DNA Fingerprinting and Quantitative Assessments of Toxigenic Microcystis Assemblages and Their Environmental Drivers in the San Francisco Estuary Delta

Timothy Otten, University of North Carolina at Chapel Hill, ottentim@email.unc.edu
Hans Paerl, University of North Carolina at Chapel Hill, ottentim@email.unc.edu

Microcystis is a toxin-producing (microcystin) harmful algal bloom forming cyanobacterium that is suspected of being involved in the pelagic organism decline occurring throughout the San Francisco Estuary. In order to characterize the toxigenicity of these blooms, individual colonies of *Microcystis* were isolated and genotyped on the basis of their 16S-23S rDNA ITS and microcystin synthetase gene (*mcyB*) sequences. During the summer of 2011, *Microcystis* colonies were observed to appear as one of two morphologies; 1) densely packed and web-like, or 2) large, loosely packed flakes. Although only two morphotypes were observed, the colonies were genetically variable and comprised at least 11 unique operational taxonomic units, with both subtypes being potentially toxic (*mcyB* possessing). Quantitative PCR was utilized to enumerate the total and the toxigenic *Microcystis* populations from six unique sites that were each sampled six times during the summer of 2011. Additionally, water was collected from each sampling site and used in bioassay experiments investigating the role of temperature and light intensity on *Microcystis* growth and toxigenicity. The spatiotemporal patterns and environmental factors likely promoting *Microcystis* throughout the delta is discussed.

Keywords: *Microcystis*, microcystin, toxin, cyanobacteria, management, spatiotemporal, light, temperature

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (I) – Order 1

Monitoring Cyanobacteria, Microcystis, and Microcystin in the San Joaquin River Estuary

William Stringfellow, Ecological Engineering Research Program, University of the Pacific, wstringfellow@lbl.gov
Mark Brunell, Department of Biological Sciences, University of the Pacific, mbrunell@pacific.edu
Teemu Koski, Turku University of Applied Sciences, Teemu.Koski@turkuamk.fi
Jeremy Hanlon, Ecological Engineering Research Program, University of the Pacific, jershanlon@gmail.com
Chelsea Spier, Ecological Engineering Research Program, University of the Pacific, cspier@pacific.edu

The Bay-Delta is experiencing a decline in pelagic fish populations, in part due to disruption of the estuarine food-web. One concern is the apparent increase in blooms of cyanobacteria, which have low food-quality and may produce toxins harmful to fish and other species. The abundance and distribution of cyanobacteria and cyanotoxins in the San Joaquin River estuary (south-east Bay-Delta) was investigated. Multiple techniques for measuring harmful cyanobacteria were tested and compared to results from direct microscopic measurements with the objective of identifying rapid methods for monitoring harmful cyanobacteria in surface waters. Using microscopic analysis, it was found that *Microcystis sp.* were the dominant cyanobacteria in all samples and cyanobacteria biomass was higher in areas of the estuary with low turbidity and soluble N:P ratios less than 10. *Microcystis sp.* are classified as harmful algal species and are associated with the production of the toxin microcystin. ELISA kits were used to measure toxin concentrations and microcystin concentrations had a linear correlation with total cyanobacteria biomass and *Microcystis* concentration. ELISA kits were found to be sensitive and reproducible and are recommended as a rapid alternative or supplement to traditional microscopic enumeration. Sondes for the *in-situ* measurement of phycocyanin from two different manufacturers were also tested and compared to results from microscopy. Phycocyanin is a pigment produced by cyanobacteria and sondes for the *in-situ* measurement of this pigment are used in Finland and elsewhere for the monitoring of harmful algae blooms in freshwater lakes and coastal regions. In this study, we found that the sondes were not sensitive to low concentrations of cyanobacteria and are therefore not recommended for monitoring cyanobacteria in this region. In conclusion, chemical methods for measuring the cyanotoxin microcystin were found to be a reliable and rapid method for the monitoring of harmful algae in this region.

Keywords: harmful algae blooms; water quality; pelagic organism decline POD

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (I) – Order 2

Occurrence and Abundance of Other Toxin-Producing Cyanobacteria in the San Francisco Bay Delta

Dolores Baxa, UC Davis, dvbaxa@ucdavis.edu
Tomofumi Kurobe, UC Davis, tkurobe@ucdavis.edu
Cécile Mioni, Institute of Marine Sciences, University of California, Santa Cruz, cmioni@ucsc.edu
Peggy Lehman, Department of Environmental Science, California Department of Water Resources, plehman@water.ca.gov
Swee Teh, UC Davis, sjteh@ucdavis.edu

The management of blooms in the San Francisco Bay Delta (SFBD) has focused mainly on *M. aeruginosa* being the major species associated in recurring algal blooms. Due to the information gap on the occurrence of other toxigenic species in the SFBD, the relevance of the cyanotoxins that they produce has been rarely investigated particularly on the implications of overlapping blooms and life stages of zooplankton and fish. We have previously demonstrated variations on the abundance of toxic and nontoxic *Microcystis* across sites in the San Francisco Bay Delta during the 2007 blooms. Although water and algal samples that were examined showed the persistence of microcystins, their concentrations did not correlate with the frequency of toxin-producing *Microcystis*. These results may be due to the 1) potential occurrence of other microcystin-producing genera in addition to *Microcystis*, and 2) difficulty of detecting other toxin-producing cyanobacterial species using traditional morphological identification.

To address the disparity in microcystin levels and the abundance of toxin-producing *Microcystis*, molecular techniques (16S rDNA sequencing and qPCR) were employed to determine the presence of other toxin-producing species on archived genomic DNA from the 2007 algal samples and compared to species present in the 2011 blooms. Our findings will show the identification, quantification, and variations on the abundance of other toxin producing cyanobacteria such as *Anabaena, Aphanizomenon,* and *Lyngbya* in addition to *Microcystis* as determined from selected sampling sites and dates from the 2007 and 2011 blooms.

Relevance: Knowledge on the shift in bloom composition and abundance will help promote the sustainability of the delta ecosystem and its resident fisheries by regulating the key environmental factors that may alter the toxicity of local cyanobacterial harmful algal blooms (CyanoHABs).

Keywords: toxin-producing, microcystin, qPCR, CyanoHABs, San Francisco Bay delta, taxon shift

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (I) – Order 3

Determining Environmental Controls and Ecological Impacts of CyanoHABs in the San Joaquin-Sacramento Delta – A Multidisciplinary Approach

Cécile Mioni, UCSC, cmioni@ucsc.edu
Dolores Baxa, UCD, dvbaxa@ucdavis.edu
Lenny Grimaldo, USBR, LGrimaldo@usbr.gov
Kendra Hayashi, UCSC, khayashi@ucsc.edu
Wim Kimmerer, SFSU, kimmerer@sfsu.edu
Raphael Kudela, UCSC, kudela@ucsc.edu
Tomofumi Kurobe, UCD, tkurobe@ucdavis.edu
Lisa Lucas, USGS, llucas@usgs.gov
Hans Paerl, UNC, hans_paerl@unc.edu
Alex Parker, SFSU, aeparker@sfsu.edu
Scott Waller, DWR, swaller@water.ca.gov
Frances Wilkerson, SFSU, fwilkers@sfsu.edu

Harmful cyanobacteria (CyanoHABs), such as *Microcystis aeruginosa*, and the toxins they produce are a growing concern as a source of impairment in California water bodies. CyanoHAB distribution, abundance and environmental conditions promoting their proliferation and toxin production are not well characterized.

Total cyanobacteria biomass has increased since 1975 throughout the San Joaquin-Sacramento Delta coincident with a decline in diatom biomass. Recurrence of seasonal CyanoHABs in the Delta since 2000 coincided with the decline of various pelagic organisms and their copepod prey, suggesting that these cyanoHABs may at least in part be responsible for this decline. The increase in CyanoHABs in the Delta coincided with several environmental changes that are known to favor their growth including increasing temperature. These environmental changes also appear to correlate with the decline of pelagic fish species.

Since 2008, we have conducted multidisciplinary, multi-agencies collaborative monitoring programs in the Delta with the goal of gaining a better understanding of the environmental drivers controlling cyanoHAB occurrence and toxicity. Here, we will present results from our multiannual seasonal monitoring of spatial and temporal distribution of cyanoHAB species and associated toxins throughout the Delta. Our preliminary results indicate that cyanoHAB abundance and toxicity in the Delta are controlled by several interacting environmental factors. Surface water temperature and nutrient availability, especially nitrogen sources, appear to be key drivers of cyanoHAB composition and toxicity, but additional environmental stressors specific to individual cyanoHAB taxa may also play a significant role. Our results also indicate that in addition to *Microcystis*, other potentially toxigenic cyanobacteria such *Aphanizomenon, Anabaena* and *Synechococcus*, may contribute to bloom toxicity in the Delta. Furthermore, we find evidence for microbial consortia which may mediate toxin production within the cyanoHAB assemblage.

Keywords: Cyanobacteria, *Microcystis*, *Aphanizomenon*, CyanoHABs, toxins,

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (I) – Order 4

Carbon and Nitrogen Uptake Rates Associated with Cyanobacterial Blooms in the San Francisco Delta

Alexander Parker, Romberg Tiburon Center, SFSU, aeparker@sfsu.edu
Allison Johnson, Romberg Tiburon Center, SFSU, allison95376@gmail.com
Jamie Lee, Romberg Tiburon Center, SFSU, jamielee00@gmail.com
Adam Pimenta, Romberg Tiburon Center, SFSU, pimenta.adam@gmail.com
Cecile Mioni, University of Californa, Santa Cruz, cmioni@ucsc.edu
Frances WIlkerson, Romberg Tiburon Center, SFSU, fwilkers@sfsu.edu

Blooms of cyanobacteria have been observed during the summer in the San Francisco Estuary Delta (Delta). These blooms have the potential to disrupt estuarine food webs and may pose a risk for human health through the production of toxins. During this study, depth-integrated measurements of carbon and nitrogen uptake were made at several sites within the central Delta where cyanobacteria were present in high abundances. It was hypothesized that both light and nutrient concentrations were drivers of cyanobacterial blooms. Because these cyanobacteria form blooms that are concentrated at the water surface, measurements were made using water collected at the surface (cyanobacteria-dominated) and at a depth of 3m (where other phytoplankton taxa-dominated) to allow comparison between cyanobacteria and other autotrophs. Stable isotope tracers of ^{13}C and ^{15}N (NH_4, NO_3 and urea) were added to bottles incubated at irradiances of 5 to 50% of surface photosynthetically active radiation. Results show a strong positive response of light on C and N uptake rates for communities in both surface and 3m samples. NH_4, NO_3 and urea uptake were observed in incubations suggesting that all N substrates were potentially important in these habitats. This work provides insight into light and nutrient controls on cyanobacterial blooms that may guide management strategies in the future.

Keywords: *Microcystis*, Cyanobacteria, primary production, nitrogen uptake, ammonia, nitrate, flooded island

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (I) – Order 5

Stable Nitrate Isotopes Reveal Different Nitrate Dynamics in the San Joaquin River under Changing Flow Conditions

Megan Young, U.S. Geological Survey, Menlo Park, CA, mbyoung@usgs.gov
Carol Kendall, U.S. Geological Survey, Menlo Park, CA, ckendall@usgs.gov
William Stringfellow, University of the Pacific, Stockton, CA, wstringfellow@lbl.gov
Randy Dahlgren, U.C. Davis, Davis, CA, radahlgren@ucdavis.edu
Steve Silva, U.S. Geological Survey, Menlo Park, CA, srsilva@usgs.gov

The dual nitrate isotope composition ($\delta^{15}N$ and $\delta^{18}O$-NO_3) of water from the San Joaquin River (SJR) and tributaries was measured approximately monthly between March 2005 and December 2007 in order to trace dominant nitrate sources and processes under different flow conditions. 2007 was a dry year in comparison to both 2005 and 2006. In the SJR, $\delta^{15}N$-NO_3 and nitrate concentrations showed similar patterns related to flow during 2005- 2006, and distinctly different behavior during the summer and fall of 2007, indicating a shift in the processes controlling nitrate sources and cycling. Higher flows in the SJR were associated with lower nitrate concentrations and lower $\delta^{15}N$-NO_3, while periods of lower flows showed higher nitrate concentrations and higher $\delta^{15}N$-NO_3. The changes in $\delta^{15}N$-NO_3 indicate shifts in the dominant nitrate sources to the river associated with flow changes, since dilution with lower nitrate concentration water containing nitrate from the same source will not cause a change in isotopic composition. The higher $\delta^{15}N$-NO_3 observed during low flow conditions suggests significant inputs of human or animal waste to the river. During 2005- 2006, $\delta^{15}N$-NO_3 typically decreased downstream in the SJR, and isotope mass balance calculations showed that the nitrate isotope patterns in the SJR were primarily controlled by downstream inputs of lower nitrate concentration water from the Tuolumne and Stanislaus Rivers. During the very low flow conditions of summer and fall 2007, both $\delta^{15}N$- and $\delta^{18}O$-NO_3 showed coupled downstream increases which could not be accounted for by inputs from the monitored tributaries. In 2007, both the $\delta^{15}N$- and $\delta^{18}O$-NO_3 also increased over time as chl-a concentration increased, suggesting that in contrast to conditions in 2005- 2006, algal uptake, in addition to other inputs from high $\delta^{15}N$-NO_3 sources, significantly influenced the isotopic composition of nitrate in the mainstem SJR during the summer and fall of 2007.

Keywords: nutrients; nitrate; San Joaquin River; stable isotopes

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (II) – Order 6

Using Multi-Isotope Techniques to Estimate the Relative Uptake of Ammonium and Nitrate by Phytoplankton for Sites in Sacramento River and Delta

Carol Kendall, U. S. Geological Survey, ckendall@usgs.gov
Megan Young, U. S. Geological Survey, mbyoung@usgs.gov
Steve Silva, U. S. Geological Survey, srsilva@usgs.gov
Marianne Guerin, Resource Management Associates, maguerin@rmanet.com
Calla Schmidt, U. S. Geological Survey and UCSC, callaschmidt@gmail.com
Alex Parker, Romberg Tiburon Center, SFSU, aeparker@sfsu.edu

High NH4 concentrations have been hypothesized to suppress phytoplankton blooms, contributing to pelagic organism decline in the San Francisco Estuary. One test of this hypothesis is to compare chlorophyll levels with the natural abundance stable isotope ratios of phytoplankton, NH4, and NO3. Isotope techniques are a useful tool for this test because we have found that the $\delta^{15}N$ of NH4, NO3, and phytoplankton (as estimated from bulk seston composition) at most sites are isotopically distinctive because of the effects of progressive downstream nitrification on the $\delta^{15}N$ of residual NH4 and new NO3.
To provide an independent test of this hypothesis, samples for isotopic analysis were collected along ~30 transects of the Estuary from March 2009 to December 2012. Many transects piggybacked on the sampling efforts of other teams and collected samples from 12-25 sites depending on transect. All samples have been analyzed for seston $\delta^{15}N$, $\delta^{15}N$, $\delta^{34}S$, and C:N. Analysis for NH4-$\delta^{15}N$, NO3 $\delta^{15}N$ and $\delta^{18}O$, DOC-$\delta^{13}C$, and water $\delta^{18}O$ and $\delta^{2}H$ are in various stages of completion; we expect that the presentation will include most of the dataset. Calculations made using the subset of total samples with complete $\delta^{15}N$ data for seston, NH4, and NO3 show few sites and dates where the $\delta^{15}N$ values definitely support a single source of N to uptake. Instead, the $\delta^{15}N$ data are consistent with variations in proportions of NH4 and NO3 to uptake at different sites and dates, with strong downstream trends in relative proportions of N source to phytoplankton. One main complication is the estimation of how much of the uptake of N is actually by bacteria, not phytoplankton. Our approach shows promise as a direct measure of in-stream uptake rates that can be piggybacked onto routine monitoring programs for habitat characterization and estimating the impacts of N loads from different sources.

Keywords: nitrate, ammonium, phytoplankton, habitat-assessment, isotopes, uptake, assimilation

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (II) – Order 7

Cyanobacterial Toxins, Environmental Drivers, and Watershed Connectivity: How Serious is the Threat?

Raphael Kudela, Institute of Marine Sciences, UC Santa Cruz, kudela@ucsc.edu
Cecile Mioni, Institute of Marine Sciences, UC Santa Cruz, cmioni@ucsc.edu
Corinne Gibble, UC Santa Cruz, corinnegibble@gmail.com
Kendra Hayashi, UC Santa Cruz, khayashi@ucsc.edu
Karen Worcester, Central Coast RWQCB, Kworcester@waterboards.ca.gov
Tara Schraga, United States Geological Survey, tschraga@usgs.gov

Harmful cyanobacteria (CyanoHABs), such as *Microcystis*, *Aphanizomenon, Anabaena,* and *Planktothrix* are of increasing concern in California's watersheds, estuaries, and coastal waters. While CyanoHABs are routinely monitored, there is increasing evidence that bloom formation and presence of toxins are not necessarily well correlated, in part because the toxins are environmentally stable and easily transported downstream, with or without cells.

New methods for toxin detection,such as Solid Phase Adsorption Toxin Tracking (SPATT) provide evidence for persistent, chronic environmental exposure to toxins in many water bodies. SPATT can detect both lower levels and ephemeral toxin exposure because it passively concentrates toxins and integrates over the duration of deployment (typically 7-30 days). Comparison of this integrated toxin data with environmental drivers such as nutrients and temperature suggest that previously identified correlates are tracking the seasonal occurrence of large bloom events but do not provide much information about the chronic toxin levels. Spatially extensive sampling of Central California watersheds has identified some "hotspot" locations such as the Bay Delta, but has also identified many other water bodies with easily detectable levels of toxins.

The imminent release of new guidelines from the Office of Environmental Health Hazard Assessment (OEHHA) will set lower recommended exposure levels for recreation and ecological impairment. Downstream transport of toxins coupled with spatial and temporal separation of toxins and cells strongly suggests that many potential negative consequences are unrecognized or unreported. We suggest that the OEHHA guidelines, together with the increasing evidence for chronic exposure not captured by traditional grab sampling (particularly during "non-bloom" periods) requires the scientific and management communities to re-assess the emerging threat of CyanoHABs. While hotspots are clearly an area of immediate concern, the widespread occurrence and transport of toxins from the coastal watershed to the land/sea interface could ultimately pose a greater and more difficult to mitigate threat.

Keywords: CyanoHAB, *Microcystis*, microcystin, blue-green algae, toxin, harmful algal bloom

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (II) – Order 8

Are Nutrients a Driver of Cyanobacterial Abundance in the San Francisco Estuary Delta?

Adam Pimenta, San Francisco State University - Romberg Tiburon Center for Environmental Studies, pimenta.adam@gmail.com
Cecile Mioni, Institute of Marine Science University of California at Santa Cruz, cmioni@ucsc.edu
Alex Parker, San Francisco State University - Romberg Tiburon Center for Environmental Studies, aeparker@sfsu.edu
Frances Wilkerson, San Francisco State University - Romberg Tiburon Center for Environmental Studies, fwilkers@sfsu.edu

The San Francisco Estuary Delta is a complex system characterized by a variety of chemical and hydrodynamic inputs from the Sacramento and San Joaquin rivers and Suisun Bay. Blooms of cyanobacteria have been increasingly common in this system with *Microcystis aeruginosa* observed starting in 1999 and *Aphanizomenon sp.* observed starting in 2011. The relationship between cyanobacteria and nutrient concentrations has been observed in many freshwater and estuarine systems, with high concentrations of dissolved inorganic nitrogen (N) and phosphorus (P) and relatively high N : P ratios (~30:1) associated with bloom development. During the July-September period in 2011 and 2012, 3 paired sets of stations, selected to represent the different environments common in the Delta (small tributary, major river and flooded island) were sampled to test the hypothesis that nutrients were important drivers of cyanobacterial blooms. Observed N concentrations were greater than 6 µM and observed P concentrations were above 1 µM at all stations, sufficient to support cyanobacterial blooms. Sites associated with *Microcystis* had relatively low (~1µM) ammonium-N concentrations, compared to non-*Microcystis* sites (except at flooded island sites where ammonium-N concentrations were uniformly low). Water column nutrient N:P was lower than the Redfield ratio (16:1) at all of the sites. These results are contrary to the conditions hypothesized to promote cyanobacteria requiring an evaluation of nutrient impacts. Characterizing the nutrient conditions associated with cyanobacteria in the Delta are critical to develop strategies to prevent or control future blooms.

Keywords: Cyanobacteria, Nutrients, Delta, Flooded Island

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (II) – Order 9

Monitoring Toxin-Producing Cyanobacteria in Clear Lake and Sacramento-San Joaquin Delta by DNA Barcoding and Development of Quantitative PCR Assays

Tomofumi Kurobe, University of California, Davis, tkurobe@ucdavis.edu
Dolores Baxa, University of California, Davis, dvbaxa@ucdavis.edu
Cécile Mioni, University of California, Santa Cruz, cmioni@ucsc.edu
Raphael Kudela, University of California, Santa Cruz, kudela@ucsc.edu
Thomas Smythe, Lake County Water Resources Department, Tom.Smythe@lakecountyca.gov
Scott Waller, California Department of Water Resources, swaller@water.ca.gov
Swee Teh, University of California, Davis, sjteh@ucdavis.edu

Accurate and consistent identification and monitoring of harmful cyanobacteria using traditional morphological taxonomy are challenging tasks due to their sizes and high degree of phenotypic plasticity in natural assemblages. To overcome the limitations associated with microscopic identification, we utilized DNA barcoding for identification of potentially toxin producing cyanobacteria in Clear Lake and in the Sacramento San Joaquin Delta in Northern California. Species specific DNA fragments, also known as "DNA barcodes" were successfully obtained and showed high similarity to major toxin producers: *Anabaena, Aphanizomenon, Lyngbya, Microcystis* and *Synechococcus*. The DNA barcodes for *Anabaena, Aphanizomenon, Lyngbya,* and *Microcystis* were also used to develop high-throughput quantitative PCR assays. Sensitivities and specificities of the assays were evaluated and all the assays showed high amplification efficiency (>98%) with excellent analytical sensitivity, as low as 10 copies of the target DNA in a single reaction. The assays showed good analytical specificities and no evidence of cross-reaction was observed among other genera obtained through DNA barcoding.

Relevance: DNA barcodes and specific qPCR assays for toxin producing cyanobacteria are useful tools for identifying the species composition of blooms. These techniques may be incorporated in monitoring recurring blooms and for determining the environmental drivers triggering bloom toxicity in the Sacramento San Joaquin delta and Clear Lake.

Keywords: Cyanobacteria, DNA barcoding, quantitative PCR

Thursday, October 18, 2012: Room 308-310, Understanding Cyanobacterial Blooms in the San Francisco Estuary Delta (II) – Order 10