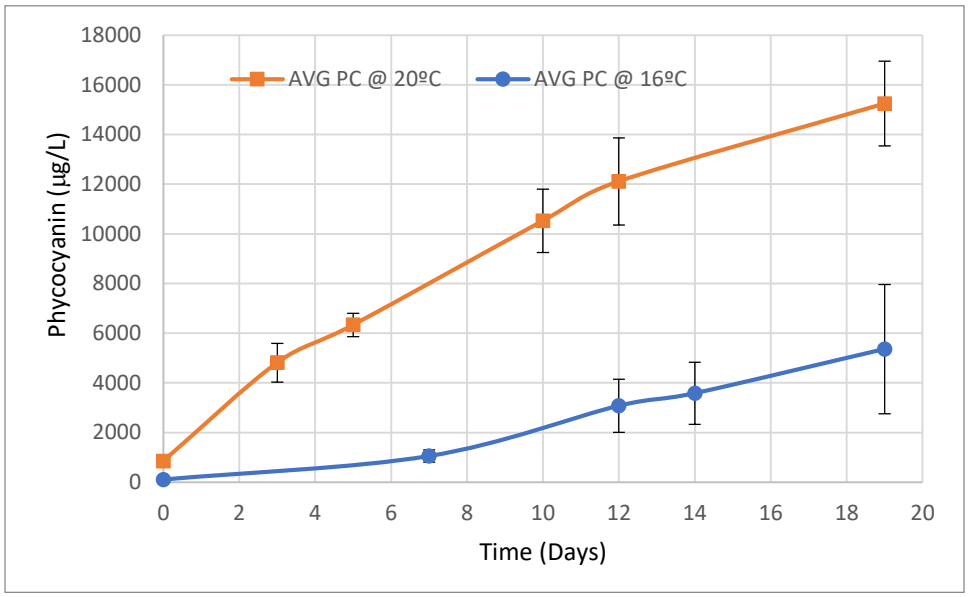
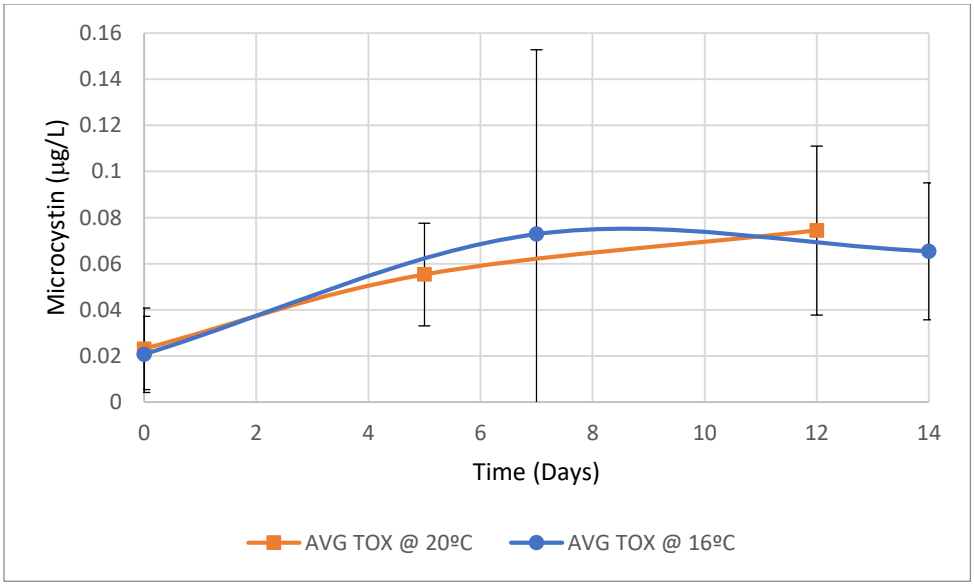


Figure 2: Phycocyanin concentrations (µg L^{-1}) for *Microcystis* cultures grown at temperatures of 16°C and 20°C. Plotted points represent the rate change of phycocyanin concentration over time.

It should be noted that there is a difference between 16°C and 20°C at Day 0. This is due to each culture being acclimated to their respective temperature variance and demonstrated different growth rates before the experiments started.

Microcystin Results

Microcystin is a toxin produced by *Microcystis* and few other toxic cyanobacteria species. Figure 3 describes the overall particulate microcystin toxin production by *Microcystis* cultured at 16°C and 20°C. The toxin production increased during the first couple of days in both 16°C and 20°C grown cultures with increasing biomass. There was a slightly higher increase in total microcystin levels in cultures grown at 16°C from day 4 to 10 followed up a gradual decrease over time, while total toxin amount continued to increase at lower rate in cultures grown at 20°C (Fig. 3). The analysis of microcystin toxin does not account for total biomass that produced the toxin in a given time, so while the toxin concentrations are similar, *Microcystis* cells grown at 16°C produced nearly ten times more microcystin than *Microcystis* cells grown at 20°C, as seen in Figure 4.



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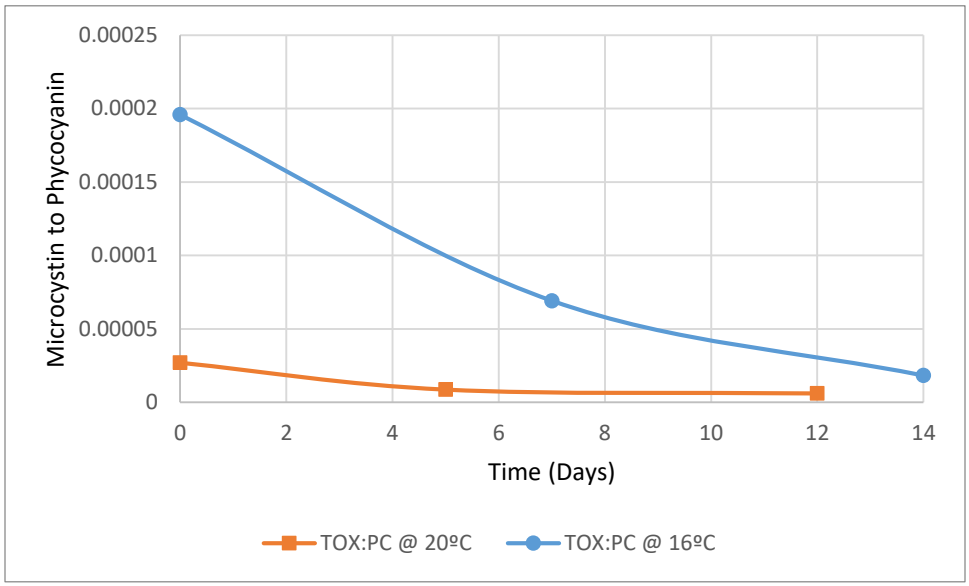

Figure 4: Microcystin concentrations (µg L^{-1}) produced per one unit of phycocyanin for *Microcystis* cultures grown at temperatures 16°C and 20°C.

Discussion

Phytoplankton successions occur with changing environmental conditions with different species adapting to these changes at different times and dominating the phytoplankton community. In Lake Pontchartrain, phytoplankton groups of chlorophytes and diatoms tend to become abundant during nutrient enriched spring months, when water temperature is still low and water is turbid due to external discharges that brings nutrient enriched fresh water and sediment (7, 9). This phytoplankton community changes over time becomes more cyanoabacteria dominated when temperature starts to increase and when water become calmer and low in bioavailable nutrients (7, 9, 10). If nitrogen is still available for biological uptake, potentially

toxic *Microcystis* is usually the first cyanobacteria species to respond, and then nitrogen-fixing species, such as *Dolichospermum*, takes over after nitrogen becomes limited significantly, especially in late summer months (7). *Microcystis* is not a nitrogen-fixing bacterium and requires the nitrogen for their growth. However, the organism's uptake is very efficient due to its small size, so even if a small amount is nutrient present, *Microcystis* can be abundant in the system. On the other hand, when water temperatures are warm and nutrient enrichment occurs, then both *Microcystis* and *Dolichospermum* can co-exist (Bargu personal communication).

The Bonnet Carré Spillway's openings into Lake Pontchartrain varies from year to year based on the water level in Mississippi River and the flood threat to surrounding residential areas. If Spillway opens late winter/early spring, then diatoms will be likely the first phytoplankton group to take advantage of nutrient enrichment and grow. By the time *Microcystis* is comfortable enough with warmer temperatures, there will not be enough nutrients for its bloom due to the previous nutrient use. Because of this, toxin production in the lake will be lessened until nitrogen-fixing toxic cyanobacteria, such as *Dolichospermum*, replace *Microcystis*. The opposite will occur if Spillway opens later in the spring and introduces nutrients at warmer water temperatures. *Microcystis* will have a chance to grow for a longer time, and consequently more toxin will be produced.

Increase of total microcystin levels was expected when *Microcystis* was grown at both 16°C and 20°C simply due to increase in biomass over time. The more *Microcystis* is found in an area, the more microcystin toxin will be potentially produced and remained in the system. We have demonstrated that *Microcystis* was grown faster at warmer temperature treatment and reached at higher biomass (Fig. 2). However, despite the growth of *Microcystis* at 20°C being over twice that at 16°C, cells at 16°C produced toxin levels nearly equal or higher in

concentration to that of those cultured at 20°C (Fig. 3). This indicates that *Microcystis* grown at 16°C produced more microcystin toxin per cell than that those cultured at 20°C, and even though low temperature suppresses *Microcystis* growth, it somehow stimulates them to be more toxic. Based on our findings, when Spillway opens earlier in the year, that delays or limits *Microcystis* growth due to low temperatures, and it might also promote higher cellular toxin production. However, their presence might be short lived in the system. On the other hand, if Spillway opens later in the spring, that might promote higher *Microcystis* biomass with high persistence, but cellular toxin might stay low.

The main anticipated outcome of this study is a comparative assessment of *Microcystis* growth in response to climate change and the timing of the Spillway openings and the effect of these changes on toxin production. The understanding and prediction of CyanoHABs in Lake Pontchartrain Estuary will greatly attribute to the overall future health and understanding of not just Lake Pontchartrain but the connected estuaries and lakes as well. Management strategies targeting the specific environmental conditions that lead to cyanobacteria bloom development and the associated toxin production are critically needed. Studying the environmental dynamics of blooms in Lake Pontchartrain in the present offers the further knowledge of implications for inevitable events in the future.

References

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