Monitoring a toxic cyanobacterial bloom in a shallow, hypereutrophic lake on California's Central Coast

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Abstract

In this study, we monitored a highly toxic cyanobacterial bloom in Pinto Lake (Monterey Bay, CA). We tracked changes in density of cyanobacterial taxa and intracellular microcystin (MC) toxins in association with environmental factors. We documented an increase of cyanobacterial biomass in association with increases in dissolved nutrients and water temperature, and evidence for a broadened bloom period. This research provides continuing data concerning the ecology and proliferation of toxic cyanobacteria in the Monterey Bay area and informs local, regional and state agencies in formulating strategies for the monitoring and management of toxic cyanobacterial blooms.

Background

MC-producing cyanobacteria

- Several cyanobacterial taxa have been documented to produce MCs, including species of *Anabaena*, *Microcystis*, *Planktothrix* and *Oscillatoria*

MC structure and properties

- Small monocyclic seven amino acid peptide (Fig. 1)
- Water soluble and stable, persisting intact in the environment from several weeks to months
- Over 90 variants , varying at 2nd and 4th positions

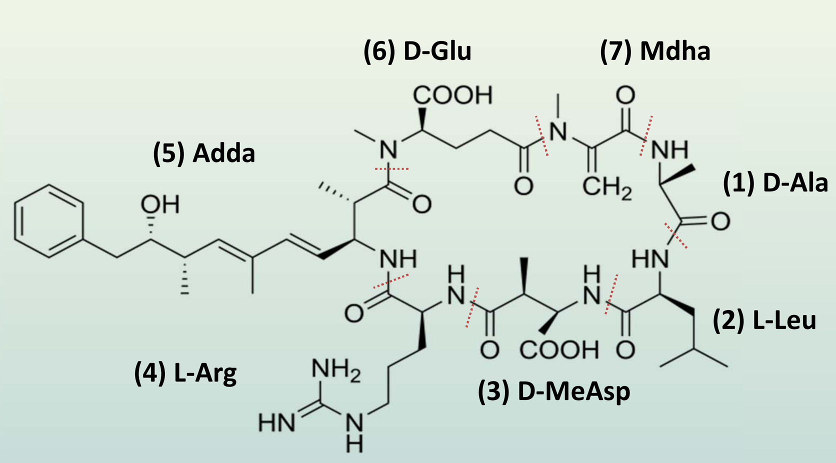

Figure 1: Microcystin-LR and amino acid positions

MC toxicity

- Uptake via bile acid transport system
- Inhibition of protein phosphatase 1 and 2A
- Acute exposure can cause intrahepatic hemorrhage and liver failure
- Chronic low dose exposure is linked with increases in liver and colorectal cancer

Environmental Factors

- Cyanobacterial toxicity can vary depending on environmental factors including :
  - dissolved nutrients
  - dissolved organic carbon
  - increased pH
  - ambient temperature
  - solar radiation
  - water column stability
  - seasonal runoff rates
  - phytoplankton competition
  - zooplankton predation

Study site

Pinto Lake, located on California's central coast , is a shallow eutrophic freshwater lake draining into the Monterey Bay National Marine Sanctuary (MBNMS) (Fig. 2). The lake is a major recreational resource for the low-income community of Watsonville, with over 100,000 visitors annually. The lake exhibits seasonal CHABs and high cyanotoxin levels (Figs. 2 - 4). In 2010, the deaths of 21 endangered sea otters (*Enhydra lutris*) in the MBNMS were linked to MC poisoning, and Pinto Lake was identified as a putative toxin source (Miller *et al.*, 2010). However, the link is as yet unconfirmed, and the association between CHABs and contributing environmental factors remain unclear. Pinto Lake has been listed as impaired by the State of California for cyanobacterial blooms and cyanotoxins. The following study constitutes a continuation of the first comprehensive analysis of the environmental factors associated with toxic CHABs in Pinto Lake.

Figure 2: Pinto Lake located on the Monterey Bay (Watsonville, CA)

Methods

Water quality and environmental parameters

- Measured *in situ* water quality parameters including temperature and pH with a Hydrolab DS1 multiprobe
- Recorded Secchi depths
- Quantified dissolved ammonium, nitrate + nitrite and orthophosphate using the colormetric method

Cyanobacteria identification and enumeration

- Preserved subsamples in Lugol's iodine, stored at 4°C
- Concentrated preserved samples by sedimentation
- Examined sample concentrate in Sedgwick-Rafter counting cells with an inverted microscope at 100x magnification
- Enumerated cyanobacteria using the natural unit method based on average cell and colony sizes
- Identified cyanobacteria morphologically to genera (Komárek 2003) (Fig. 3)

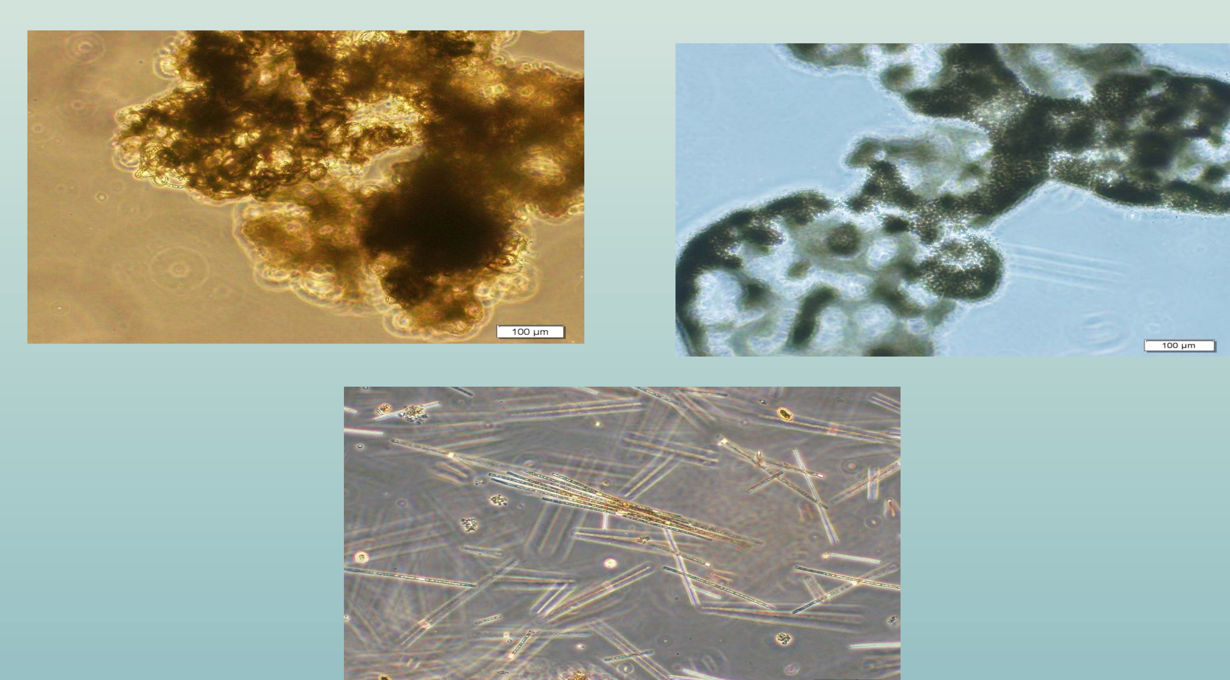

Figure 3: Environmental samples (left to right) *Anabaena* spp., *Microcystis* sp. and *Aphanizomenon* sp.

Quantifying intracellular microcystins

- Extracted filtered cyanobacteria in 50% methanol diluted to 5% methanol to avoid assay interference
- Applied sample extracts and MC-LR standards to enzyme-linked immunosorbent assay (ELISA) plates (Envirologix Inc.)
- Measured intracellular MCs spectrophotometrically

Acknowledgements

I would like to thank UROC for providing undergraduate interns, and my advisors for their input to this project.

This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-38422-31204 from the USDA National Institute of Food and Agriculture.

Results

We identified several bloom-forming cyanobacteria including *Anabaena*, *Aphanizomenon*, and *Microcystis* spp. by microscopy (Fig. 3). We documented an increase of cyanobacterial biomass and intracellular MCs from late summer through early winter (Fig. 4). This is in contrast to previous years when MCs decline in the late autumn. The MC levels were also more than double previous years. An early peak in *Aphanizomenon* and *Anabaena* in April 2012 was accompanied by an early peak in MCs in the absence of *Microcystis.* This may indicate preliminary support for the first evidence of a non-*Microcystis* MC producer in the lake. Later peaks in intracellular MCs appear to correspond with increases in *Microcystis* bloom density. The overall increase in cyanobacteria abundance in this period also corresponded with seasonally-associated increased air and water temperature, and water column stability.

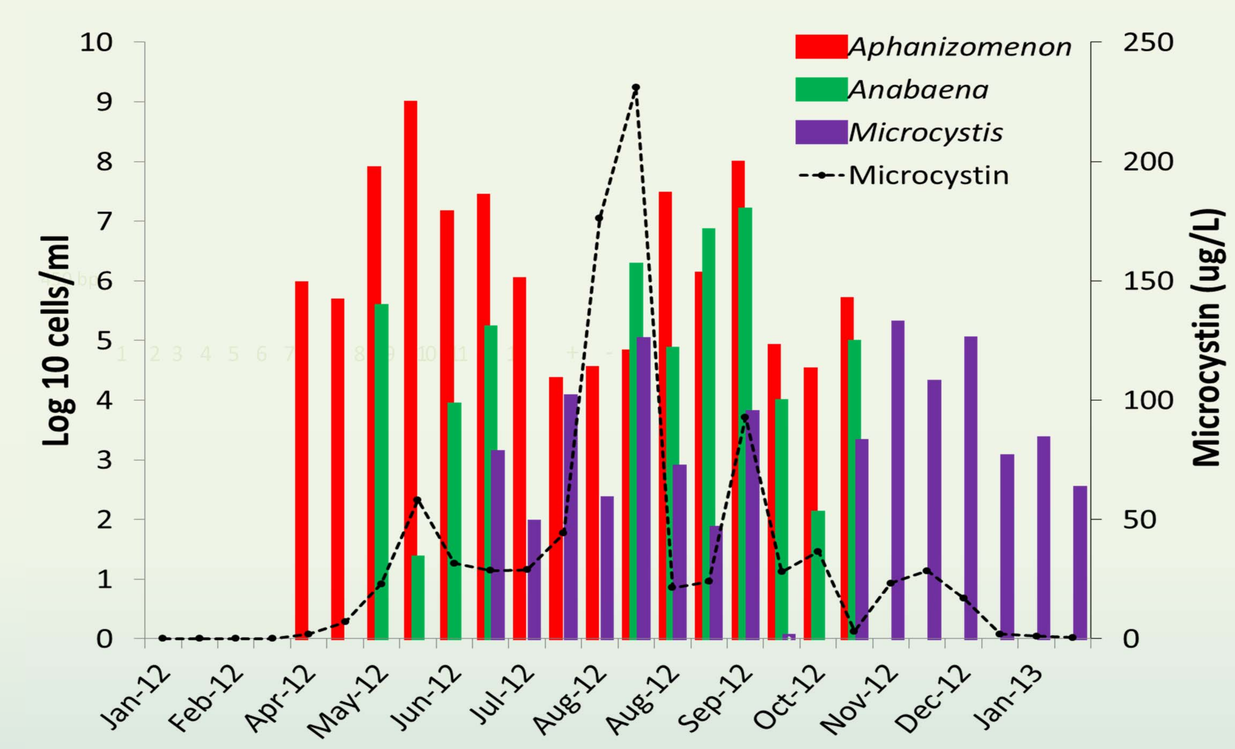

Figure 4: Pinto Lake cyanobacterial abundance (log cells·mL^{-1}) and intracellular microcystins throughout January 2012-February 2013.

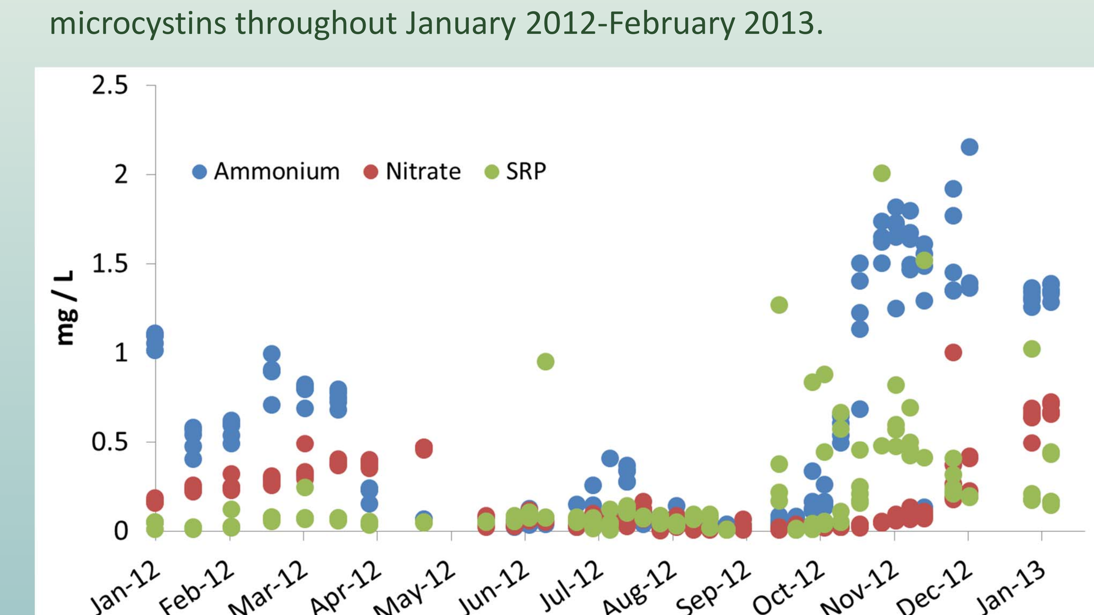

Figure 5: Pinto surface dissolved nutrient concentrations (mg · L^{-1}) January 2012 - January 2013.

To explore environmental factors associated with the cyanobacteria abundance and microcystin levels, we performed several correlation analyses with log transformed data. We observed a strong negative correlation between dissolved surface nitrate and *Anabaena* (r=-0.58, p=.002) and *Aphanizomenon* (r=-0.73, p<.001). We also documented a positive correlation between phosphate below the thermocline and *Aphanizomenon* (r=0.58, p=.005) and *Microcystis* (r=0.62, p=.002). *Microcystis* abundance also moderately positively correlated with surface ammonium (r=0.45, p=.03). The negative relationship between cyanobacterial abundance and dissolved surface nitrate may be indicative of the ecological advantage of nitrogen-fixing cyanobacteria when nitrogen levels are low. Furthermore, *Microcystis* benefits from other cyanobacterial nitrogen fixation and is adept at utilizing other nitrogen species, including ammonium.

While previous DNA sequencing supports *Microcystis* as the microcystin producer in Pinto Lake, we observed moderate correlation between microcystin levels and *Microcystis* abundance (r=0.37, p=.05) and microcystin levels and *Anabaena* abundance (r=0.48, p=.01). Surprisingly, we documented a strong correlation between microcystin levels and *Aphanizomenon* abundance (r=0.63, p<.001).