Microcystis Blooms and Controlling Factors during Two Successively Severe Drought Years in San Francisco Estuary

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The frequency, intensity and duration of drought and associated cyanobacteria blooms are expected to increase in California with future climate change. As the third (2015) and fourth (2014) most severe drought years on record, the 2014 and 2015 drought years provided an opportunity to determine if successive severe drought years vary in their impact on the structure and function of *Microcystis* blooms in San Francisco Estuary. Field sampling was conducted bi-weekly at 10 stations during the bloom season for a suite of physical, chemical and biological factors. Primary producer biovolume, community composition, toxin production, and growth rate were quantified by chlorophyll *a* concentration, qPCR, microscopy, ELISA assays, and carbon uptake measurements. Inorganic nitrogen sources were determined using stable isotopes. The magnitude of the bloom and associations among physical, chemical and biological variables during the *Microcystis* bloom differed between the two successively severe drought years. Contrary to expectations, *Microcystis* biovolume and chlorophyll *a* concentration were greater in 2014 than 2015, the drier year climatically. Primary producer community composition was also spatially and temporally more variable and contained a greater percentage of total cyanobacteria in 2015 than 2014. Differences between the two years were related to the high frequency spatial and temporal variation in environmental conditions that were more favorable to *Microcystis* during the bloom season in 2014 than 2015, including warmer water temperature, more light in the euphotic zone, increased water residence time and availability of ammonium. Correlation and ordination analysis also confirmed that the two severe drought years demonstrated different functional associations. We concluded that understanding the impact of drought on *Microcystis* blooms requires an evaluation of the impact of high frequency spatial and temporal variation and functional associations of physical, chemical and biological variables for each drought year.

Keywords: *Microcystis,* drought, biovolume, isotope analysis, cyanotoxins, qPCR

Poster Cluster Title: *Microcystis* Blooms in San Francisco Estuary during Drought Conditions: Field and Laboratory Studies Associated with *Microcystis* spp from 2014 to 2016

Spatiotemporal Dynamic Changes in Cyanobacteria Assemblages and Emerging Cyanotoxins in the San Francisco Estuary

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Harmful algal blooms (HABs) are a major environmental concern due to the production of toxins and hypoxic environments. Harmful cyanobacteria can also outcompete nutritious forms of green algae and reduce nontoxic food availability for higher trophic levels. It is therefore crucial to understand the dynamics of the cyanobacterial blooms. In collaboration with the Department of Water Resources and Fish and Wildlife, we have analyzed water samples from ten different sites in the San Francisco Estuary from 2014 to 2016, including the severe drought years. Throughout sampling we discovered a shift in cyanotoxin composition. In 2014 and 2015 microcystins were the only cyanotoxin detected, however in 2016 microcystins were detected less frequently while anatoxin-a and saxitoxin were also detected. In addition, we successfully determined anatoxin synthetase gene from the local algal samples and we are currently measuring abundance of anatoxin producing cyanobacteria using algal samples archived in our laboratory. The spatiotemporal distribution pattern of cyanobacteria will be further discussed in the presentation.

Student Award Competition: Yes

Keywords: Harmful algal blooms, Cyanobacteria, Cyanotoxins, Emerging Contaminants

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Biodiversity of Cyanobacteria and Presence of Multiple *Microcystis* Genotypes in the San Francisco Estuary

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Blooms of harmful cyanobacteria can result in water quality deterioration by the production of cyanotoxins. Since the blooms began in 1999, the largest biomass of *Microcystis* was observed during the severe drought in 2014, with median chlorophyll *a* concentration reaching levels that were 13 and 9 times greater than in previous wet and dry years. Apparently, cyanobacteria assemblage seems to be changing. In 2014 and 2015, microcystins were the only cyanotoxin detected, however in 2016, microcystins were detected less frequently while anatoxin-a and saxitoxin were detected. To better understand cyanobacteria assemblages in the San Francisco Estuary, we investigated biodiversity of cyanobacteria by shotgun metagenomic analysis on algal samples collected from 2014 to 2016. Our data indicate that *Microcystis* was the dominant genus found in the SFE from 2014 and 2015 while the relative abundance of other cyanobacteria increased in 2016. Besides *Microcystis*, various cyanobacteria were detected in 2016, including *Anabaena*, *Aphanizomenon*, *Cyanothece*, *Lyngbya*, *Nostoc*, *Planktothrix*, *Pseudanabaena*, and *Synechococcus*. Additional screening also revealed anatoxin and saxitoxin synthetase genes and the presence of multiple *Microcystis* genotypes. These *Microcystis* genotypes may belong to different species within the genus. Interestingly, some of the *Microcystis* genotypes showed high DNA sequence similarity to *Microcystis* detected at other geographical locations such as China. Our qPCR results indicate that the distribution pattern of the two *Microcystis* genotypes found in the SFE was slightly different. We will discuss the ecological niche of the *Microcystis* genotypes.

Keywords: cyanobacteria, *microcystis*, genotypes, cyanotoxin synthetase genes

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Establishment of Pure Algal Cultures from the San Francisco Estuary for Testing Differing Water Qualities

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Phytoplankton are the main primary producers in aquatic environments and are critical for productive and healthy ecosystems. Establishment of pure cultures is essential for investigating environmental drivers for algal growth and for testing effects of contaminants (e.g., herbicides), however, only a few pure cultures are currently available, collected from the San Francisco Estuary (SFE). To address the issue, we established a total of 21 phytoplankton cultures, including cyanobacteria (e.g. *Microcystis*, *Anabaena,* etc.), diatoms (*Entomoneis*, *Aulacoseira,* etc.), and green algae (*Volvox*, *Chlamydomonas,* etc.) from algal samples collected in the SFE. Identification of phytoplankton was determined to genus or species level by morphology or genetic sequencing. In addition, we optimized growth test conditions for the cultures, using Erlenmeyer flasks for cluster forming phytoplankton (*Microcystis*, *Anabaena*, *Volvox,* etc.) and 96 well culture plates for single cell phytoplankton (*Thalassiosira*). The growth test using 96 well culture plate is very powerful because the method allows us to test a large number of water samples or various environmental conditions simultaneously and inexpensively (>200 treatments per test). Using the 96 well plate method, we are investigating effects of different salinity levels and nutrient conditions on growth of *Thalassiosira*. Furthermore, using the same method, we are testing suitability of field water for phytoplankton growth, collected from different sites in 2017.

Keywords: Cyanobacteria, water quality, nutrients, phytoplankton, *Microcystis*, Anabaena, herbicides, algae

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Detection of Microcystins in Tissue Samples via Gas Chromatography Coupled With MMPB Extraction

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Microcystins, toxins secreted by cyanobacteria, and are hepatotoxic, neurotoxic, and cytotoxic. Upon entry into the liver they covalently bind to protein phosphatase making them difficult to detect with ELISA (enzyme linked immunosorbent assay) or PPIA (protein phosphatase inhibition assay). This results in a drastic underestimation of toxin concentration in samples such as animal tissues and sediments. Detection and quantification of microcystin is crucial when considering bioaccumulation and food safety. We have developed a method of detecting bound microcystins by cleavage of Adda, a unique amino acid found in all microcystins, to yield 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB). MMPB was identified using gas chromatography coupled with mass spectrometry. This method is currently being optimized for detection in tissue and sediment samples. Optimization of this method can allow for accurate detection of microcystins enabling management and policy makers to make effective decisions to improve the health of the estuary. Developments in this technique will be further discussed.

Student Award Competition: Yes

Keywords: Cyanotoxins, Harmful algal blooms, Bioaccumulation, Gas Chromatography, Mass Spectrometry

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