

Understanding Nitrogen Assimilation and Inhibition in Cultures of *Microcystis aeruginosa*: Implications for Cyanobacterial Bloom Development in the San Francisco Delta

Jamie Lee*, Romberg Tiburon Center, SFSU, jamielee00@gmail.com
Alexander E. Parker, California Maritime Academy, aparker@csum.edu
Frances P. Wilkerson, Romberg Tiburon Center, SFSU, fwilkers@sfsu.edu

The magnitude of toxigenic *Microcystis aeruginosa* blooms have increased in the San Francisco Delta (Delta) over the last decade. Although never directly tested in the Delta, the availability of different chemical forms of nitrogen (N) may play a role in promoting *Microcystis* blooms. Our field studies show that *M. aeruginosa*-dominated communities have higher rates of ammonium (NH₄) uptake than nitrate (NO₃), urea or glutamate, suggesting a competitive advantage over phytoplankton such as diatoms, which have high NO₃ uptake rates. NH₄ often inhibits algal NO₃ uptake but it is unknown whether inhibition occurs with *M. aeruginosa*. If *M. aeruginosa* does not experience NH₄ inhibition, this would provide a growth advantage over other algal species since it could equally access both NO₃ and NH₄ pools. The goal of this study was to investigate inhibition phenomena in *M. aeruginosa*. Short-term (0.5 hour) experiments were conducted using non-toxic and toxic strains of *M. aeruginosa* to study whether NH₄ inhibited NO₃ or urea uptake, or if NO₃ inhibited NH₄ uptake. Our results suggest that the non-toxic strain exhibited urea inhibition by NH₄ but no inhibition of NO₃ or NH₄ uptake. Conversely, the toxic strain showed inhibition of uptake by all forms of N; NH₄ inhibited NO₃ and urea uptake, and NO₃ inhibited NH₄ uptake. The strain-specific responses imply that there may be an interaction between toxin production and N uptake. The lack of inhibition of NO₃ and NH₄ uptake with the non-toxic strain may provide a competitive advantage for N uptake over toxic *M. aeruginosa* strains and other algal species. Understanding how *M. aeruginosa* reacts to various N species and concentrations provides insight on its potential for growth and bloom formation in environments with increasing anthropogenic N. These results can inform management in mitigating these cyanobacterial blooms that have negative effects on the Delta.

Keywords: *Microcystis aeruginosa*, nitrogen uptake, ammonium inhibition

Session Title: Food Webs III: When Things Get Toxic

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Ecotoxicological Effects of Microplastic and Priority Pollutants in a Bay-Delta Food Chain

Chelsea Rochman, UC Davis, cmrochman@ucdavis.edu

Sebastian Serrato, UC Davis, saserrato@ucdavis.edu

Swee Teh, UC Davis, sjteh@ucdavis.edu

Plastic debris has been documented in the Bay-Delta. To mitigate the problem, storm drain systems are currently undergoing modification to remove larger material, but much of this debris is likely microplastic. Because plastic debris is associated with large concentrations of persistent, bioaccumulative and toxic (PBT) chemicals, the combination of microplastic and PBTs are likely ingested by Bay-Delta organisms. Ingestion of microplastic provides a pathway for PBTs to biomagnify in higher trophic levels, with uncertain health effects. There is no information on the extent to which ingesting microplastics may enhance biomagnification or cause ecological impacts in aquatic foodwebs. To provide a fundamental understanding of how microplastic affects PBT transfer into Bay-Delta food chains and how microplastic affects the health of Bay-Delta organisms we designed an experiment using Asian clams (*Corbicula fluminea*) and white sturgeon (*Acipenser transmontanus*). We aim to quantify the pathway for microplastics to transfer sorbed PCBs to prey, measure the chemical transfer of PCBs from prey to a predator and determine how ingestion of various polymers, with and without PCBs, affects organismal health. Asian clams will be exposed for 30 days to separate treatments of microplastic (polyethylene terephthalate, polyethylene, polyvinyl chloride and polystyrene) with and without sorbed PCBs. Next, diets will be formulated using purified ingredients and clams from the first exposure and will be fed to their predators (sturgeon) for 30 days. Chemical analyses (GC/MS) will track concentrations of PCB congeners from microplastic in animal tissues, allowing us to measure bioaccumulation and biomagnification. Toxicological assays will provide important understanding about whether ingesting microplastic with and without PCBs affects their health. Our work will provide original understanding of the capacity of different microplastics and PCBs to act as multiple stressors in Bay-Delta foodwebs. Experiments are currently under way, and results to date will be presented.

Keywords: plastic debris, Asian clam, sturgeon, food web, PCBs, bioaccumulation, microplastic

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The Future of *Microcystis* spp. in the San Francisco Estuary Delta: Investigations into the Role of Temperature and Salinity Tolerance on Growth

Allison N. Johnson*, Romberg Tiburon Center for Environmental Studies-SFSU,
allison95376@gmail.com

Alexander E. Parker, Cal Maritime Academy, aparker@csum.edu

Frances P. Wilkerson, Romberg Tiburon Center for Environmental Studies-SFSU,
fwilkers@sfsu.edu

Increases in both water temperature and salinity predicted under climate change are hypothesized to promote cyanobacteria blooms in estuaries globally. In the San Francisco Estuary Delta (Delta) such a shift to cyanobacterial dominance may already be occurring. Since 1999 blooms of the toxigenic cyanobacterium *Microcystis aeruginosa* have been observed during the summer, with negative consequences for water quality and the estuarine food web. With the goal of understanding how variations in water temperature and salinity may influence present and future cyanobacteria success in the Delta, a series of small bottle experiments were conducted using field collected phytoplankton, including cyanobacteria-dominant assemblages. The influence of temperature and salinity on phytoplankton community biomass and composition was assessed by phytoplankton size structure, direct microscopy and photosynthetic pigment analysis using high-performance liquid chromatography. Of the phytoplankton groups, cyanobacteria grew at higher temperatures and were tolerant of substantially higher salinities than ambient conditions in the Delta habitats where they were observed. These results linking cyanobacteria to conditions associated with predicted climate change provide insight into potential future habitat expansion and microbial community shifts toward cyanobacterial dominance in the Delta.

Keywords: *Microcystis aeruginosa*, temperature, salinity, climate change, HPLC, cyanobacteria

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The Distribution, Ecology and Genetics of *Microcystis* Blooms Throughout the San Francisco Bay Delta

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

Problem statement: The on-going decline of the planktonic food web and several important Bay Delta fishes, collectively termed the pelagic organism decline (POD), temporally coincides with the establishment and persistence of cyanobacterial harmful algal blooms (CyanoHABs) that have occurred annually since the early 2000's. We aimed to assess how these CyanoHABs may be directly or indirectly influencing the food web structure within the Delta.

Approach: One component of this work was to better understand the ecology and physiological potential of these cyanobacterial assemblages. We employed a suite of molecular analyses, including: quantitative PCR to track the distribution of toxic and nontoxic cells over time and space, DNA fingerprinting by 454 pyrosequencing to track the origins and fates of *Microcystis* sub-populations (strains), and shotgun metagenomics and genome assembly to assess their genetic capabilities.

Results: This work identified the presence of at least eight *Microcystis* strains within the Delta, as well as the genetic pathways required to produce a wide variety of secondary metabolites thought to negatively affect the fitness and fecundity of herbivorous zooplankton. Additionally, cyanobacterial blooms were observed to influence microbial community structure within the Delta, resulting in a nearly 50% reduction in total microbial diversity observed during bloom events.

Conclusions/Relevance: Future studies are needed to directly quantify the impacts of cyanobacterial secondary metabolites on keystone zooplankton within the Bay Delta. Additionally, we posit that competitive displacement of more nutritious algae such as diatoms, flagellates and green algae may also confer indirect negative effects on zooplankton fitness, which in turn may influence the POD. Restoration and management strategies should aim to reduce the magnitude of cyanobacterial blooms sustained within the Bay Delta by limiting total nitrogen and phosphorus inputs and by increasing the flow rates through the flooded islands, which appear to be "hot beds" for cyanobacterial blooms.

Keywords: Cyanobacterial blooms, toxicity, genetics, pelagic organism decline, zooplankton, food web

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Pesticide Mixture Toxicity Assessments Differ Between Single Species Tests and Mesocosm Studies

Simone Hasenbein*, School of Veterinary Medicine, Department of Anatomy, Physiology and Cell Biology, University of California, shasenbein@ucdavis.edu

Sharon P. Lawler, Department of Entomology and Nematology, University of California, splawler@ucdavis.edu

Jürgen Geist, Aquatic Systems Biology Unit, Technische Universität München, Germany, geist@wzw.tum.de

Richard E. Connon, School of Veterinary Medicine, Department of Anatomy, Physiology and Cell Biology, reconnon@ucdavis.edu

Water pollution is a major threat to biological diversity worldwide. Increased pesticide use, and their application as mixtures, is one of many drivers affecting habitat health. Most data used in risk assessment of pesticides are based on single species tests using single substances, at concentrations that are usually not environmentally realistic, with few assessments including sublethal endpoints.

In order to bridge the gap between laboratory toxicity tests using individual chemicals, and the effects of mixture toxicity on aquatic ecosystems, we first conducted 10-day toxicity assessments of single and combined exposures of three commonly used insecticides: two pyrethroids; lambda-cyhalothrin and permethrin, and one organophosphate; chlorpyrifos, on lethal and sublethal effects on *Chironomus dilutus* and *Hyalella azteca*, two important ecotoxicological testing organisms. We then evaluated these conditions on community composition within a 6-month multi-species field study using mesocosms, composed of naturally developed invertebrate communities.

In the laboratory-based single-species tests, growth and motility were significantly affected at ecologically relevant concentrations. The effects of mixture exposures, at relative toxic concentrations, were less severe than those observed in the single exposures. In the mesocosms, the effects of a series of applications of tertiary contaminant mixtures resulted in significant decrease of *H. azteca* population, and zooplankton species such as copepods and cladocera. The lethal concentrations determined in single-species tests in the laboratory do not necessarily reflect the effects predicted in the environment. By using lab-based toxicity tests it is possible to determine ecologically relevant sublethal effects under controlled conditions within a very short period of time. Mesocosms on the other hand allow us to evaluate long-term community and food-web effects. Both approaches provide essential information for understanding mixture toxicity and evaluating their effects on aquatic ecosystems, which can be used in risk-assessments of contaminants of concern.

Keywords: aquatic communities, mesocosms, pesticide mixtures, single-species testing, toxicity tests, food-webs

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