THE ROLE OF MICROCYSTIS IN THE DELTA: A FUNCTIONAL APPROACH FINAL REPORT

Delta Stewardship Council Grant #2044

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Project Overview

Identification of the Problem

The Pelagic Organism Decline (POD) occurred around 2002 (Sommer et al. 2007, Thomson et al. 2010), roughly coincident with an apparent increase in frequency and intensity of *Microcystis* blooms centered in the freshwater Delta (Lehman et al. 2005). Several potential mechanisms may link these two temporal trends (Lehman et al. 2008). Although direct toxicity to fish is unlikely at observed toxin concentrations, indirect effects on fish may arise through the planktonic food web. Specifically, larvae and juveniles of POD species feed mainly on copepods, and copepod survival may be impaired by blooms (Ger et al. 2010a). Thus, a thorough investigation of the mechanisms forming *Microcystis* blooms and their effects on the pelagic food web would quantify the link between blooms and fish and help to identify management strategies.

Project Goal and Objectives

The goal of this project was to determine the environmental conditions leading to *Microcystis* blooms, their toxicity and their impact on the pelagic food web of the Delta.

- Objective 1. Determine the biotic and abiotic factors controlling *Microcystis* bloom formation and toxin production in the Delta.
- Objective 2: Determine the role that *Microcystis* strains play in resulting toxicity associated with blooms.
- Objective 3: Determine the role of *Microcystis* in the Delta pelagic food web and its effect on the POD through zooplankton grazing.

Conceptual Model

Harmful algal blooms (HABs) have become a significant nuisance in freshwaters, estuaries, and coastal waters worldwide (Paerl 1988, Paerl et al. 2001). Blooms of cyanobacteria (CHABs) including *Microcystis* species have been on the increase in freshwaters globally, particularly in warm, stratified, eutrophic waters of long residence time (Paerl et al. 2001). All HABs can alter nutrient and biogeochemical cycles and deplete oxygen, but the most troublesome attribute of CHABs is the production of cyanotoxins that can impair food web function and fishery production as well as human uses (Paerl et al. 2001). Climate change, particularly warming and droughts, may favor further increases in the prevalence of CHABs (Paerl and Huisman 2008, 2009).

Microcystis is a colony-forming, non-N_2 fixing cyanobacterium and one of the most common species of cyanobacteria worldwide, dominating in eutrophic and hypereutrophic aquatic ecosystems (Christoffersen 1996). Several strains of *Microcystis* produce hepatotoxins including microcystins and microviridins (Dawson 1998, Rohrlack et al. 2003). The life cycle of *Microcystis* includes overwintering resting stages that remain dormant in sediments, with a shift to pelagic forms triggered by increases in water temperature and irradiance or a change in dissolved oxygen concentration (Stahl-Delbanco et al.2003). High nutrient concentrations combined with low N:P ratios can favor maximum recruitment of resting cysts (Stahl-Delbanco et al, 2003). While benthic colonies are near neutral buoyancy, photosynthesis prompts positive lift (Reynolds 1981). *Microcystis* colonies tend to concentrate at the surface, especially in turbid waters, due to vertical migrations and the ability to regulate buoyancy (Reynolds 1981).

Likely drivers of *Microcystis* blooms and resulting impacts on estuarine food webs are summarized in Fig. B.1. Our conceptual model contrasts non-bloom (left) with bloom (right) conditions to illustrate the factors that might facilitate the transition to a bloom, and the influences that the bloom might have on the pelagic food web. The diagram also depicts the topics that were addressed in the project research.

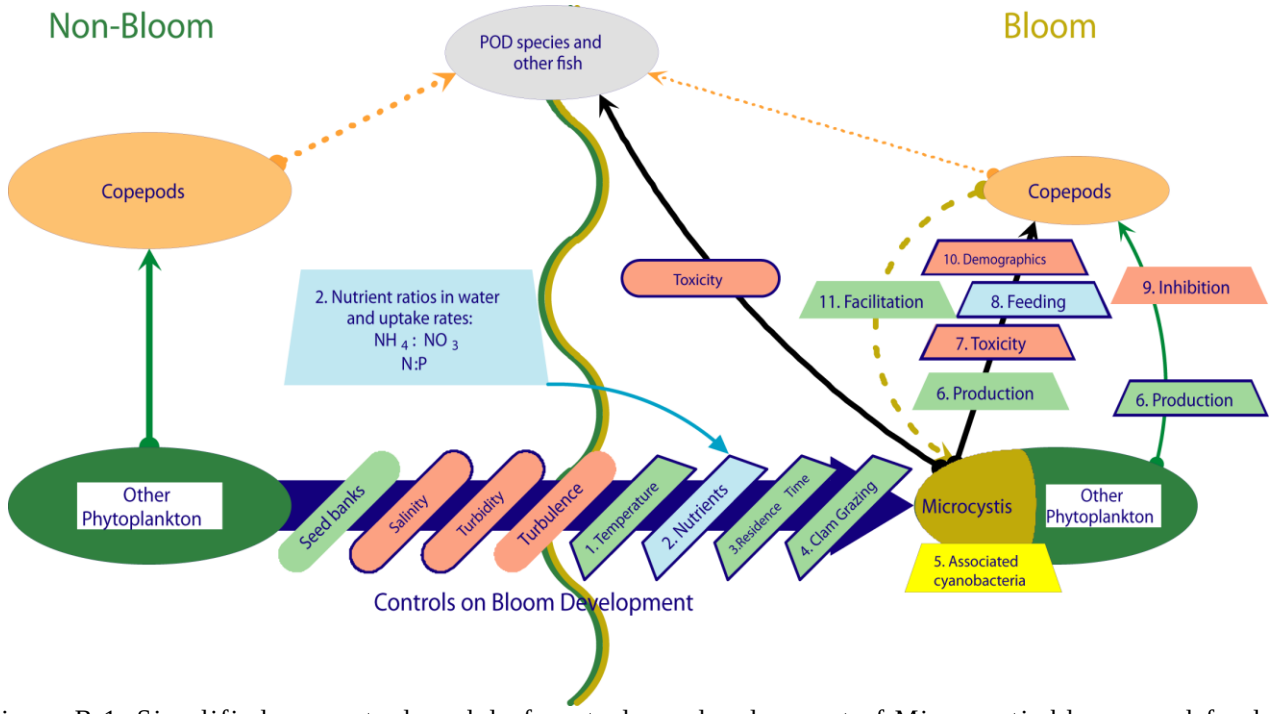
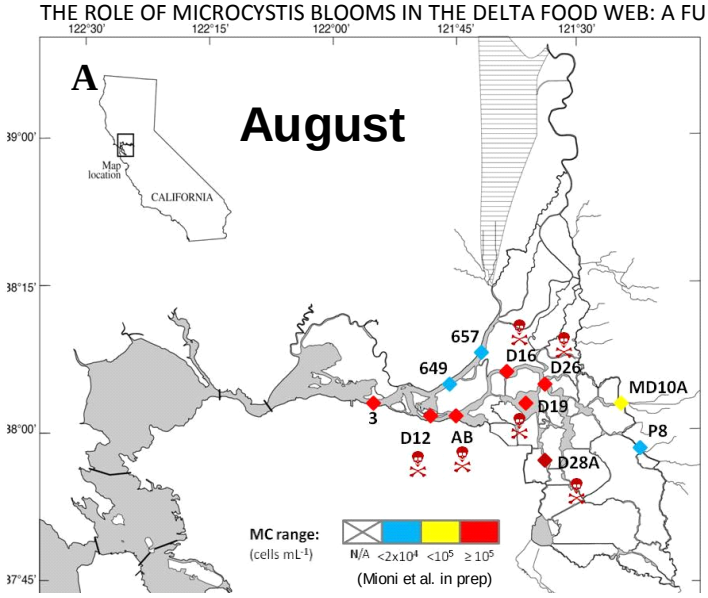


Figure B.1: Simplified conceptual model of controls on development of Microcystis blooms and food web consequences in the Delta. Left side of wavy line: non-bloom conditions with high phytoplankton biomass supporting high biomass of copepods. Right side: bloom conditions including some potential food web effects. Heavy blue arrow indicates bloom development; small boxes indicate controls on bloom development or influences on the food web.

Microcystis in the Sacramento-San Joaquin Delta

Microcystis was first documented in the SFE in 1999 and has become widely distributed among freshwater to brackish reaches of the estuary (Lehman et al., 2005, 2008). While the distribution of Microcystis in the SFE includes the upper estuary (i.e. Suisun Bay) identified "hotspots" of *Microcystis* abundance is centered in the central Delta in the San Joaquin River (Fig. B.2).



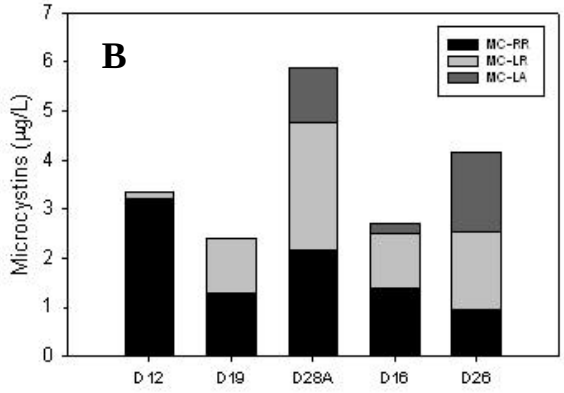


Figure B. 2: A. Distribution of Microcystis at the climax of the bloom (August) in the Delta. The skulls indicate that microcystins were present. B. Microcystins concentrations as determined by LC-MSMS. Mioni et al. in prep.

Long residence time appears to be an important precursor for *Microcystis* bloom development (Paerl et al. 2001). Residence time in sub-regions of the Delta vary from days to months depending on location and flow conditions (Kimmerer and Nobriga 2008). This suggests a basis for the strong spatial variability in bloom formation previously reported (Lehman et al. 2008).

Because inorganic nutrients are generally abundant in the SFE (Hager & Schemel 1996) nutrient availability has been largely overlooked as a driver of phytoplankton dynamics in the SFE. Recent experimental evidence as well as analysis of long-term trends of nutrients and phytoplankton suggests a role for nutrients in determining phytoplankton abundance (Van Nieuwenhuyse et al. 2008), frequency and timing of phytoplankton blooms (Wilkerson et al. 2006; Dugdale et al. 2007) and species community shifts (Dugdale et al. 2007; Glibert et al. 2011; Glibert et al. 2015) in the SFE. Substantial N loading to the Delta (and specifically increased NH_4 concentrations) has been hypothesized to sustain *Microcystis* growth in the ecosystem (Meyer et al. 2009).

While the overall toxicity of the Delta population has not been documented, microcystin-LR comprises the greatest percent of the total microcystins at most stations (Lehman et al. 2004, 2008, 2010). The highest toxin concentrations is usually observed in the surface layer of the western and Central Delta stations (Antioch and Old River), which is characterized by lake-like flooded islands, including agricultural channels diverting water to storage reservoirs and the State Water project (Lehman et al. 2004, Lehman et al. 2010, Mioni et al. in prep).

Microcystis has relatively slow growth rates, so blooms result more from biomass accumulation than rapid growth (Lehman 2008). Low total primary production in the Delta may exacerbate the influence of *Microcystsi* during summer and fall when it is abundant. However, the contribution of *Microcystis* to total primary production appears to be small. Primary production estimates for phytoplankton of the size range of *Microcystis* colonies (>75 µm) ranged from 19 to 141 ng C m^2 h^{-1} using PI curves for whole water determined by the light/dark bottle oxygen technique (Lehman et al 2008). Assuming a 13-hr day-length in August, corresponding daily rates would be 0.2 to 1.8 µg C m^2 d^{-1}. In contrast, Jassby et al. (2002) reported mean gross primary production rates of 132,000 to 201,000 µg C m^2 d^{-1} for the

Central Delta and Lidstrom (2008) reported 155,000 μg C m^2 d^{-1} in the western Delta during summer where *Microcystis* was not found.

If *Microcystis* is affecting the pelagic food web, the most likely mechanism for effects is through copepods, notably *Pseudodiaptomus forbesi*. This copepod is the most abundant calanoid in the freshwater-brackish habitat of *Microcystis*, and is important food for early life stages of nearly all fishes in the Delta, as well as juveniles of some and all stages of delta smelt. Previous experiments have demonstrated an effect of *Microcystis* on *P. forbesi*, apparently through toxicity not related to microcystin (Ger et al. 2010a). The cyclopoid copepod *Limnoithona tetraspina* is much more abundant in brackish water than is *P. forbesi*, but is much less fed upon by fish because of its small size and cryptic behavior (S. Slater, CDFG, and L. Sullivan, SFSU, pers. comms. To W. Kimmerer). Furthermore, *L. tetraspina* feeds only on motile cells such as ciliates (Bouley and Kimmerer 2006, Gifford et al. 2007) and therefore is unlikely to consume *Microcystis*.

Project Organization: Identification of Project Tasks and PIs

The Project addressed several areas of inquiry into the development and implications of *Microcystis* blooms in the San Francisco Estuary Delta. These areas were arranged by task from Task 2 to 5 (Task 1 was the overall management of the project). In the report that follows we provide a reporting of project status by task highlighting **Key Findings**, provide a **Task Narrative** and **Task Deliverables**.

1. Controls on Bloom Development: Nutrients – Task 2: Parker and Wilkerson Task 4 Pearl and Otten

2. Controls on Bloom Development: Climate – Task 2: Parker and Wilkerson, Task 4 Pearl and Otten

3. Toxin Production and Microcystis Strains – Tasks 3 Mioni and Kudela, Task 4 Paerl and Otten

4. Microcystis Food Web Effects - Task 5 Kimmerer and Ger

Summary of Key Project Outcomes by Task

TASK 1: PROJECT MANAGEMENT

PI: Parker

KEY FINDINGS

1. Completed a total of 24 sampling days, occupying 72 stations in the Delta between 2011 and 2012. Completed five cruises of 8 to 10 stations in the San Joaquin River in 2013.

2. Convened a one day project meeting for state and federal agency representatives and other stakeholders during June 2013.

3. Held a one day project data workshop in June 2013 for PIs and associated staff; several additional PI meetings held throughout project period.

4. PIs were served in technical advisory roles for state and federal cyanobacteria harmful algal bloom management.

5. Project resulted in successful mentorship of three San Francisco State University MSc students, with PIs serving as graduate committee members. Mentorship of one U. North Carolina PhD student.

TASK NARRATIVE

Project Implementation

The field component of the project was implemented between July and October during 2011 and 2012, with participation of all project tasks and follow-up with sampling by Task 2 during the summer of 2013, in collaboration with the University of the Pacific (Table 1.1). The project team completed a total of 24 sampling days in the Delta, visiting six stations (Table 1.2, Fig 1.1) on six occasions during 2011 and 2012 and on five dates in 2013. Sampling in 2011 and 2012 was carried out aboard the RV Questuary. At each station the water column was profiled using a Seabird Electronics SBE-32 rosette fitted with a Seabird SBE-19 plus CTD and Wetlabs fluorometer, Licor 4Π PAR sensor, and a D &A OBS turbidity sensor for temperature, specific conductance (salinity), fluorescence, PAR, and transmittance.

Stations were selected to complement rather than replicate existing monitoring programs by state agencies (Table 1.1, Fig 1.1). Three pairs of stations were visited with each station pair representing two sites that are similar in terms of residence time and hydrology, have close geographic proximity, but contrast in terms of historical *Microcystis* abundance. Station pairs included:

Franks Tract (relatively low *Microcystis* and has bivalves) vs. Mildred Island (high *Microcystis* abundance and no bivalves). This station pair represents the shallow water habitat of the Central Delta, where residence time is relatively high and hydrology is more similar to a lake than compared to a river. **Rio Vista (low *Microcystis*) vs. Antioch (high *Microcystis*).** This station pair represents the large river channel habitat of the Western Delta region of the SFE. Both areas are dominated by high flows. Rio Vista represents Sacramento River water and Antioch represents San Joaquin water. **Mokolumne River (relatively low *Microcystis*) and Old River (high *Microcystis*).** This station pair represents Central Delta's small channel habitats.

During 2011 and 2012 a series of experiments were conducted following the transect work, using water collected from sites within the Delta (Table 1.1). During 2011 a total of six replicated 96-hr enclosure experiments were conducted; three experiments investigated temperature effects and three experiments addressed irradiance effects on phytoplankton nutrient drawdown and biomass increase in environments dominated by cyanobacteria, including *Microcystis* and *Aphanizomenon.* This research led to the development of an SFSU Master's thesis work (discussed further under Task 2).

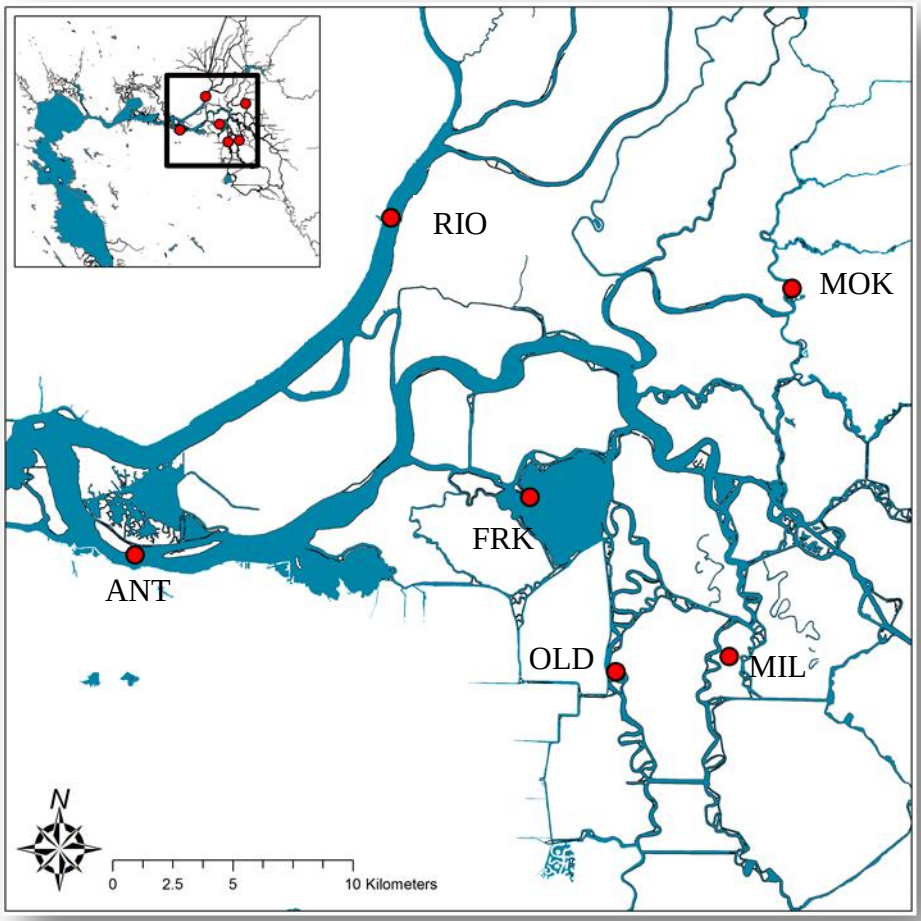
Table 1.1: Sampling dates of the RV Questuary (2011 and 2012) and the U of Pacific collaboration (2013). Nutrient bioassay and enclosure results are described in Task 2 (Nutrients and Rates) section of the report.

Year	**Sampling Date**		
2013	June 13		
	July 11		
	July 25		
	Aug 8		
	Aug 22		
		Nutrient Bioassays	**Bioassay Stations**
2012	July 23-24 (M/T)	July 25-27 (W/F)	RIO and. ANT
	Aug 6-7 (M/T)	Aug 8-10 (W/f)	MOK and. OLD
	Aug 26-27 (Su/M)	Aug 28-30 (T/Th)	FRK and. MIL
	Sept 10-11 (M/T)	Sept 12-14 (W/F)	RIO and ANT
	Sept 24-25 (M/T)	Sept 26-28 (W/F)	MOK and OLD
	Oct 8-9 (M/T)	Oct 10-12 (W/F)	FRK and MIL
		Enclosure Dates	**Enclosures Type**
2011	July18-19 (M/T)	July 20- 24 (W-Su)	RIO vs. ANT (temp)
	Aug 1-2 (M/Tu)	Aug 3-6 (W-Sa)	MOK vs. OLD (temp)
	Aug 8-9 (M/Tu)	Aug 10-13 (W-Sa)	FRK vs. MIL (temp)
	Aug 29-30 (M/Tu)	Aug 31-Sept 3 (W-Sa)	RIO vs. ANT (light)
	Sept 8-9 (Th/F)	Sept 10 – 13 (Sa-Tu)	MOK vs. OLD (light)
	Sept 19-20 (M/Tu)	Sept 21 – 24 (W – Sa)	FRK vs. MIL (light)

Table 1.2: Station names and locations for MIC 2011 and 2012 sampling aboard the RV Questuary. Stations were selected based on paired hydrographic environments and historical abundances of *Microcystis*

Hydrographic environment	***Microcystis* Site**	**Low(non)-*Microcystis* Site**
Flooded Island	Mildred Island (MIL) 37.98734N,121.52324W	Frank's Tract (FRK) 38.0438889N,121.6136111W
Large River	Antioch (ANT) 38.05190N,121.80273W	Rio Vista (RIO) 38.148N,121.688W
Small River	Old River (OLD) 37.9827778N,121.5772225W	Mokolomne River (MOK) 38.1261111N,121.4952778W

Figure 1.1 Map of locations of sampling stations occupied for the project in 2011 and 2012.



During 2012 six nutrient bioassay experiments were conducted to investigate the effect of nutrient (NO3, NH4, Urea and PO4) on phytoplankton biomass increase and cyanobacterial toxin production.

During 2013 a series of cruises led by Dr. William Stringfellow at the University of the Pacific were conducted with Task 2 of this project to investigate biomass, rates of C and N uptake and nutrient concentrations within the San Joaquin River near Stockton, a region regularly impacted by high abundance of Microcystis during the summer.

Task Integration / PI Collaboration (Table 1.3)

Prior to the start of the project the PIs met on 11 April 2011 at the Romberg Tiburon Center for discussion of sampling and experimental design of laboratory-based studies and to ensure good coordination between sampling components.

Hans Paerl and Tim Otten (Task 4) collaborated with Task 2 in both transect surveys and also laboratory-based experiments conducted in 2011 and 2012. Drs. Paerl and Otten visited the Romberg Tiburon Center on two occasions during each field season, between 8 – 13 August 2011 and 5 - 9 August 2012 to facilitate their collaboration in field work and lab-based experiments.

Project members met regularly throughout the project period to discuss project findings and status of information synthesis across tasks. A two-day project workshop was held in Tiburon between 26 to 27 June 2013. This meeting included presentations by representatives from each task as well as graduate student theses presentations.

Table 1.3 Table of dates of project PI and stakeholder meetings held as part of project task integration and synthesis.

Dates	Locations	Purpose	PI Participants
November 2013	Coastal and Estuarine Research Federation Conference, San Diego, CA	Informal discussion of project findings.	Kimmerer, Paerl, Parker
June 2013	Romberg Tiburon Center, Tiburon CA	Two day stakeholder / Data workshop	Kimmerer, Paerl, Parker, Mioni
December 2013	Romberg Tiburon Center, Tiburon CA	Informal discussion of project findings.	Paerl, Parker Paerl, Kimmerer
December 2014	Romberg Tiburon Center, Tiburon CA	Project synthesis, MS thesis committee meeting: A. Johnson	Kimmerer, Paerl, Parker, Wilkerson

TASK DELIVERABLES

Project Reporting

All semiannual project reports were completed on time to the Delta Science Program.

Synergistic Activities: Dessemination of Project Results and Coordination with the Broader Scientific Community and Stakeholders

1. During summer and fall 2011 Parker presented an overview of the project at two venues: the Biennial State of the Estuary Conference in Oakland, CA and at the summer Estuarine Ecology Team (EET) meeting held at the Romberg Tiburon Center, Tiburon, CA.

2. Parker and Mioni acted as session chairs for a special session entitled: "Understand Cyanobacterial Blooms in the San Francisco Estuary Delta" at the 7th Biennial Bay-Delta Science Conference 16-18 October 2012.

3. Parker, Paerl and Kudela presented in the Cyanotoxin Workshop hosted by the San Francisco Regional Water Quality Control Board and the San Francisco Estuary Institute 28-29 November 2012 in Oakland, CA.

4. On 26 June 2013, representatives from the Delta Stewardship Council / Science Program, Interagency Ecological Program, US Bureau of Reclamation, the CA Department of Water Resources and the San Francisco Estuary Institute attended a series of presentations, and were able to provide feedback to the research team about project relevance to management issues in the SFE Delta.

5. Parker and Wilkerson attended a one day meeting the Central Valley Water Quality Control Board, organized by Chris Foe on 9 September 2014.

6. Parker presented project findings as part of an invited presentation at a one-day seminar on lower trophic levels in the Delta hosted by UC Davis to be held on 18 February 2014.

7. Parker and Kudela served on Technical Advisory Group for The Central Valley Regional Water Quality Control Board on nutrients and cyanobacteria in the Delta during May to August 2015.

TASK 2: NUTRIENTS, PRIMARY PRODUCTION, AND NITROGEN UPTAKE

PIs Parker and Wilkerson

KEY FINDINGS

1. Phytoplankton blooms in the Delta were spatially and temporally variable; but overall sampling approach of contrasting +Microcystis and –Microcystis stations appeared to be achieved with the stations that were selected.
2. We observed Microcystis concentrations were lower overall then reported by previous studies in the Delta.
3. Nutrient concentrations were consistent across years with large variations in nitrogen and phosphorus concentrations by station; Old River was characterized by low dissolved inorganic N allowing for nutrient bioassay and N kinetic studies to be performed.
4. Primary production and assimilation numbers were higher in the Delta stations compared to other studies in the low salinity zone of Suisun Bay, likely reflecting clearer waters in the Delta.
5. Ammonium was the primary form of nitrogen used even when nitrate concentrations were as much as 20-fold higher than other forms of nitrogen.
6. Sampling in 2013 in the San Joaquin River revealed substantially elevated concentrations of chl-a, and C and N production compared to samples collected at stations in 2011 and 2012.
7. Nitrogen kinetics studies carried out by J. Lee (lee et al. 2015 Harmful Algae) found highest rates of N uptake (Vmax) for NH_4, followed by NO_3, urea and glutamic acid. Some indications of non-Michaelis-Menten kinetics observed including inhibition kinetics for glutamic acid
8. No evidence of strong NH_4 inhibition of NO_3 uptake using cultured strains of Microcystis.
9. Temperature and salinity effects on phytoplankton community composition (Johnson in prep.) suggest that temperature will select for cyanobacteria and that phytoplankton collected from the freshwater Delta could be shifted with salt intrusion; diatoms and cryptophytes are most susceptible to elevated salinities (>9 ppt) while cyanobacteria did not show a clear salinity effect at salinities up to 18ppt.
10. Nutrient bioassay experiments show elevated biomass production for Microcystis dominated communities when grown on NH_4 relative to NO_3 and urea, which produced similar amounts of biomass. Phosphorus additions alone did not result in chl-a concentrations that were different from controls. No evidence of increased microcystin production was observed in any nutrient-amended treatment.

TASK NARRATIVE

Delta Surveys 2011 to 2013

Personnel associated with Task 2 took the lead in logistical and field support for station sampling in the SF Delta as described under the task narrative for Task 1. .

General conclusions from the 2011 station sampling included an increase in large (>20-µm) chlorophyll containing cells in late summer/ early fall at 4 of the 6 stations surveyed (Fig 2.1). The large size fraction was a combination of *Microcystis* and *Aphanizomenon* colonies During the peak chl-a in 2011

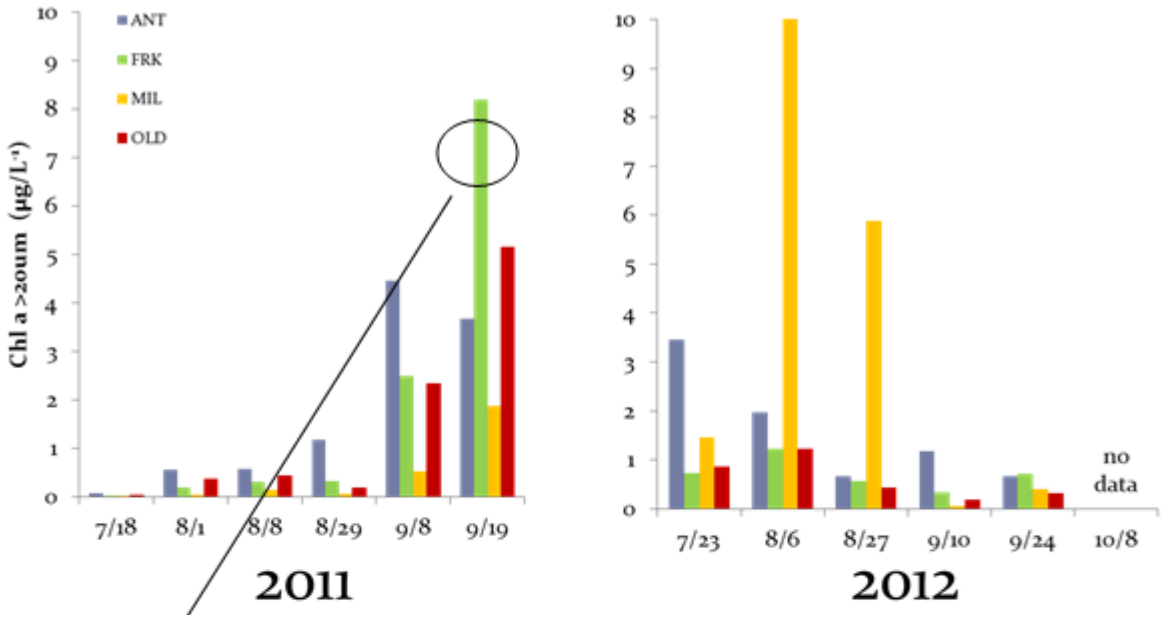


Figure 2.1: Time series of chlorophyll-a contained in cells greater than 20-µm at selected stations in 2011 and 2012. The overall trends in chl-a were increasing in 2011 and decreasing in 2012.

(circled) 95% of the phytoplankton community was cyanobacteria. During 2012 biomass was higher early in the field season and declined as the season progressed; the initial early biomass was associated with a brief period of elevated air temperatures in the Delta. The three stations hypothesized to support *Microcystis* blooms showed this trend, whereas only 1 of the 3 stations (Frank's Tract) hypothesized to NOT support *Microcystis* blooms showed an increase in the >20-µm size fraction. This suggests that our general research approach of contrasting *Microcystis* versus non-*Microcystis* stations was valid. An examination of samples collected from near surface versus water at 3m samples suggests that the total chlorophyll concentrations were generally similar between depths, however the phytoplankton community composition in near surface samples was different than composition at 3m, with cyanobacteria dominating in the surface samples (when they were present in the environment; data collected under Task 3, not shown).

From our analyses completed in the Central Delta during 2011 and 2012 our biomass estimates for *Microcystis* and other colonial cyanobacteria is lower than reported by others previously in the SFE Delta (e.g. Lehman et al. 2005; 2008). This suggests that the relative importance of cyanobacteria highly spatially and temporally variable (Fig. 2.2).

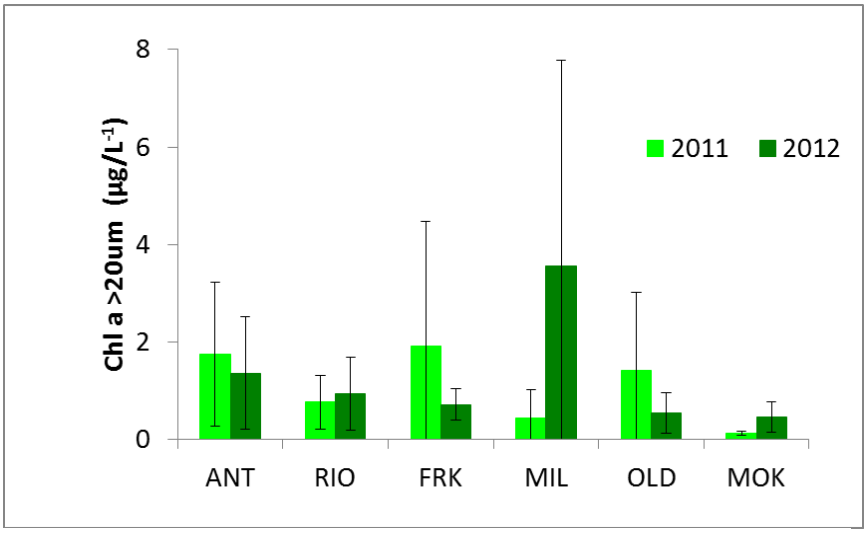
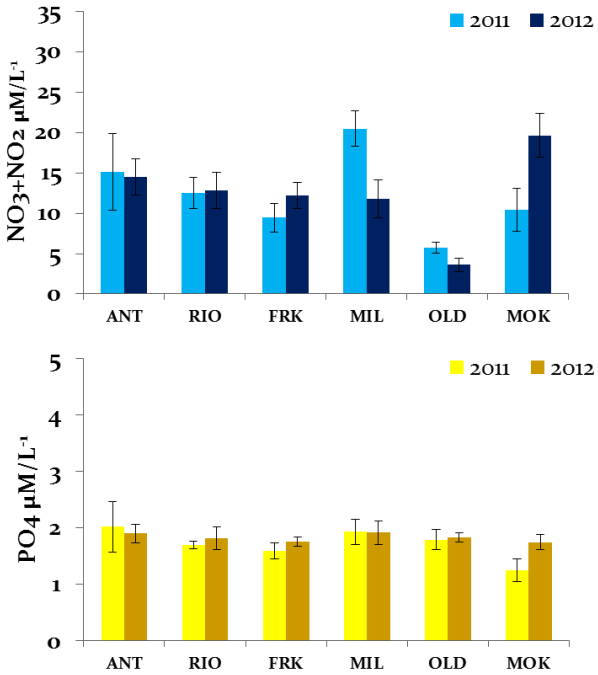


Figure 2.2: Mean chlorophyll-*a* at sampling stations for 2011 and 2012.

In general, nutrient concentrations were consistent between years but varied across stations (Fig 2.3). For example, Rio Vista and Mokolumne River had the highest NH_4 concentrations (reflecting the contribution of Sacramento River water to these sites) whereas, at all other sites, NH_4 concentrations were low relative to many other locations in the SFE (Wilkerson et al 2006). PO_4 concentrations were relatively uniform across stations at ~2µM. NO_3 concentrations were more variable (data not shown) but were relatively low (<5µM) at Old River. These conditions suggest the possibility that N limitation may occur under some conditions providing justification for the nutrient bioassay experiments (see section below) completed during 2012. The low NH_4 and urea concentrations also allowed for nitrogen kinetics experiments from field-collected samples that were carried out by Lee (see section below).



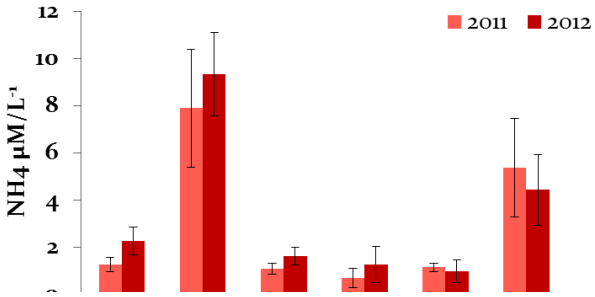


Figure 2.3 Spatial variability in NO_3+NO_2, NH_4, and PO_4 concentrations during surveys conducted in the Delta during 2011 and 2012.

A comparison of depth-integrated primary production at the Delta sites surveyed in this project to those published for the low salintiy zone (Suisun Bay) by Kimmerer et al.(2012) and Parker et al. (2012) indicate that the Delta is generally more productive and supports higher phytoplankton biomass compared to the LSZ (Table 2.1). This may in part be due to deeper photic zone depths.

	LSZ ('06/'07)	**2011 All stations**	**2012 All stations**	**2011 cyano stations***
Chlorophyll-*a* (µg L^{-1})	2.1 – 2.3	3.7	4.8	9.4
Zp (m)	1.6 – 1.8	4.4	4.6	5.3
PP (mg C m^{-2} d^{-1})	107 – 171	604	536	660
P^B_M (mg C (mg chl-*a*)$^{-1}$ d^{-1}	62.8 – 68.1	91.3	56.2	29.7

Table 2.1: Comparison of phytoplankton parameters at the Delta stations sampled in this project during 2011 -2012 with parameters for the low salinity zone collected during 2006 and 2007 by Kimmerer et al. 2012 and Parker et al. 2012. Cyano stations in 2011 were Antioch, Franks Tract and Old River on 19 September.

During the field season in 2011 as the phytoplankton bloom developed with an increased contribution by cyanobacteria (i.e., increasing contribution of chl-a in cells >75-µm diameter), the assimilation number (P^B_M, primary production normalized to chl-a) decreased, suggesting lower efficiency by the autotrophic community. An example of this is seen for Antioch during 2011 (Figure 2.4). This situation was observed at all of the Delta sites in the survey, including those where cyanobacteria abundance was low (e.g., Rio Vista) making the link between cyanobacteria and low autotroph efficiency difficult.

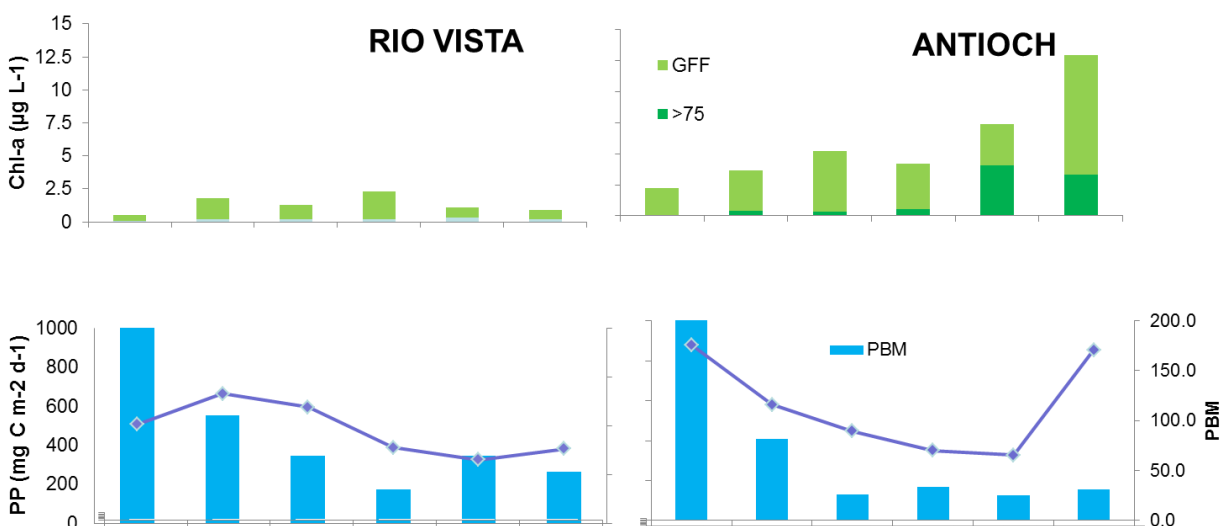


Figure 2.4: Chlorophyll-*a* (>0.7-µm and >75-µm), depth-integrated primary production (PP) and assimilation number (P^B_M) at a lower abundance cyanobacteria station (Rio Vista) versus a higher abundance cyanobacteria station (Antioch).

Nitrogen uptake rates were consistent in magnitude to the carbon uptake (ie balanced growth by the autotrophic community) suggesting that NH_4 and NO_3 uptake (which werer measured using stable isotope tracers) represented the bulk of the N used by the community. Generally, NH_4 was the inorganic N substrate used by the autrotophic community, even when NO_3 concentrations were substantially (several fold) higher than NH_4. This most likely reflects the well-established phenomenon of NH_4 inhibition of NO_3 uptake (Dugdale et al. 2007, but see next section on N kinetics).

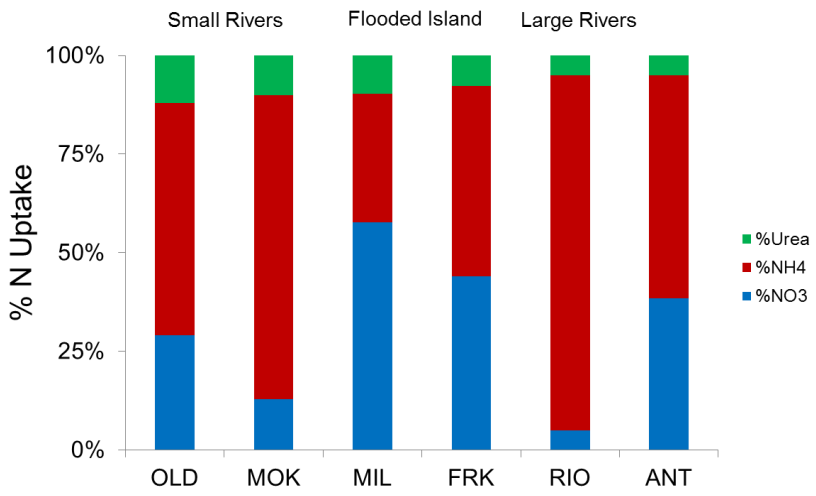


Figure 2.5: Percentage of nitrogen uptake attributed to NH_4, NO_3, and urea-N at each of the stations sampled averaged over the two field season.

During 2012 estuarine CO_2 concentrations were measured on four of the six surveys. For two surveys where direct measurements were not available, CO_2 concentrations were calculated from the pH and dissolved inorganic carbon data. The excess or deficit of estuarine CO_2 relative to the ambient atmospheric concentration provides a qualitative assessment of net respiration or production. By calculating the flux to/from the atmosphere we can obtain a quantitative assessment of the total respiration or production during the surveys at each station. Our results were consistent with the earlier studies showing highly variable CO_2 concentrations generally exceeding atmospheric levels (Table 2.2). Our results provide greater detail than the previous studies and reveal significant geographic variability. For example our measurements for the Mokelumne River (1670-2100 µatm partial pressure) were well above ambient atmospheric (≈400 µatm) while the waters of the Old River were significantly and consistently well below atmospheric (140-220 µatm) (Table 2.2).

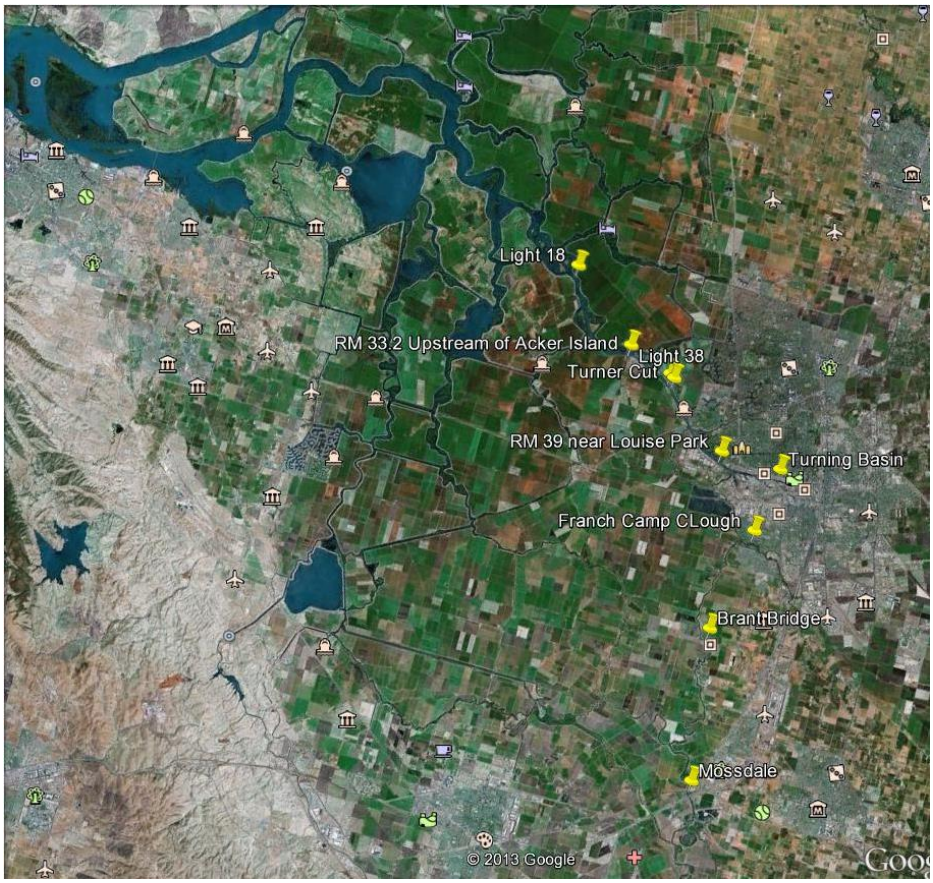
	ANT	RIO	MOK	OLD	FRK	MIL
MIC12_1	750	1448	2019	136	714	1120
MIC12_2	800	1480	1669	157	626	963
MIC12_3	693	1332	2016	166	729	972
MIC12_4	568	1940	2102	216	704	1068
MIC12_5	914	1399	1438	298	1075	969
MIC12_6	847	915	1678	730	553	1029

Table 2.2: pCO_2 measurements (µatm) measured during regular surveys in 2012 (see Table 1.1 for dates of each survey). Ambient atmospheric pCO_2 is approximately 400µatm.

The surveys conducted during 2013 were carried out in the San Joaquin River and provide a contrast in *Microcystis* abundance (i.e, substantially higher) relative to the locations surveyed in 2011 and 2012. A total of 51 stations were occupied.

Five additional field surveys in the San Joaquin River were completed in collaboration with the University of the Pacific (Dr. William Stringfellow) during summer 2013 (Table 1.1). These cruises were at minimal cost to the project (graduate student time). The objective of these surveys was to capture environmental conditions and associated *Microcystis* physiological rates during conditions of high cyanobacterial abundance. Cruises were completed between June and August 2013. Each survey occupied between 8 and 11 stations (Fig. 2.6). Inorganic nutrients, dissolved inorganic carbon and chlorophyll-a concentrations, and ^{13}C/ ^{15}N uptake rates were analyzed.

Figure 2.6: Map of sampling stations occupied between June and August 2013 as part of *Microcystis* sampling in collaboration with the University of the Pacific.



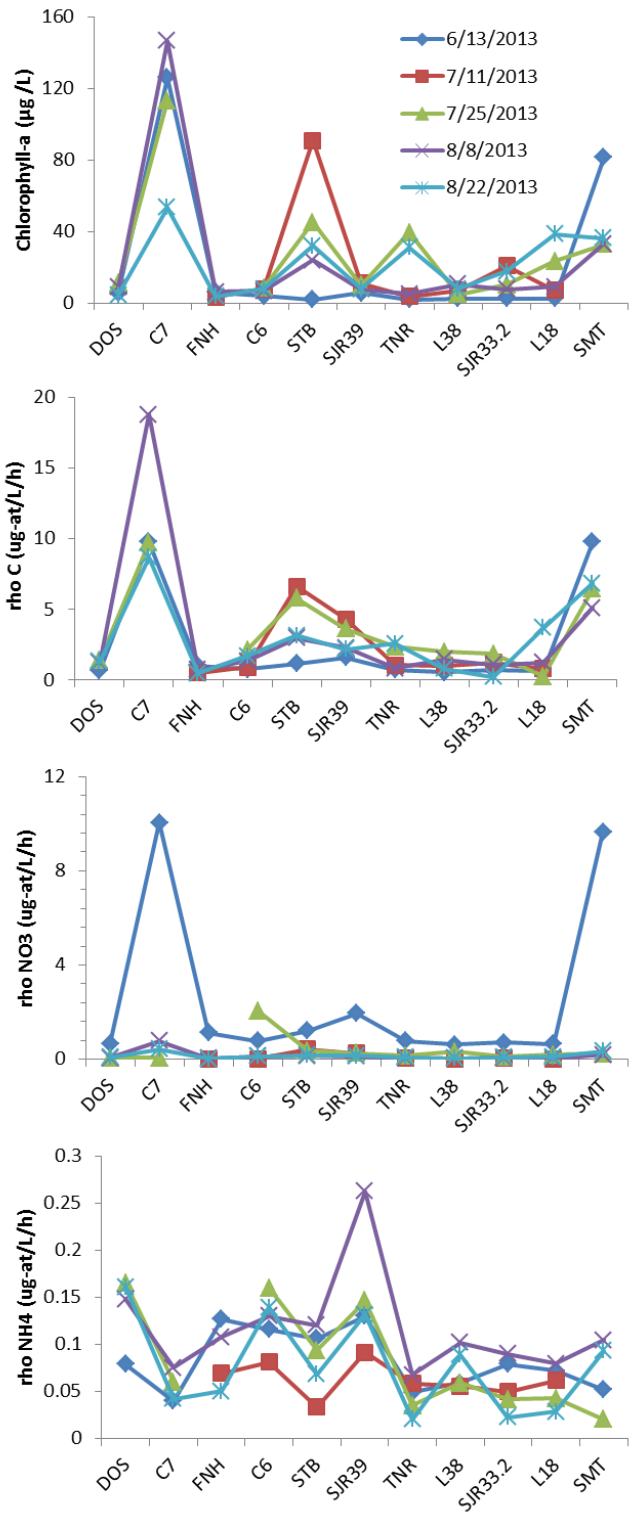


Figure 2.7: Chlorophyll-a, primary production (rho C), NO_3 uptake and NH_4 uptake (rho) at stations in the San Joaquin River sampled in 2013.

Peaks in chlorophyll-a were observed centered at C7, the Stockton Turning Basin (STB), and at Smith Canal at Yosemite Lake (Figure 2.7). These occurred on 13 June, 25 July and 8 August. These peaks were at least an order of magnitude larger than observed at our stations in 2011 and 2012 and are consistent with observations made by Kress et al. 2012. Similarly, peaks in carbon uptake were centered at the same locations and the same dates. Nitrate uptake was observed to be associated with the peaks in chl-a and carbon uptake, consistent with the findings of Kress et al. (2012) . The apparent reason for the utilization of NO_3 is that NO_3 concentrations were high at these stations (>100 μM) and NH_4 relatively low (often ≤5 μM). These data will be combined with results for toxin concentrations and microscopy conducted at the University of the Pacific (W. Stringfellow) for further synthesis and publications.

Nitrogen Kinetics of Natural Populations of CyanoHABs and Microcytis Cultures.

San Francisco State University masters student, Jamie Lee completed her theses entitled: "Nitrogen uptake kinetics of *Microcystis aeruginosa* in the San Francisco Delta" in December 2014. Her thesis, which explores the nitrogen kinetics of natural phytoplankton populations dominated by *Microcystis spp.* as well as *Microcystis* in culture, was published in the Journal Harmful Algae in June 2015.

The paper provides insight into how different chemical forms of organic and inorganic nitrogen may promote *Microcystis* growth, across the nutrient-limiting to nutrient-replete continuum in the Delta. *Microcystis* collected from sites in the Delta showed the highest uptake potential for NH_4, followed by NO_3^-, urea, and an amino acid, glutamic acid (e.g., Fig. 2.8). Based upon the analysis of half-saturation constants and ambient N concentrations obtained from Delta sites during the two years of survey work, it appears that ambient N concentrations at some of the field sites may be below saturating concentrations for N uptake (Table 2.3). This conclusion challenges long-held hypotheses that the SFE is nutrient-replete at all locations (e.g. Cloern, 1991; Jassby et al., 2002) and raises the possibility that continued N loading has the potential to support more *Microcystis* growth. At sub-saturating N concentrations, the specific affinity (α) was highest for NH_4 and the lowest for NO_3 , indicating that increases in NH_4 could impact *Microcystis* blooms the most of all the N forms tested.

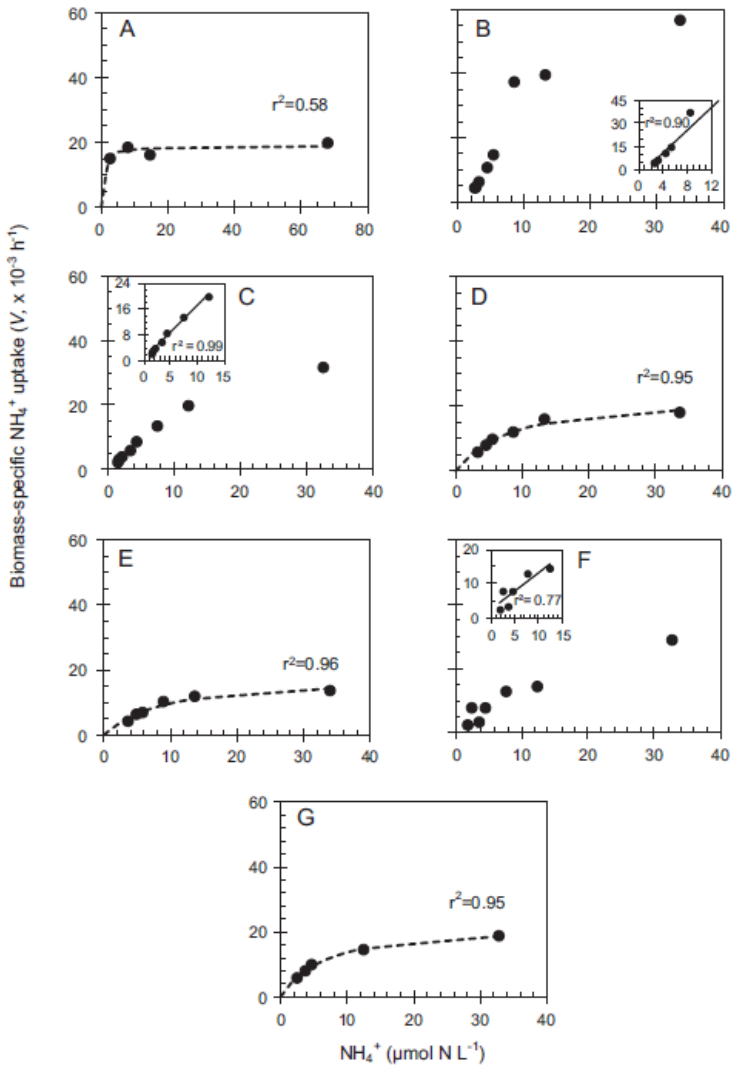


Figure 2.8: Example of N kinetics for NH_4 using natural phytoplankton populations dominated by *Microcystis.* From Lee et al. 2015

Table:2.3 Summary of kinetics parameter, specific affinity, obtained for N substrates during this study and compared to literature values. From Lee et al. 2015.

Algal species/community	α ($\times 10^{-3}$ h^{-1}) (μmol N L^{-1})$^{-1}$				Source
	NH_4^+	NO_3^-	Urea	Glutamic acid	
Cyanobacteria					
Microcystis, SF Delta, CA	*1.1–26.4*[a]	*0.2–4.2*[a]	*0.8–2.8*[a]	*0.8–6.9*[b]	*This study*
M. aeruginosa cultures	*443–2542*[a]	*1.7–2.2*[a]	*0.6*[a]	*9.9–24.7*	*This study*
Microcystis, Lake Kasumigaura, Japan	2.0–30.7[a]	5.3–280	52.6		Takamura et al. (1987)
M. aeruginosa, prairie lake, Manitoba, Canada	0.005–0.012[a]				Murphy and Brownlee (1981)
Anabaena flos-aquae, Smith Lake, Alaska	229.7	125	32.2		Gu and Alexander (1993)
Aphanizomenon flos-aquae, prairie lake, Manitoba, Canada	0.005–0.028[a]				Murphy and Brownlee (1981)
Diatoms					
Chaetoceros, Thau Lagoon, S. France		151.5			Collos et al. (1997)
Pseudo-nitzschia australis culture	13.2	37.3	2.8[a]	11.1[d]	Cochlan et al. (2008)
Pseudo-nitzschia spp., South Africa	13.4	12.4			Seeyave et al. (2009)
P. fryxelliana culture	8.7–20.6	16.4–17.6	32.5		Auro and Cochlan (2013)
Skeletonema costatum, E. China Sea	227.0	0.7	4.1	3.1[c]	Li et al. (2010)
Dinoflagellates					
Alexandrium catenella, Thau Lagoon, S. France	7.6	5.2	1.4		Collos et al. (2004)
A. catenella, South Africa	5.9		5.4[e]		Seeyave et al. (2009)
Ceratium, prairie lake, Manitoba, Canada	0.04				Murphy and Brownlee (1981)
Lingulodinium polydedrum, Southern California	13.7	8.2	10.7		Kudela and Cochlan (2000)
Prorocentrum minimum, Chesapeake Bay	16.4–67.0	2.0–5.5	1.4–7.7		Fan et al. (2003)[f]
P. donghaiense, E. China Sea	179.5		3.2	14.2	Li et al. (2010)
P. donghaiense culture	10.5		332.9	2.5[c]	Hu et al. (2014)
Karenia mikimotoi, E. China Sea	102.5	0.4	14.8	6.5	Li et al. (2010)
K. brevis, West Florida Shelf and in culture	53.4–134.9	40.8–44.1	15.3–42.3	0.13–0.34[c]	Killberg-Thoreson et al. (2014)
Other					
Heterosigma akashiwo culture	12.2–26.1	10.2–13.4	6.9		Herndon and Cochlan (2007)
California current and coastal Alaska (eutrophic)	27.9	3.9–36.8			MacIsaac and Dugdale (1969)
California current and coastal Alaska (oligotrophic)	10.4–58.0	65–190			MacIsaac and Dugdale (1969)
Washington (USA) coast	9.6	116	5.9		Dortch and Postel (1989)

[a] Includes α estimated from lower portion of uptake curves.
[b] Calculated only up to 6.25 μmol N L^{-1}.
[c] Amino acid mixture used.
[d] Glutamine used.
[e] V_{max} estimated from the mean uptake at four concentrations considered to be saturated.
[f] As cited in Kudela et al. (2010).

Another notable finding from the study was a lack of strong ammonium inhibition of nitrate uptake for *Microcystis* in culture (Fig. 2.9). The potential for interaction between various forms of nitrogen and N uptake appeared to vary by strain type (toxic versus nontoxic strains) and we reported < 50% inhibition of N uptake in various combinations (i.e. NH_4 inhibition of NO_3 or urea uptake, NO_3 inhibition of NH_4 uptake). Lower inhibition potential may offer a competitive advantage for *Microcystis* as it would have access to multiple pools of N simultaneously, while species that experience more profound NH_4 inhibition of NO_3 uptake, as has been reported for the bulk phytoplankton community in the SFE (e.g. Dugdale et al. 2007) would not be able to utilize NO_3 until most of the NH_4 was exhausted

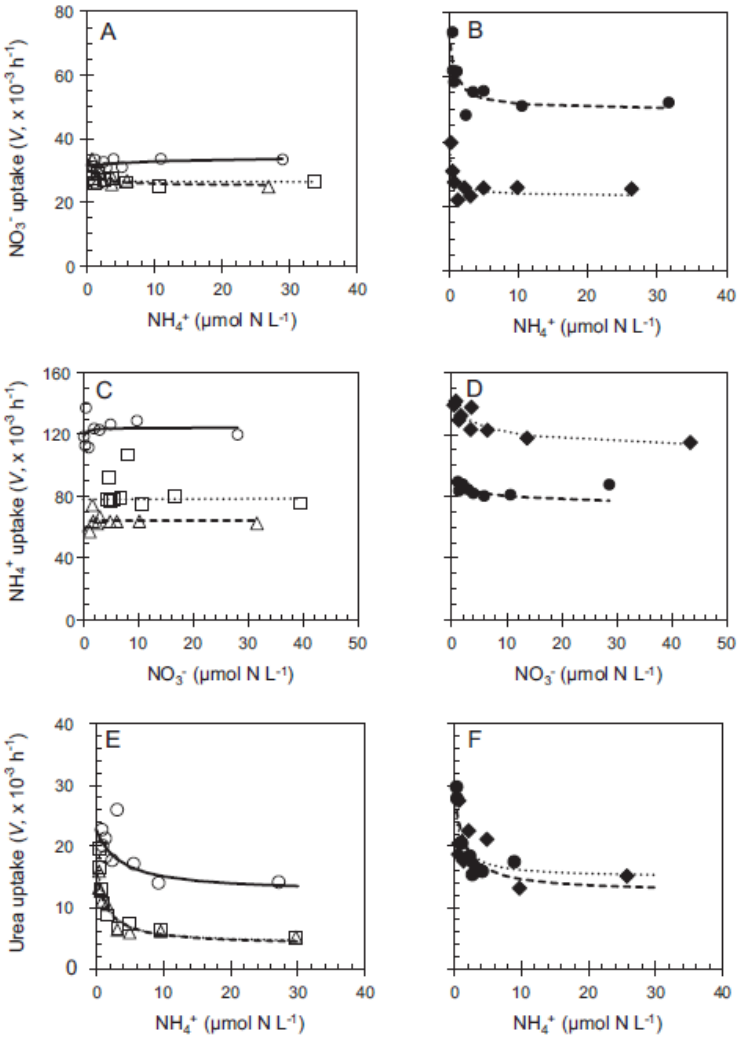


Figure 2.9 : Results of inhibition kinetics testing NH_4 inhibition of NO_3 uptake (A,B), NO3 inhibition of NH_4 uptake (C,D) and NH_4 inhibition of urea uptake (E,F). From Lee et al. 2015.

Other notable findings from the study included an apparent inhibition of N uptake with increasing concentrations of the amino acid, glutamic acid (Fig. 2.10). This is consistent with findings for some other HAB species grown on glutamic acid, (Hu et al., 2014; Bronk et al., 2004) and other amino acids (Mulholland et al., 2002).

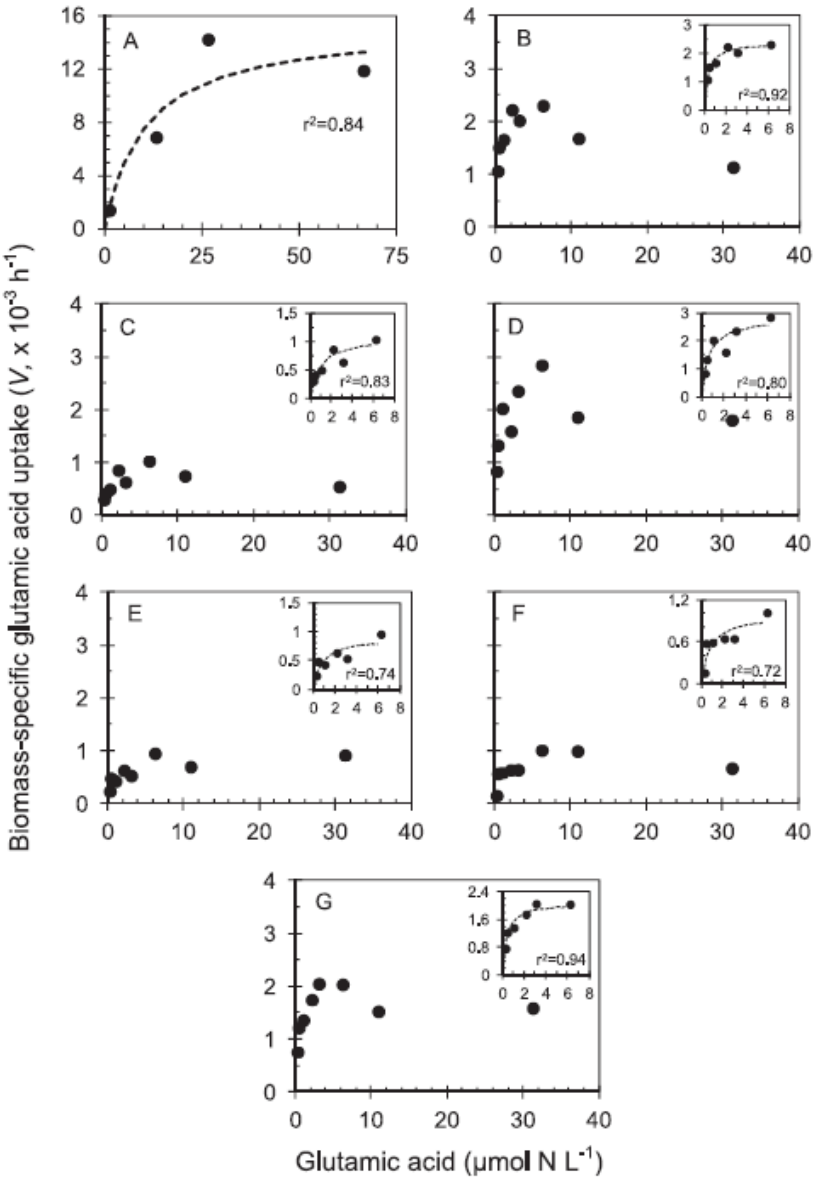


Figure 2.10: Example of N kinetics for glutamic acid using natural phytoplankton populations dominated by *Microcystis.* From Lee et al. 2015

Effect of Temperature and Salinity on phytoplankton Community Composition

Allison Johnson is completing analysis of phytoplankton community compositional shifts, including shifts to cyanobacteria, in the Delta as a result of temperature and salinity changes. Her thesis topic grew out of initial experiments conducted in 2011 in which temperature manipulations revealed strong temperature regulation of phytoplankton growth in the Delta. Ms. Johnson has synthesized her data from the 2012 and 2013 *Microcystis* field seasons and defended her thesis in October 2015. Her thesis will also be submitted as a manuscript, likely to the journal Estuaries and Coasts during the first half of 2016. Ms. Johnson was awarded First Place in the Student Presentation Award competition at Bay Delta Science Conference in 2014 and presented her research at two conferences hosted by the Association for Scientists in Limnology and Oceanography (ASLO) in New Orleans, LA in 2013 and in Granada, Spain in 2015.

The goal of Ms. Johnson's study was to investigate the influence of salinity and temperature on the relative abundance of major algal taxa in the Delta by addressing the questions: 1. How will increased Delta water temperature that are predicted to result from climate change affect phytoplankton community composition?, 2. How will increased salinity due to salt intrusion associated with climate change affect phytoplankton community composition?, 3. What synergistic effects on phytoplankton may result from both water temperature and salinity shifts under future Delta scenarios?

To answer these questions 20L surface water and phytoplankton samples for temperature and salinity manipulation experiments were collected between August and October in 2012 and 2013 at Antioch and Mildred Island. In some experiments a surface net tow was used to concentrate surface "scum", made of primarily *Microcystis* colonies. Three temperature treatments were established based upon the average summer water temperature regime of the Delta: 1) "low" temperature (16± 2.00°C), 2) "ambient" temperature (~23 ±1.86C), 3) "high" temperature (~28±1.75°C). Water temperatures were monitored every 15 minutes over the duration of the incubation (HOBO Water Temp Pro v2) and were maintained to within ±2°C for the duration of the experiment. Phytoplankton were exposed to a series of increasing salinity treatments from 6 to 18ppt using additions of sodium chloride brine. Salinity treatments were exposed to each of the three experimental temperatures treatments with phytoplankton growth determined by the accumulation of chlorophyll-a and accessory pigments after 72 hr. Incubations were conducted outdoors under ambient irradiance attenuated to 50% of incoming solar radiation with neutral density screening. All incubation water collected was amended with NH_4Cl, NO_3 (KNO_3) and PO_4 (KH_2PO_4) to avoid nutrient limitation during the incubation period (Parker et al. 2012).

The accumulation of chlorophyll-a and accessory pigments varied in reproducible patterns across temperature and salinity treatments. In control treatments chlorophyll-*a* (chl-*a*) increased between two and 18-fold over 72 hr, from an initial concentration of 3 to 17 µg L^{-1} (Fig. 2.11). Consistent across all experiments, more chl-*a* accumulated in the high temperature treatment compared to the ambient or low temperature treatments. . In all temperature treatments a net gain of chl-*a* was observed at salinities less than 8.5ppt and in high temperature treatments, exposure to low salinity levels (control to 7.4ppt) resulted in the most chl-*a* accumulation. In contrast, in a majority of experiments (a,b,d) the low temperature treatments with salinities between 10 and 14ppt, resulted in a net loss of chlorophyll-a (16-75%)

The accessory pigment zeaxanthin, indicative of cyanobacteria consistently increased with increasing temperature in all four experiments (Fig. 2.12). Fucoxanthin, indicative of diatoms, also showed a trend

of increasing pigment with increasing temperature. Unlike zeaxanthin, a consistent pattern of declining fucoxanthin concentrations were observed at salinities greater than 9ppt. Net gain of fucoxanthin was found at all salinities in experiment "a" and "b". In experiment "c" all salinities lower than 15.2ppt showed net gain but at salinity treatment 15.2ppt at 18°C net loss of pigment of 0.18μgL^{-1} was seen. At salinities above 9.3ppt all temperature treatments in experiment "d" showed net loss of pigment <0.2μgL^{-1}. Increase in alloxanthin, indicative of crypotophytes, was seen between low and ambient temperature treatments with increases of 18 to 90% (Fig. 2.13). The response of allozanthin concentrations to increasing salinity varied somewhat between temperature treatments with maximum concentrations observed in the low temperature treatment at salinities below 5ppt, whereas in the ambient temperature treatment salinities between 5.7 to 6.4ppt supported the greatest increase in alloxanthin while in high temperature treatments the greatest accumulation of alloxanthin was observed between 3 and 7.5 ppt. In all experiments net loss of alloxanthin was observed at salinities above 8ppt regardless of temperature (Figure 5). Chlorophyll-*b*, indicative of chlorophyte biomass, showed no consistent pattern across experiments. Chl-*b* concentrations generally increased in the high temperature treatment and at salinity treatments equal to and below 12ppt.

Warming will lead to longer periods of elevated water temperatures worldwide. This trend favors expanding phytoplankton bloom periods, particularly dominance by CHABs (Paerl and Huisman 2007,2009) A recent example is the proliferating toxic *Microcystis* spp. blooms that caused a drinking water supply crisis at Lake Taihu, China (Qin et al. 2010). Here, blooms have increased in magnitude due to increased N and P loading, and duration most likely due to a multi-decadal warming trend. Summer droughts appear to be increasing in intensity and duration, possibly another symptom of global warming (IPCC 2007). This, combined with increased use of freshwater for irrigation, has led to rising salinities around the world.

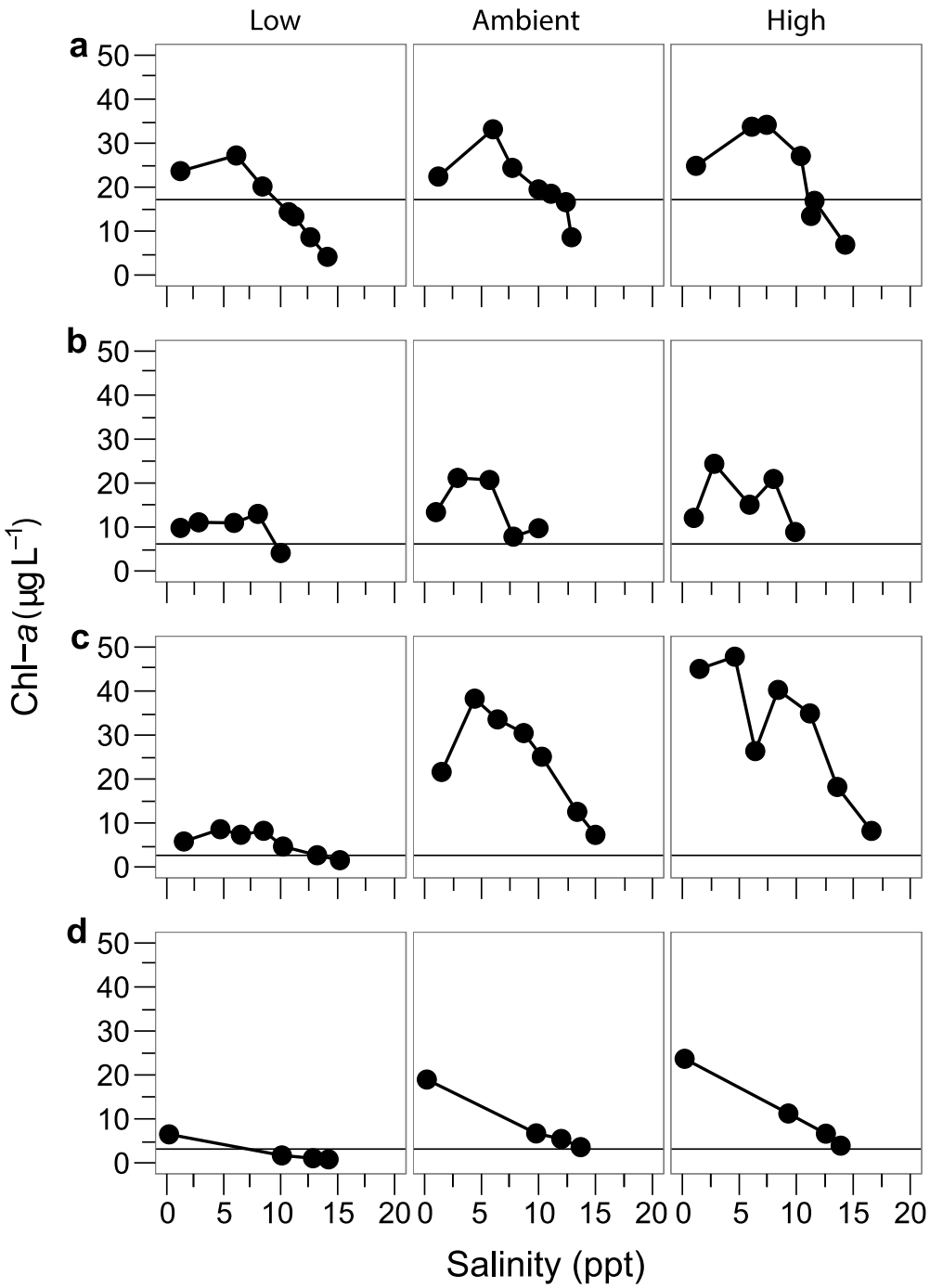


Figure 2.11. Chlorophyll-a versus salinity in three experimental temperatures after 72-hr incubation period. Phytoplankton for the experiments were collected in the Delta.

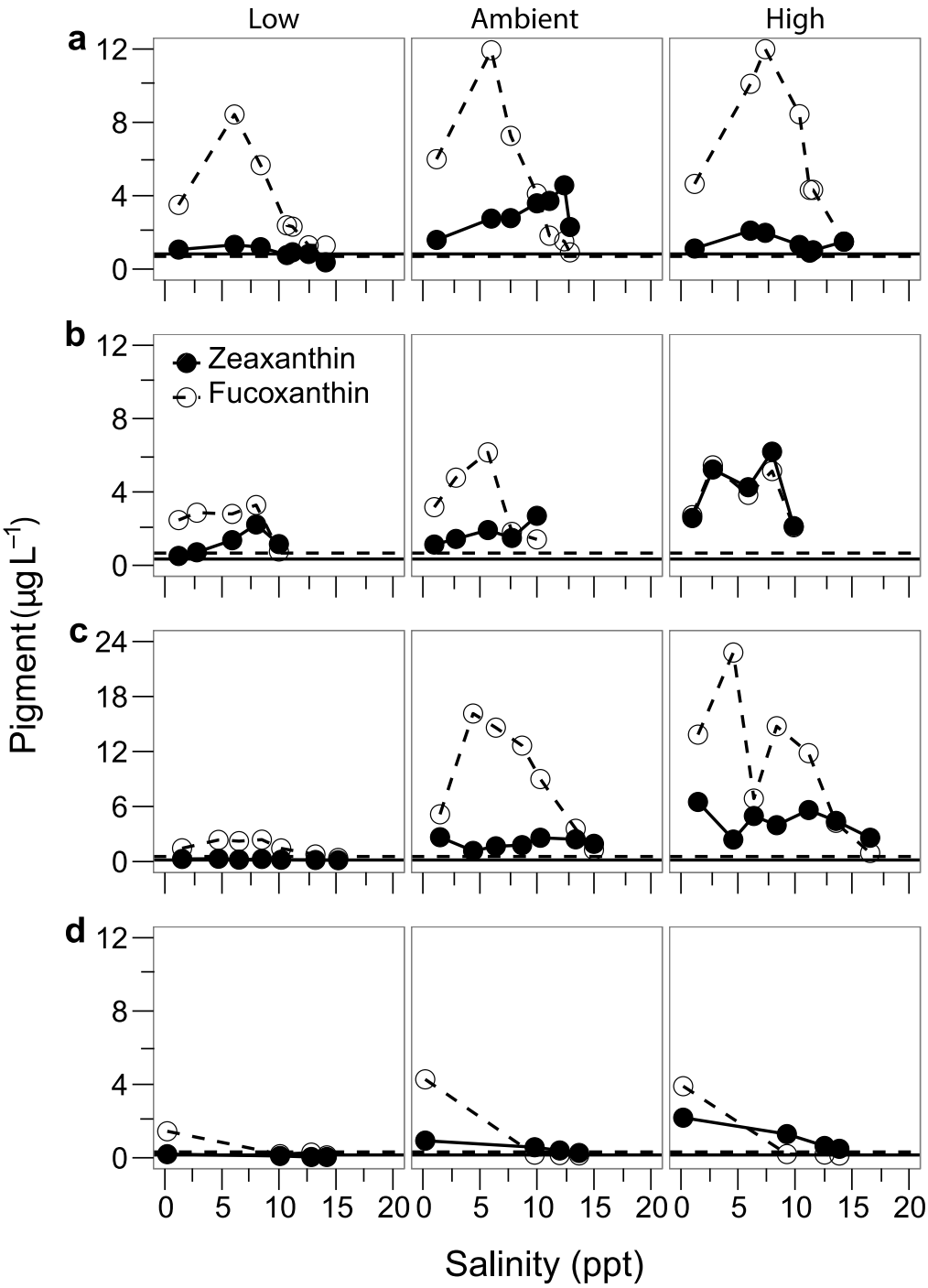


Figure 2.12. Fucoxanthin (diatoms) and Zeaxanthin (cyanobacteria)versus salinity in three experimental temperatures after 72-hr incubation period. Phytoplankton for the experiments were collected in the Delta.

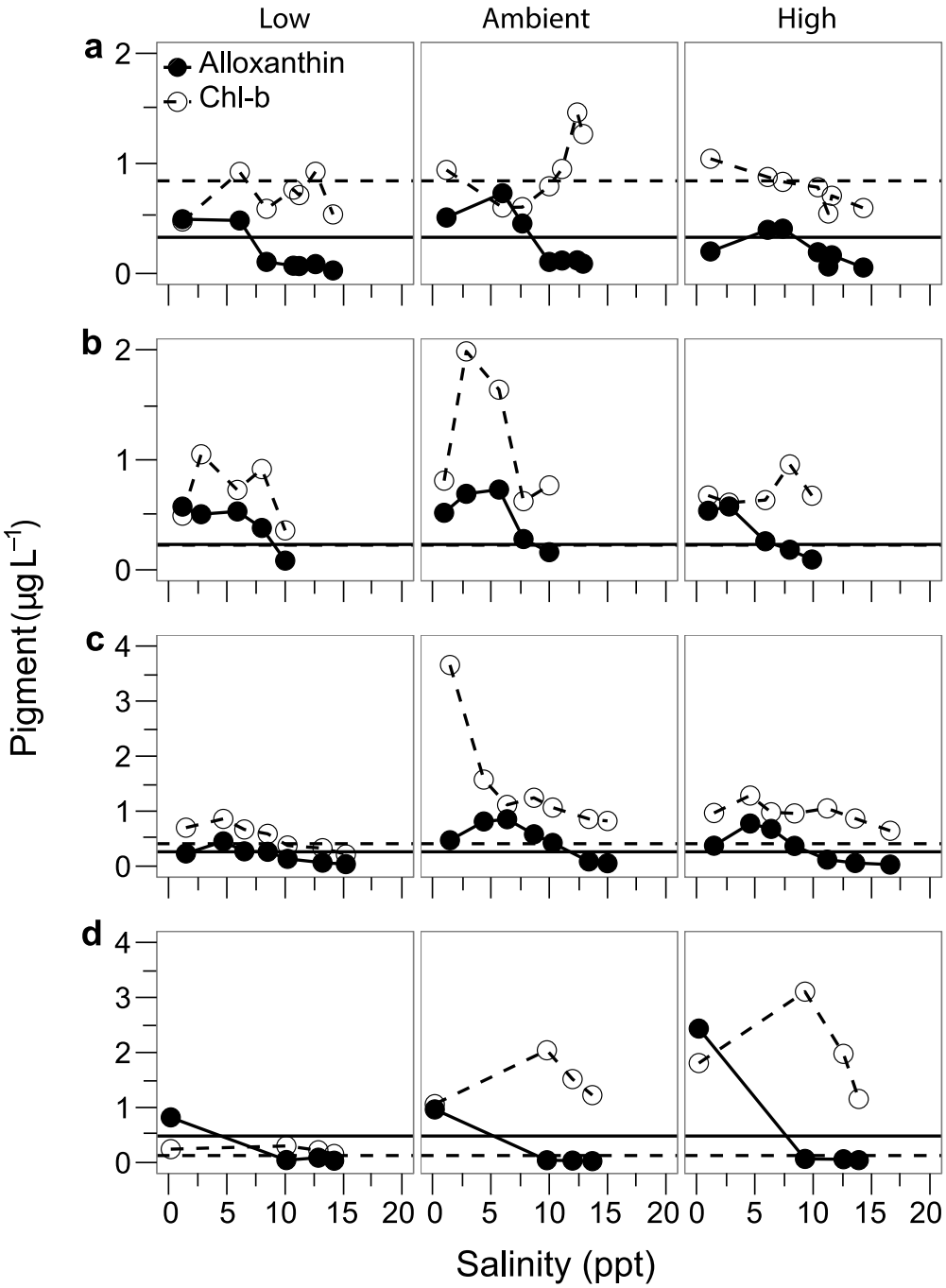


Figure 2.13. Alloxanthin (cryptophytes) and chlorophyll-b (chlorophytes) versus salinity in three experimental temperatures after 72-hr incubation period. Phytoplankton for the experiments were collected in the Delta.

Nutrient Bioassays 2012

A series of nutrient addition bioassays were conducted using water collected from Old River during 2012. The station location was selected because it has been associated with cyanobacterial (i.e. *Microcystis aeruginosa*) blooms for the past decade and based upon the survey work, nitrogen concentrations were low (≤5 µm N). The objective of these experiments was to determine the potential magnitude of autotrophic response (e.g. chlorophyll-a production) to nitrogen and phosphorus amendments after a 48-hr incubation period. We especially wanted to test the hypothesis that N and not P additions would result in biomass increases, and that the form of N (i.e. ammonium, nitrate, urea) added would result in differences in the biomass response.

Water for nutrient addition bioassays was collected from the surface using an acid-washed clean bucket and dispensed into 20-L carboys. A 53-µm mesh plankton net tow was deployed horizontally at the surface for five minutes to concentrate the microbial community (i.e. *Microcystis* colonies). The concentrated surface tow was stored in a separate carboy and was returned to the Romberg Tiburon Center for experiments.

Treatment	DIN	P	N:P
	µM	µM	Mol:Mol
Control	2	1.5	1.3
$+NO_3$	30	1.5	20.0
$+NH_4$	30	1.5	20.0
+UREA	30	1.5	20.0
$+PO_4$	2	5	0.4
$+NO_3/PO_4$	30	10	3.0
$+NH_4/PO_4$	30	10	3.0
$+UREA/PO_4$	30	10	3.0

Table 2.4: Nutrient amendments made for bioassay experiments conducted during 2012 field season.

to nutrient additions, inorganic nitrogen and phosphorus concentrations were measured in carboy samples in order to determine nutrient additions needed to achieve desired initial nutrient concentrations and N : P ratios. Water in the carboy was continually homogenized by magnetic stirrer. Water was amended with concentrated surface tow; the level of enrichment varied across experiments, in some cases representing a several fold increase in ambient chlorophyll-a concentrations. After cyanobacterial concentrate was added to the carboy the water was dispensed into forty eight 600ml HDPE bottles. Triplicate bottles were then amended with experimental additions of the inorganic nutrients, N and P (Table 2.4). After nutrients were added, initial samples for inorganic nutrients, chlorophyll-a, HPLC, toxins, qPCR and taxonomy were collected. Bottles were then placed under window screening to attenuate surface PAR to either 50% (high light) or 10% (low light) and incubated in water maintained at Delta water temperature . After 48-hr incubations were terminated with samples collected for determination of inorganic nutrients, chlorophyll-a, HPLC, taxonomy, qPCR, toxins, and flow cytometry.

In several cases the resulting increases in chlorophyll-a in high light treatments were lower than the chlorophyll-a gains that were observed under low light (Fig. 2.14) however results from flow cytometry consistently showed a greater accumulation of fluorescent particles in high light treatments (Figure 2.14). This suggests that the lower chl-a found in high light treatments were due to cellular adjustment in chl-a quota rather than reduced biomass accumulation at high light. Nitrogen additions consistently yielded greater chlorophyll-a concentrations compared to the control (no nutrient amendment) treatment, while phosphorus additions alone were not different than the control. Dual amendments with both N and P yielded chlorophyll-a accumulations that were similar to N additions alone. This suggests that N and not P have the potential to limit the growth of phytoplankton at Old River. Varying the form of N

supplied appeared to have a significant impact on biomass accumulation with NH_4 additions yielding more chlorophyll-a compared to NO_3 and urea, which were often similar to one another (Fig. 2.15).

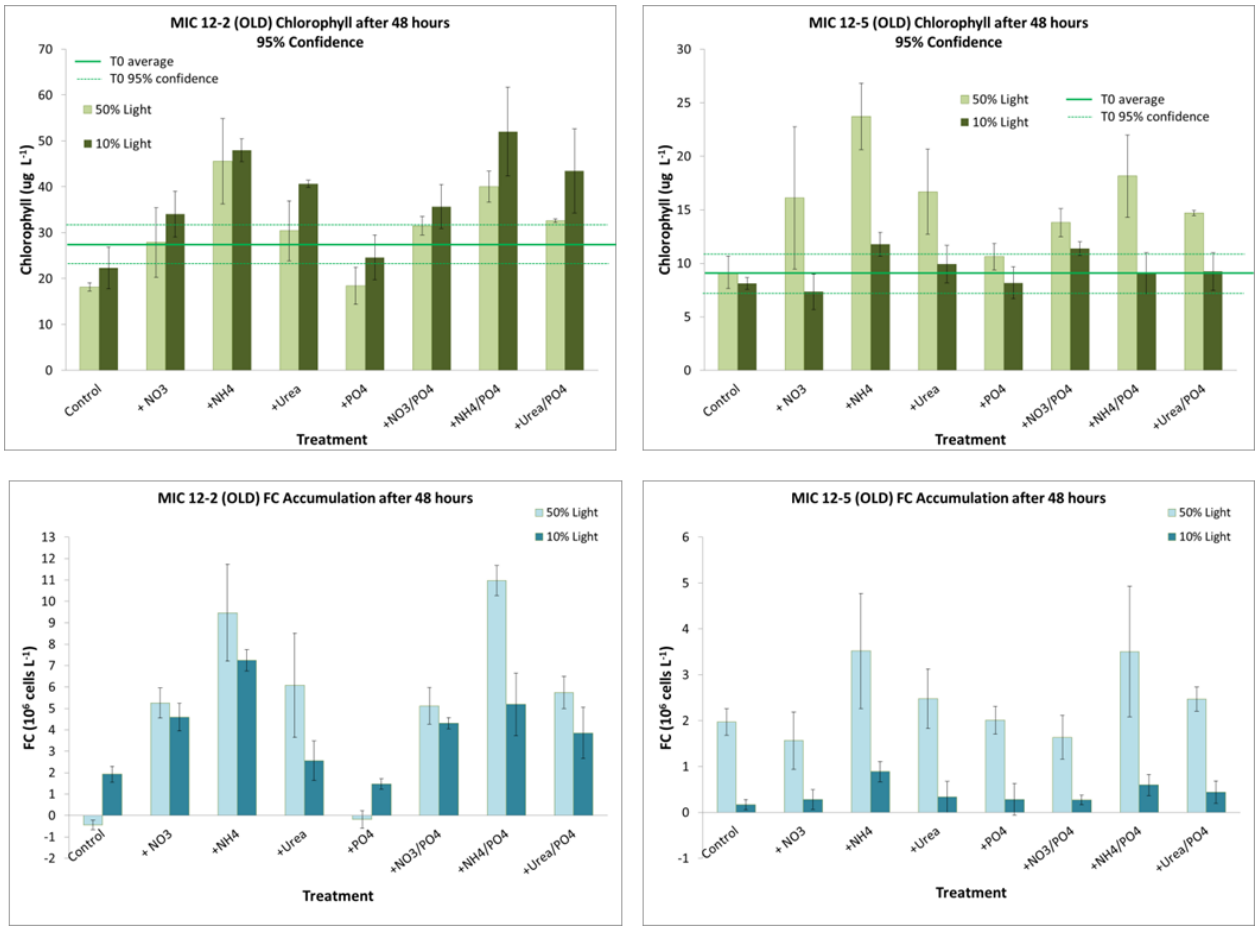


Figure 2.14. Two representative results of 48-hr bioassay experiments showing (top panel) response of chlorophyll-a versus nitrogen and phosphorus addition and (bottom panel) cell count from flow cytometry. Samples were collected at Old River during 2012.

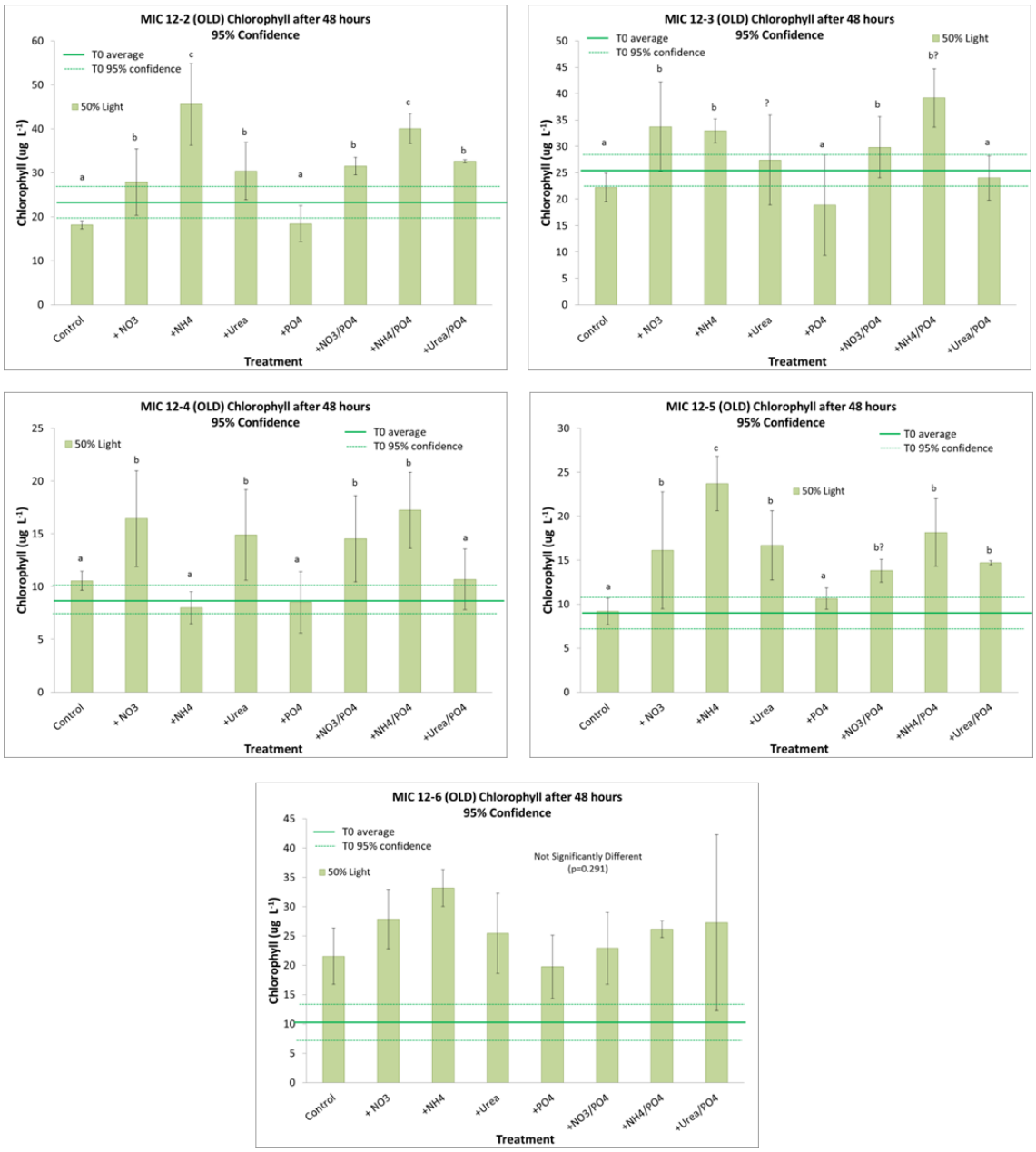


Figure 2.15. Results of five 48-hr bioassay experiments showing response of chlorophyll-a to nitrogen and phosphorus addition. Samples were collected at Old River during 2012.

Nutrient drawdown was calculated as the difference between initial and final N and P concentrations and show patterns that were consistent with the increases observed in chlorophyll-a (i.e. where chl-a increases were greatest, N and P drawdown were greatest, Fig. 2.16). These results further suggest that the increases in chlorophyll-a and fluorescent particle abundance were due to increased growth of phytoplankton. The patterns provide further evidence of differential growth on different forms of N and a lack of response by phytoplankton to P additions.

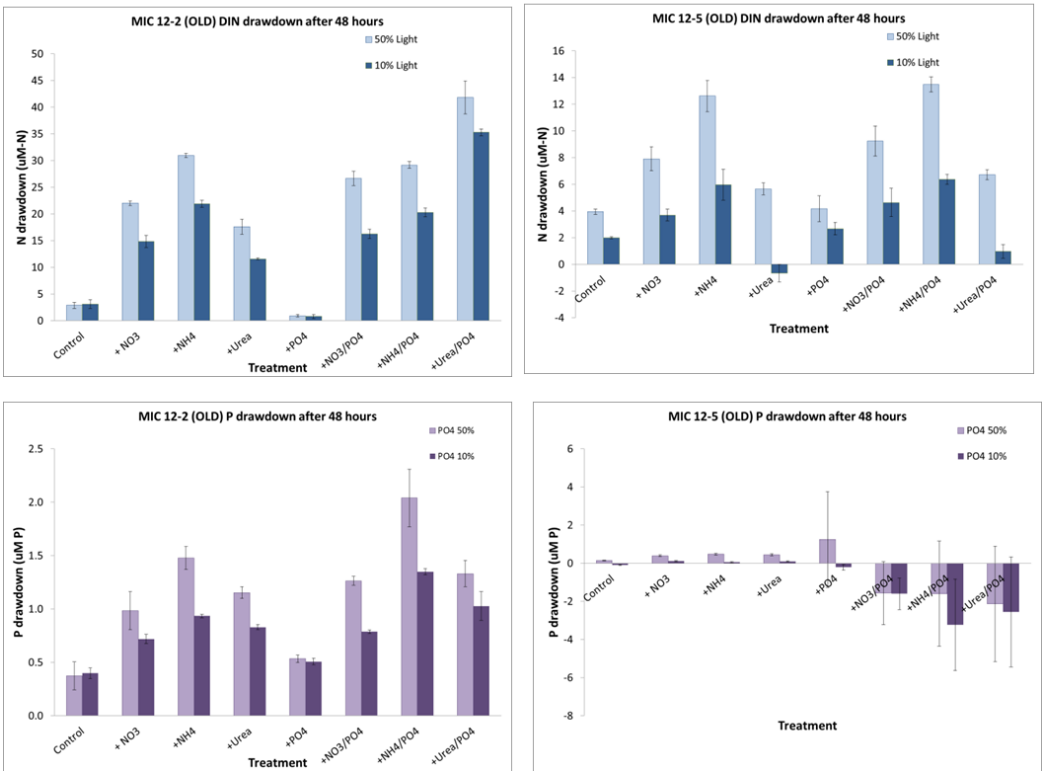
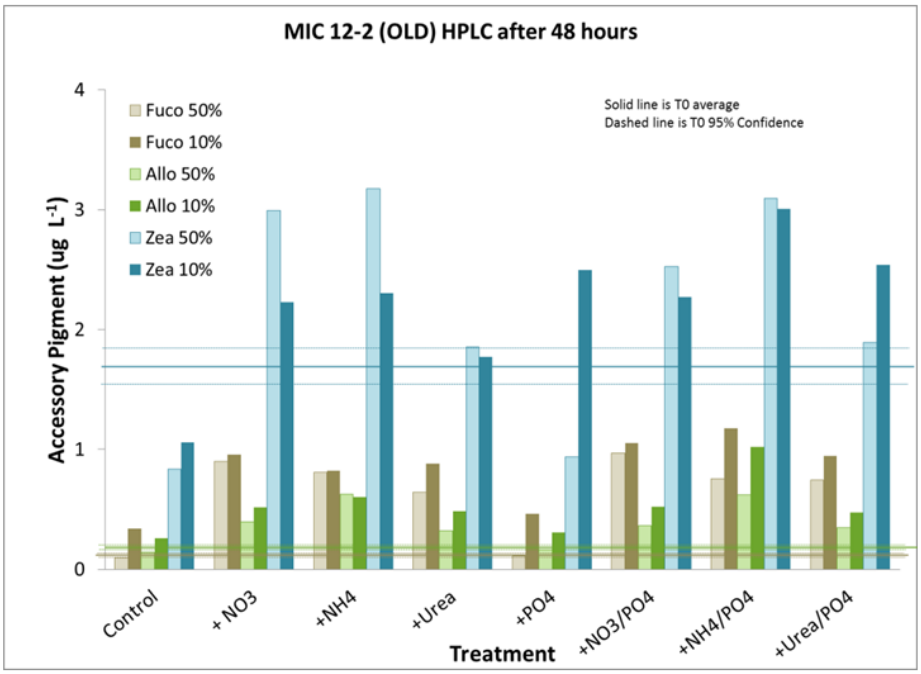


Figure 2.16. Nitrogen (top panel) and phosphorus (bottom panel) drawdown in two representative bioassay experiments. N and P drawdown was calculated as the difference between initial and final (48-hr) N and P concentrations.

We used high performance liquid chromatography to determine increases in phytoplankton accessory pigments that are diagnostic of specific algal function groups. Specifically we used zeaxanthin as an indicator of cyanobacteria, fucoxanthin as an indicator of diatoms, and alloxanthin as diagnostic of cryptophytes. All algal functional groups responded to N additions with the largest treatment responses found for cyanobacteria (zeaxanthin; Fig. 2.17). Ammonium addition treatments yielded the largest gains in all groups, although diatoms appeared less response to specific forms of N.



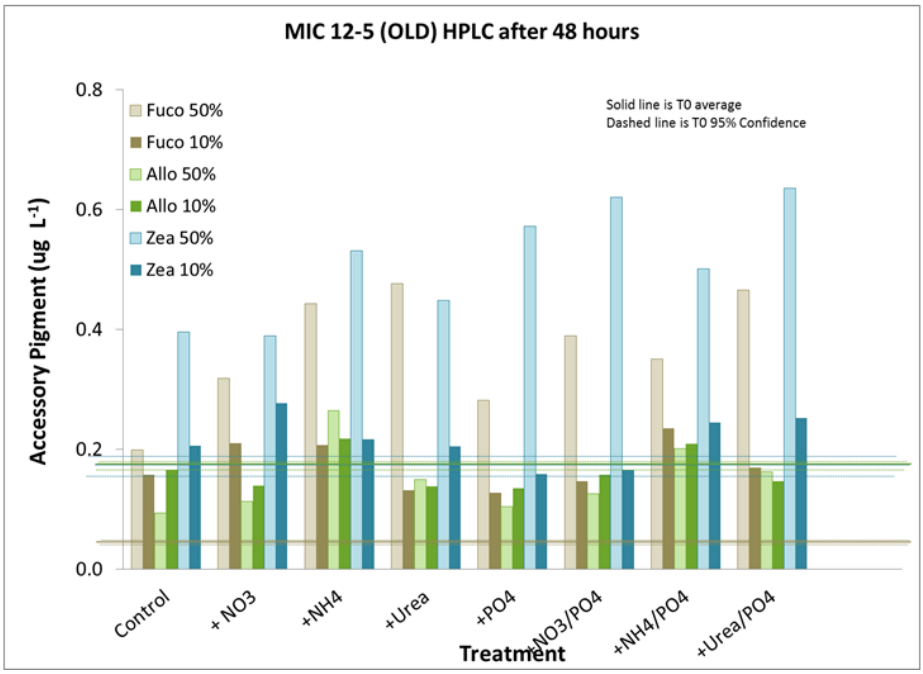


Figure 2.17. Two representative results of 48-hr bioassay experiments showing phytoplankton accessory pigments fucoxanthin (indicative of diatoms), alloxanthin (cryptophytes) and zeaxanthin (cyanobacteria).

Toxin concentrations were also measured in two of the five experiments conducted (Fig. 2.18). We did not observe a significant response in toxin production across nutrient amendment treatments, but there is some indication of elevated toxin concentration under N limited conditions (i.e. the control and P addition treatments).

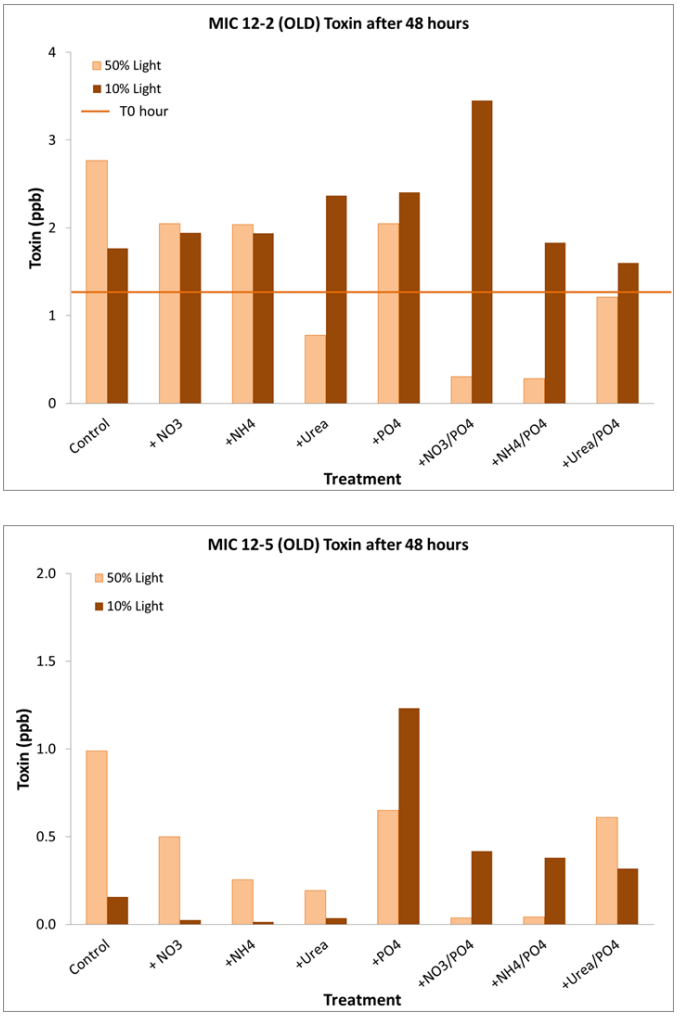


Figure 2.18. Two representative results of 48-hr bioassay experiments showing microcystin concentration versus nutrient addition treatment.

A limited number of nutrient bioassay experiments were conducted at other survey stations in the Delta. Stations with elevated nitrogen did not show patterns of nutrient (N or P) control of biomass during the 48-hr incubation period and therefore do not support strong nutrient control of cyanobacterial biomass at these other locations.

Traditionally, phosphorus (P) input reductions have been recommended to control CHABs, because P limitation is widespread in freshwaters and some CHABs can satisfy their nitrogen (N) requirements by fixing atmospheric N_2 (Schindler 1971). However, systems undergoing eutrophication are increasingly plagued with non-N_2-fixing CHABs, including *Microcystis* spp., that are N and P co-limited or even N

limited. Many of these systems are experiencing accelerating N loads, even as P loading is being controlled.

Several studies have investigated the importance of varying N and P concentrations on the growth of toxic and nontoxic strains of *Microcystis*, microcystin production, and competition with other phytoplankton (Yoshida et al. 2007, Downing et al. 2005, Marinho et al. 2007). Competition studies showed that *Microcystis* had a higher uptake capacity for N than a freshwater diatom and another cyanobacterium (Fujimoto et al. 1997, Marinho et al. 2007, Lee et al. 2015), which may help explain why *Microcystis* thrives in high N:P waters (Xie et al. 2003, Marinho et al. 2007).

TASK DELIVERABLES

Presentations

Johnson, A. A. E. Parker, F.P. Wilkerson, T. Otten. 2015. Climate change effects on the San Francisco Estuary Delta: Phytoplankton community: the role of temperature and salinity tolerance on growth. ASLO Aquatic Science Conference, Granada, Spain February 2015.

Johnson, A., Parker, A. E., Wilkerson, F. P. 2014. The future of Microcystis spp. in the San Francisco Estuary Delta: Investigations into the role of temperature and salinity tolerance on growth. Biennial Bay Delta Science Conference, Sacramento, CA October 28-30. – BEST STUDENT PRESENTATION AWARD

Johnson, A., Parker. A.E., Wilkerson, F.P. 2014. Climate change effects on the San Francisco Estuary Delta phytoplankton community: investigations into the role of temperature and salinity tolerance on growth. CAERS, Bodega Marine Lab, CA. September 26-27. BEST STUDENT PRESENTATION AWARD

Lee, J. 2014. Kinetics and inhibition of nitrogen uptake by Microcystis aeruginosa: results from culture and the San Francisco Delta. Master's Thesis defense, Tiburon, CA December 10, 2014

Lee, J., Parker, A.E., Wilkerson, F.P. 2014. Understanding nitrogen assimilation in Microcystis aeruginosa: implications for cyanobacterial bloom development in the San Francisco Delta. Bay-Delta Science Conference, Sacramento, CA. October 28-30.

Lee, J., Parker, A.E., Wilkerson, F.P. 2014. Investigating the nitrogen uptake in the cyanoHAB Microcystis aeruginosa: insight for bloom development in the San Francisco Delta. California Estuarine Research Society meeting. Bodega Marine Lab, CA. September 26-27.

Parker, A.E. 2014. Primary Production in the Delta: Nutrient Considerations to Guide Conservation. Delta Day of Science, Sacramento, CA May 16, 2014

Parker, A.E. Dugdale, R.C. Wilkerson, F.P. 2014 Consideration of forms of DIN when evaluating the impact of nutrients in the Urban Ocean: Results from the Delaware Bay and the San Francisco Estuary. ASLO Ocean Science Meeting, Honolulu, HI

Parker, A.E. 2014 Primary Production in the Delta: Nutrient Considerations to Guide Conservation: Lower Foodweb Dynamics in California's Bay-Delta Ecosystem: Current Understanding and Future Interactions with a Changing Landscape. The Delta Science Program and UC Davis Center for Aquatic Biology & Aquaculture. Davis, CA February 18, 2014

Johnson, A., Parker, A.E., Wilkerson, F. P. 2013. Climate change effects on the cyanobacterial blooms in the San Francisco Delta. Coastal and Estuarine Research Federation Meeting, San Diego, CA

Johnson, A., Parker, A.E., Wilkerson, F.P. 2013. Climate change effects on cyanobacteria blooms (Micorcystis aeruginosa) in the San Francisco Estuary Delta: evidence from experimental manipulations. State of the Estuary Conference, Oakland, CA

Lee, J., Parker, A.E., Wilkerons, F.P. 2013. Nitrogen uptake by the bloom-forming blue-green alga, Microcystis aeruginosa, in the San Francisoc Estuary Delta. State of the Estuary Conference, Oakland, CA

Lee, J. Parker, A.E., Wilkerson, F.P. 2013. Nitrogne uptake kinetics of Microcystis aueruginosa in the San Francisc Estuary Delta, CA. Coastal and Estuarine Research Federation Meeting, San Diego, CA

Johnson, A., Parker, A.E., Wilkerson, F.P. 2013. Do increased temperature and salinity influence the success of cyanobacteria in the San Francisco Bay Delta? Interagency Ecological Program Workshop in Folsom, CA.

Lee, J., Parker, A.E., Wilkerson, F.P. 2013. Ammonium vs. Nitrate Uptake by the cyanoHAB Microcystis aeruginosa in the San Francisco Estuary Delta Presented at: Interagency Ecological Program Workshop in Folsom, CA.

Johnson, A., Parker. A.E., Wilkerson, F.P. 2012. Climate change effects on cyanobacteria blooms in the San Francisco Estuary Delta. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Lee, J., Parker, A.E., Wilkerson F.P. 2012. Nitrogen uptake kinetics of Microcystis aeruginosa in the San Francisco Estuary Delta. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Parker, A.E., Johnson, A., Lee, J., Mioni, C.E., Pimenta, A., Wilkerson, F.P. 2012. Carbon and nitrogen uptake rates associated with cyanobacterial blooms in the San Francisco Delta. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Parker, A.E., Johnson, A., Lee, J., Pimenta, A., Mioni, C., Wilkerson, F.P. 2012. Carbon and nitrogen uptake rates associated with cyanobacterial blooms in the San Francisco Estuary Delta. California Estuarine Research Society, Long Beach, CA.

Pimenta, A.R. Mioni, C.E., Parker, A.E., Wilkerson, F.P. 2012. Are nutrients a driver of cyanobacterial abundance in the SFE Delta? 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

DuBose, M., Johnson, A., Parker, A.E., Wilkerson, F.P. 2012. Temperature and light availability as drivers of cyanobacteria blooms in the San Francisco Estuary Delta: evidence from experimental enclosures. Interagency Ecological Program Conference, Folsom, CA.

Parker, A.E., Dugdale, R.C., Ger, A., Kimmerer, W., Kudela, R., Mioni, C., Otten, T., Paerl, H., Wilkerson, F.P. 2011. The role of Microcystis blooms in the Sacramento-San Joaquin Delta foodweb: a functional approach. 10th Biennial State of the Estuary Conference, Oakland, CA

Parker, A.E. 2011.The role of Microcystis blooms in the delta foodweb: a functional approach. Estuarine Ecology Team Meeting - August. Romberg Tiburon Center, Tiburon, CA.

Published Papers

Lee, J., A.E. Parker, F. P. Wilkerson, R.C. Dugdale. 2015. Uptake and inhibition kinetics of nitrogen in Microcystis aeruginosa: results from cultures and field assemblages in the San Francisco Bay Delta, CA. Harmful Algae. 47: 126-140.

Papers in Prep

Johnson, A. , A. E. Parker, F.P. Wilkerson, W. J. Kimmerer, H. Paerl. Effects of changing salinity and temperature on phytoplankton community composition in the northern San Francisco Estuary, California, USA For submission to Estuaries and Coasts.

Parker, A. E., S. Blaser, F. P. Wilkerson, H. Paerl, R. Kudela. Nitrogen and phosphorus availability as a determinant of Microcystis blooms in the San Francisco Estuary Delta: results from bioassay experiments. For submission to Harmful Algae.

Synergistic Activities

Synergistic activities for Task 2 are summarized under Task 1: Management.

List of Students (Graduate/ Undergraduate) Supported

Jamie Lee, SFSU MSc Student December 2014

Allison Johnson, SFSU MSc Student October 2015

Task 3: Phytoplankton Microscopy and Toxin Characterization and Assessment of Microbial Consortia Associated with Microcystis Colonies

PIs: Mioni and Kudela

KEY FINDINGS

1. We observed a strong statistical correlation between *Microcystis* and *Aphanizomenon* abundances in the Delta.

TASK NARRATIVE

By combining observations from multiple programs, we can observe a strong statistical correlation between *Microcystis* and *Aphanizomenon* abundances (Table 3.1). For comparison with the Delta refer to Table 3.2 from the 2008-2012 study period. From the original dataset (Table 3.1), Microcystis was significantly correlated with temperature, pH, and ammonium. By expanding the dataset , pH and ammonium drop out, but there is a strong correlation to *Aphanizomenon*. This is consistent with the findings of Kudela et al. (2015) for Pinto Lake, where it was shown that *Aphanizomenon* is a good predictor of succession to *Microcystis* and elevated toxin levels.

These data are being combined with molecular information from Kurobe and Baxa, for preparation as a peer-reviewed manuscript comparing the Delta and Clear Lake ecosystems.

Table 3.1: Intra-annual correlation matrix between environmental variables and individual cyanobacterial taxa abundance for the year 2011 (June – October). Spearman coefficients (ρ) are highlighted in bold characters when correlations were significant at the 0.05 level, two-tailed (p<0.05).

	MSAE	Apha.	Anabaena	Oscillat.	Temp.	EC	Secchi	pH	PO4	NOx
Apha.	**ρ = 0.537** p < 0.001									
Anabaena	ρ = -0.086 p = 0.521	ρ = -0.102 p = 0.444								
Oscillatoria	**ρ = 0.366** p = 0.005	**ρ = 0.274** p = 0.038	ρ = 0.061 p = 0.647							
Temp.	ρ = 0.093 p = 0.485	ρ = 0.027 p = 0.838	ρ = 0.254 p = 0.054	ρ = 0.085 p = 0.527						
EC	**ρ = 0.403** p = 0.002	**ρ = 0.674** p < 0.001	ρ = -0.205 p = 0.126	**ρ = 0.300** p = 0.023	ρ = 0.090 p = 0.496					
Secchi	ρ = -0.051 p = 0.712	ρ = 0.081 p = 0.561	ρ = -0.061 p = 0.660	ρ ~ 0.00 p = 0.999	**ρ = 0.265** p = 0.046	ρ = -0.097 p = 0.471				
pH	ρ = 0.220 p = 0.101	**ρ = 0.577** p < 0.001	ρ = 0.004 p = 0.976	ρ = 0.190 p = 0.156	ρ = 0.203 p = 0.119	**ρ = 0.307** p = 0.018	ρ = 0.244 p = 0.070			
PO4	ρ = 0.125 p = 0.348	ρ = 0.090 p = 0.502	ρ = -0.059 p = 0.662	ρ = 0.222 p = 0.093	**ρ = 0.257** p = 0.045	**ρ = 0.315** p = 0.014	ρ = 0.089 p = 0.508	ρ = 0.246 p = 0.058		
NOx	ρ = -0.222 p = 0.093	ρ = -0.123 p = 0.359	ρ = -0.192 p = 0.149	ρ = -0.131 p = 0.327	ρ = -0.217 p = 0.093	ρ = 0.150 p = 0.253	ρ = -0.167 p = 0.213	**ρ = -0.330** p = 0.010	ρ = 0.251 p = 0.051	
NH4	ρ = 0.038 p = 0.778	ρ =-0.124 p = 0.356	ρ = -0.014 p = 0.916	ρ = 0.071 p = 0.595	**ρ = -0.381** p = 0.002	**ρ = -0.265** p = 0.041	**ρ = -0.355** p = 0.007	**ρ = -0.399** p = 0.002	**ρ = -0.325** p = 0.011	ρ = 0.063 p = 0.630

Table 3.2 Interannual correlation matrix between environmental variables and Microcystis (MSAE) abundance for the combined CALFED, MIC & SWAMP monitoring data (2008 - 2012). Spearman coefficients (ρ) are highlighted in bold characters when correlations were significant at the 0.05 level, two-tailed ($p<0.05$).

	MSAE	Temp.	EC	Secchi	pH	PO4	NOx
Temperature	**ρ = 0.293** p < 0.001						
EC	ρ = 0.034 p = 0.604	**ρ = -0.292** p < 0.001					
Secchi	ρ = -0.048 p = 0.461	**ρ = 0.175** p = 0.006	**ρ = -0.184** p = 0.005				
pH	**ρ = 0.271** p < 0.001	ρ = -0.078 p = 0.265	**ρ = -0.188** p = 0.007	**ρ = 0.149** p = 0.034			
PO4	ρ = 0.129 p = 0.056	**ρ = -0.233** p < 0.001	**ρ = 0.428** p < 0.001	**ρ = -0.385** p < 0.001	ρ = 0.055 p = 0.443		
NOx	ρ = -0.101 p = 0.127	**ρ = -0.507** p < 0.001	**ρ = 0.501** p < 0.001	**ρ = -0.343** p < 0.001	**ρ = -0.271** p < 0.001	**ρ = 0.539** p < 0.001	
NH4	**ρ = -0.175** p = 0.008	**ρ = -0.541** p < 0.001	**ρ = 0.417** p < 0.001	**ρ = -0.388** p < 0.001	**ρ = -0.341** p < 0.001	**ρ = 0.271** p < 0.001	**ρ = 0.538** p < 0.001

TASK DELIVERABLES

Presentations

Kudela, RM. 2015. Knowledge gaps/research priorities for HAB species. Global Solutions to Regional Problems workshop, Bowling Green State University, April 13-14, 2015.

Kudela, RM. 2015. Cyanobacterial Problems in California: More of the Same, Or Some Unexpected Surprises? American Society for Microbiology, June 2, 2015, New Orleans, LA.

Kudela, R, Peacock, M, Schraga, T, Senn D. 2014. Does San Francisco Bay have a harmful algal bloom problem? 2014 Bay-Delta Science Conference, 28-30 October 2014, Sacramento, CA.

Published Papers

Kudela, RM, SL Palacios, DC Austerberry, EK Accorsi, LS Guild, and J Torres-Perez. 2015. Application of Hyperspectral Remote Sensing to Cyanobacterial Blooms in Inland Waters. *Remote Sensing Environment*, doi 10.1016/j.rse.2015.01.025

Synergistic Activities

Hosted the State Water Board Training Academy and SWAMP program, Introduction to CyanoHABs workshop. 14 July 2015, UCSC.

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TASK 4: MOLECULAR CHARACTERIZATION OF MICROCYSTIS BLOOMS

PIs: Paerl and Otten

KEY FINDINGS

1. The *Microcystis* population is diverse throughout the Delta, comprising no less than eight strains, with at least 3-4 being microcystin producers.

2. The *Microcystis* population produces a wealth of other bioactive secondary metabolites that are poorly resolved but may be directly influencing zooplankton fitness.

3. A number of indirect stressors stemming from *Microcystis* blooms could be negatively influencing zooplankton fitness, these include: alterations to the microbial loop, outcompeting other nutritious algae or by augmenting the local environment (e.g., raising dissolved oxygen concentration, temperature, pH, etc.) making it unsuitable for zooplankton or their prey.

TASK NARRATIVE

We utilized a suite of molecular biology tools to characterize the ecology and physiological potential of HAB taxa observed from all transect sites surveyed in 2011 and 2012 (n=72). The goal of this research was to identify the bioactive metabolites these genera are able to produce and to assess how spatiotemporal dynamics of specific subpopulations (i.e., strains) influence microbial community structure and food webs.

It is well established that cyanobacteria have allelopathic effects on other phytoplankton due to the inhibitory activity of various secondary metabolites they produce [Legrand et al., 2003]. However, there has been scant scientific inquiry as to how pelagic cyanobacteria may affect non-photoautotrophic bacteria in the water column. In this study we hypothesized that: 1) there are multiple strains of *Microcystis* spp. present throughout the SFE and different sampling stations may contain unique, endemic strains; 2) that each *Microcystis* species/strain exhibit unique physiological capabilities as assessed by gene content; 3) that cyanobacterial blooms influence microbial community richness directly by augmenting dissolved organic carbon pools and indirectly by altering the physicochemical environment (e.g., increased pH, increased dissolved oxygen concentration, increased temperatures, etc.); 4) zooplankton fitness and/or abundance are negatively influenced during bloom events.

In order to assess *Microcystis* strain diversity relative to colony morphology, individual *Microcystis* colonies (n=21) from surface grab samples collected at four sites (Antioch, Franks Tract, Mildred Island, Old River) between 2011and 2012 were isolated under a dissecting microscope. In both years, the majority of colonies existed as one of two different morphologies, both similar to *M. aeruginosa* in that they were large, irregularly shaped and lacking a thick mucilaginous sheath; yet different in that some morphotypes were densely packed and web-like (termed "web"), whereas others were considerably larger and loosely packed (termed "flake") (Fig. 4.1). A third variant was occasionally observed and presented as a combination between the web and flake morphologies; we hypothesized that all three morphologies may represent the same strain, with the flake representing young colonies, the hybrid occurring later after some tearing by turbulent mixing or grazing activities, finally culminating in the web morphology.

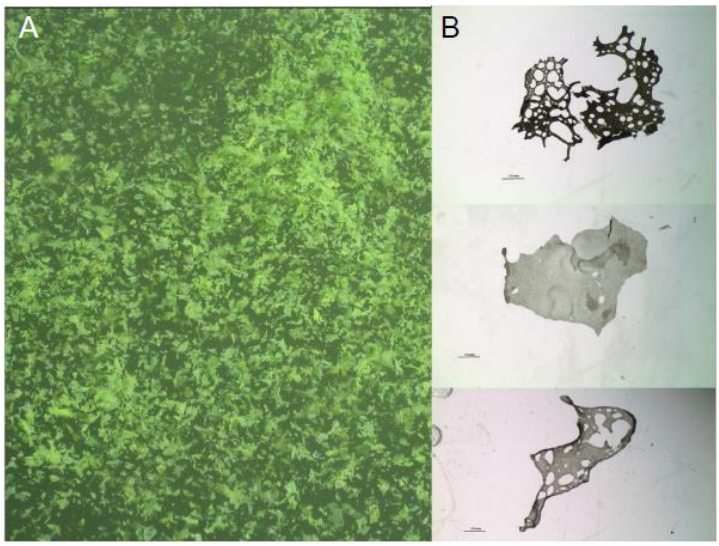
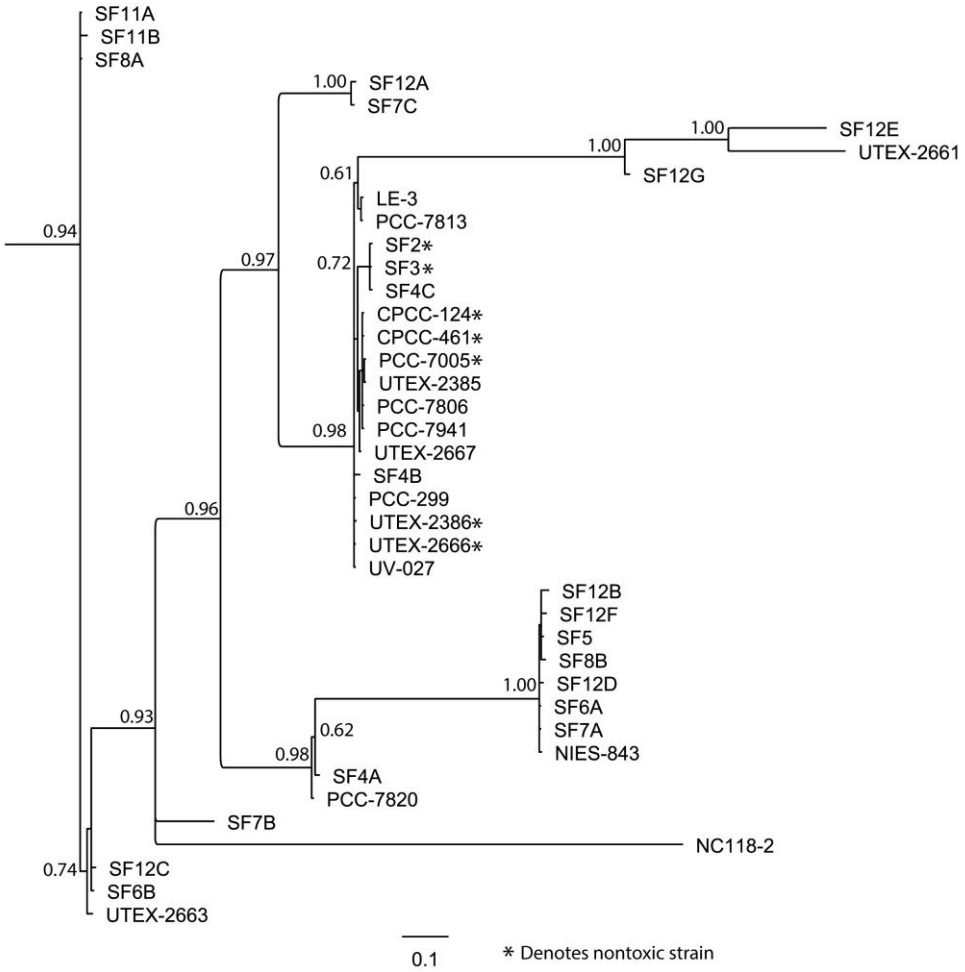


Figure 4.1: A) Photograph of a *Microcystis* bloom at Mildred Island (8-8-2011). B) Microscope images (40X mag.) of the different *Microcystis* spp. colonial morphologies observed in the SF Delta, top = "web", middle = "flake", bottom = "hybrid".

The individual isolated colonies were washed thrice in sterile deionized water and stored in PCR tubes containing 20 µL of 1X TE buffer at -20°C until further processing. Colony isolates were subjected to three freeze-thaws cycles (-80°C and 75°C) to facilitate cell lysis (to release DNA) and a portion of each was screened for potential toxigenicity using a whole-cell PCR assay targeting the *mcyB* toxin gene and the ribosomal 16S-23S rDNA internal transcribed spacer (ITS) region. All samples were positive for the 16S-23S rDNA and 20/21 were positive for the *mcyB* toxin gene. These amplicons were ligated into plasmids (pCR2.1) and bi-directionally Sanger sequenced. The phylogenies of the ITS amplicons were inferred using a Bayesian framework in MrBayes, a general time reversible substitution model with gamma distributed variation was utilized to construct a phylogram displaying strain relationships (Fig. 4.2).

By sequencing the *mcyB* genes from the colony isolates and analyzing the adenylation domain binding pocket signatures, the likely microcystin congeners produced by each variant can be deduced. From the 21 colony isolates investigated, at least three different *mcyB* gene variants were identified, indicating that at a minimum 3/8 of the *Microcystis* strains are microcystin producers since each strain is expected to only contain a single copy of this gene (i.e., no paralogs have been reported for the *mcy* genes). Delving further into these genes, we expect the principle microcystin congeners to be produced by these taxa to be MC-LR, MC-RR and MC-YR. These variants have toxicological relevance since different microcystin congeners exhibit different toxicities, for example, MC-LR is 6.4-times more toxic than MC-RR in mice assays and MC-YR falls somewhere in between (Chen et al., 2010).

Figure 4.2: Phylogenetic inference of SF Delta *Microcystis* colony isolates based on 16S-23S rDNA ITS sequencing. Included in this dendrogram are various toxic and nontoxic *Microcystis* strains from culture collections. Overall, there were 8 different clades/strains of *Microcystis* identified in the Delta, even though only 2-3 colony morphologies were observed, indicating colony morphology corresponds poorly with genotype, as has been observed in other *Microcystis* impacted systems (e.g., Lake Taihu, China; Otten and Paerl, 2011).



In addition to the colony isolation work, environmental samples were collected from all six sample sites in 2011 and 2012 (n = 72) and concentrated onto Supor membrane filters (0.2 µm pore size). The small pore size of these filters enables retention of all phytoplankton and bacteria. Total DNA from each filter was extracted using GeneRite RW03C kits and first screened for the presence of total *Microcystis* and toxigenic *Microcystis* by real-time Quantitative PCR (QPCR) amplification of the α-subunit of the c-phycocyanin gene (*cpcA*) and the microcystin synthetase E gene (*mcyE*), respectively. Plasmid standards (*mcyE* and *cpcA*) were created for the QPCR assays and serial dilutions of these plasmids were used to make standard curves spanning eight dilutions. All samples were tested for PCR inhibition with an external DNA control (salmon sperm - *Oncorhynchus keta*) and total and toxigenic *Microcystis* gene equivalents were enumerated from all environmental samples by QPCR (Fig. 4.4).

Figure 4. 3: Multiple pairwise alignment of amino acids spanning the variable adenylation domains (denoted by * above the top rows) of the *mcyB* toxin gene sequenced from the 2011 colony isolates (Antioch 8-8-11). At least three genetically distinct microcystin producers were identified to comprise the *Microcystis* population on this date based on polymorphisms within the *mcyB* coding region. The largest group (rows 1-9 & 12-13) are predicted to produce the microcystin variants MC-LR, RR and YR; the middle group (rows 10-11) are predicted to encode for MC-RR; and the bottom group (rows 14-17) are predicted to encode for MC-LR. Note that some colonies (e.g., SF4, SF5, SF6 and SF12) were comprised of more than one toxigenic genotype.

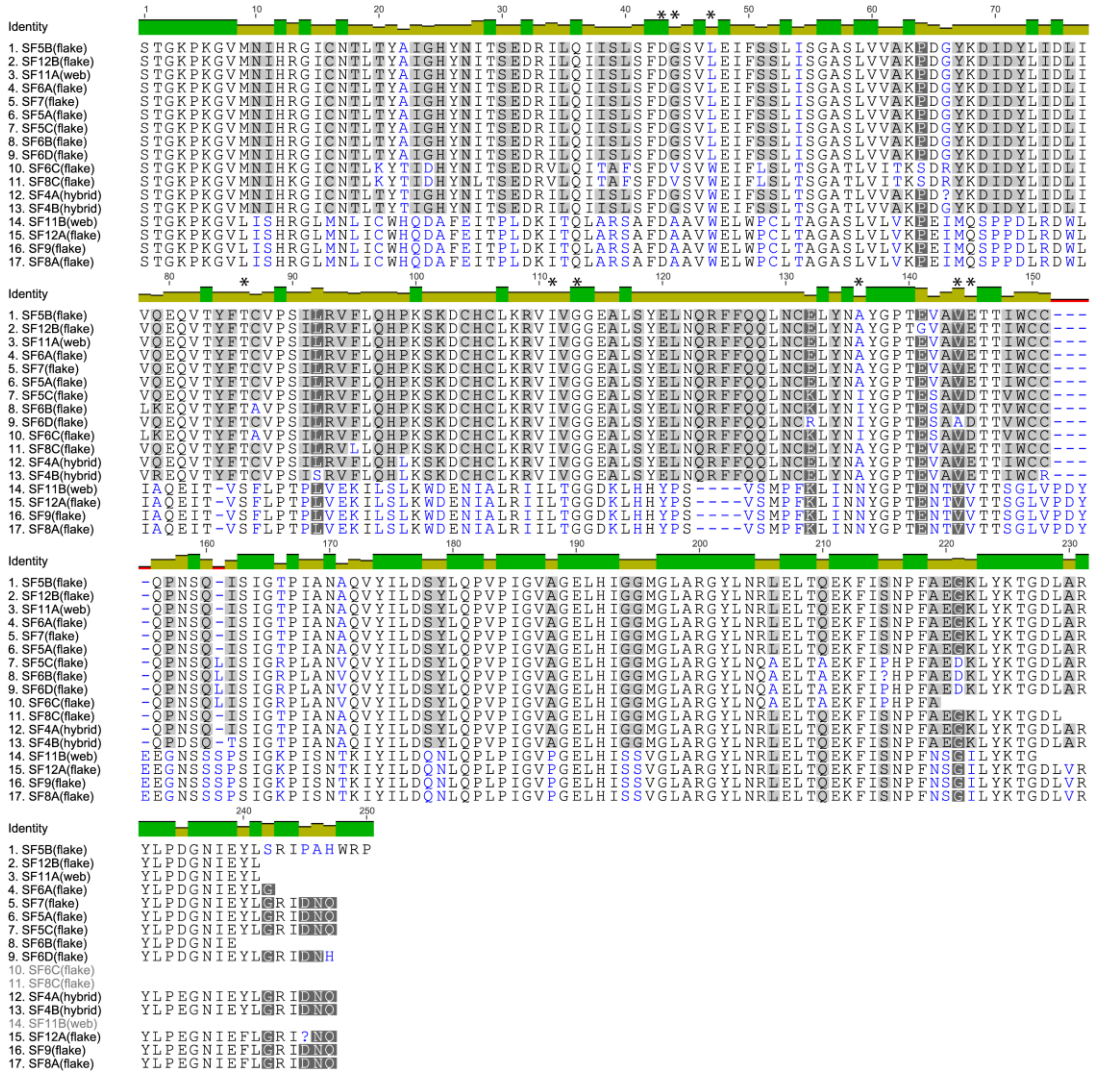
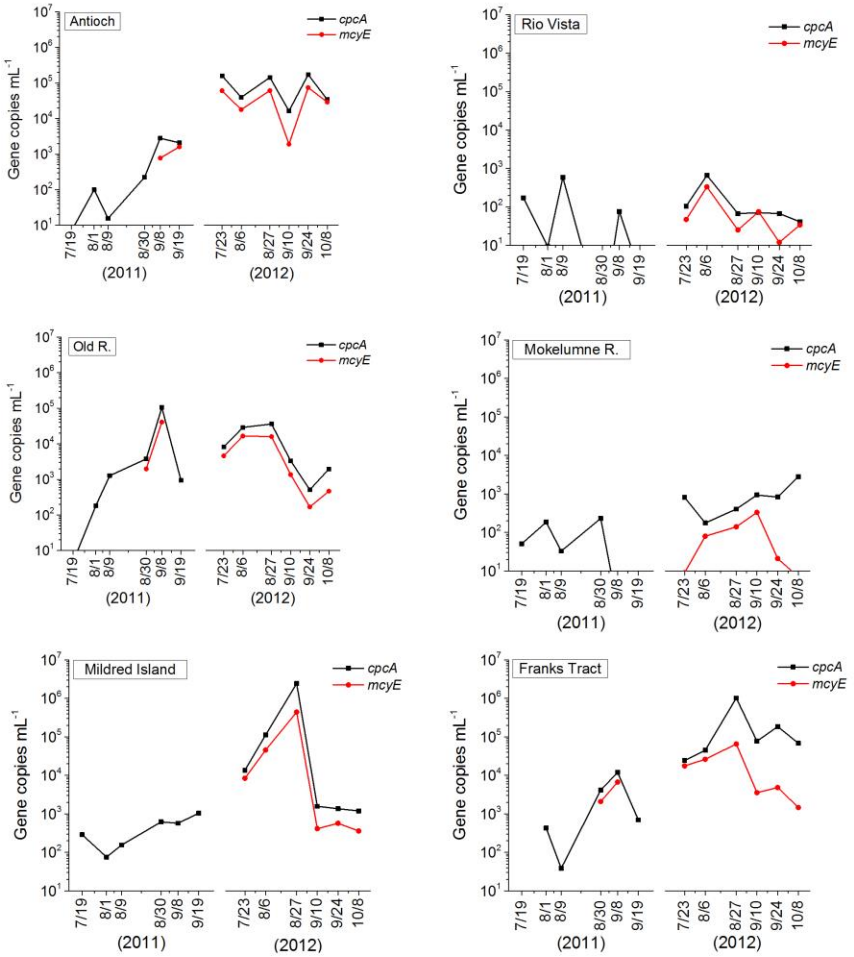
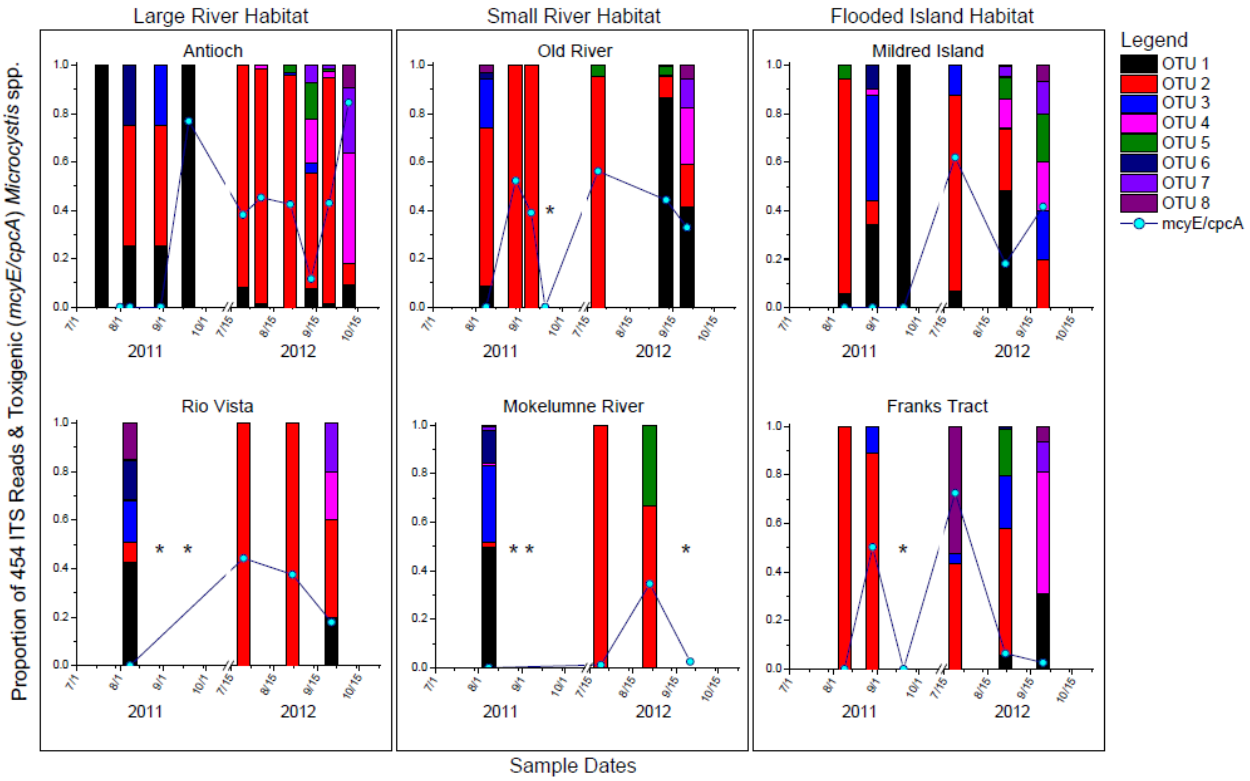


Figure 4.4: Combined 2011 and 2012 QPCR results grouped by habitat type: Top row = Large river; Middle row = Small river; Bottom row = Flooded island. Sample sites within the left column historically contain abundant *Microcystis*, whereas sites within the right column generally consist of little to no *Microcystis*. The black lines (*cpcA*) represent total *Microcystis* and the red lines (*mcyE*) represent potential microcystin producers tal (*cpcA*; black) and toxigenic (*mcyE*; red) *Microcystis* abundances throughout all study sites and sample times assessed by QPCR.



Overall, these data indicated that on average ~26% (±27%) of the *Microcystis* population consisted of toxigenic (*mcyE*-possessing) cells throughout the study period (2011-2012) across all sites. The summer of 2011 produced considerably less *Microcystis* than in 2012 throughout all sampling sites, and there also appeared to be fewer toxigenic cells–on a percentage basis–in 2011 than 2012. In an attempt to track the different *Microcystis* strains and identify which specific ones are the microcystin producers in the system, we used high throughput 454 pyrosequencing of the 16S-23S rDNA ITS locus from representative samples collected from all study sites (Fig. 4.5). We rationalized that certain strains would be consistently dominant when toxicity was high, that certain sites would have unique Microcystis populations (endemic) and that samples from the Antioch location would exhibit total Delta *Microcystis* diversity since it represents the confluence of all upstream sample sites. As such, we sequenced every sample from Antioch and every other from the other five sites in order to generate a diversity index of *Microcystis* populations.

Figure 4.5: Relative proportion of *Microcystis* operational taxonomic units (OTUs; strains) based on 454 pyrosequencing of the 16S-23S rDNA ITS locus and the relative proportion of toxigenic *Microcystis* (*mcyE*/*cpcA*) comprising these populations. Asterisks (*) denote samples that failed to amplify because there was not enough target present.



As with the colony ITS analysis (Fig. 4.2), the 454 ITS analysis also identified 8 *Microcystis* strains, or OTUs (operational taxonomic units), delineated at the 97% nucleotide identity level. The two most abundant strains, OTUs 1 and 2, were highly prevalent at all sites, suggesting that they are widely distributed throughout the Delta as opposed to being transported from a specific source upstream. Both strains occurred at times when *mcyE* genes were present, but also at times when *mcyE* genes were not detected. This suggests that toxicity cannot be predicted based on strain identifiers alone and should be assayed for directly by toxin analysis or QPCR assays targeting the toxin genes themselves.

Since cyanobacteria are well known for their ability to produce myriad secondary metabolites–in addition to microcystins–we conducted shotgun metagenomic surveys on three samples collected from Mildred Island in 2012 that corresponded to the onset of a *Microcystis* bloom, peak *Microcystis* biomass and subsequent *Microcystis* bloom collapse (Jul-23, Aug-28 and Sep-10, respectively). These three samples contained a variety of *Microcystis* genotypes based on the 454 16S-23S rDNA ITS analysis, as such the focus of the shotgun metagenomes was to identify what other secondary metabolites are produced by these taxa and also to assess how total microbial community diversity and individual organism abundances change over the course of a cyanobacterial bloom. Each shotgun metagenome produced over 35 million sequencing reads (Illumina 100 bp, paired-ends). The reads were quality trimmed and assembled using IDBA-UD. The assembled contigs were then screened for secondary metabolites using antiSMASH 2.0. This search identified a number of genes/operons involved in the biosynthesis of most of the known secondary metabolites produced by *Microcystis* (Humbert et al.,

2013), as well as several other putative secondary metabolites (both polyketide synthases and nonribosomal peptide synthetases) that are presently uncharacterized. The known compounds included the zooplankton serine protease inhibitors: cyanopeptolin and microviridin j, as well as other compounds that have been reported elsewhere, although there is little to no toxicological understanding of their effects on microbes, zooplankton or higher organisms, these included: aeruginosin, anabaenopeptin and microginin. Amazingly, the SF Delta *Microcystis* population was found to contain a global assortment of *Microcystis* secondary metabolites, underscoring that this population is genetically quite diverse. A targeted study investigating the specific effects of these individual compounds on zooplankton fitness and fecundity will be needed to unravel which–if any–of these compounds may be exhibiting direct food web impacts.

In the meantime, we attempted to characterize the community diversity (total bacteria and eukarya) using shotgun metagenomics on the three aforementioned samples. The principal findings from this analysis was that: 1) *Microcystis* represents the single most abundant organism in the water column when blooming, dwarfing most other taxa by an order of magnitude or more (Fig. 4.6); 2) most other bacteria become numerically less abundant during *Microcystis* blooms based on rarefaction curve analyses of alpha diversity (i.e., species-richness) (Fig. 4.7); and 3) the only other organisms to increase in abundance during a *Microcystis* bloom are other cyanobacterial taxa (Fig. 4.8), suggesting that either synergisms exist or they are better suited to the local environment produced by bloom events (e.g., supersaturated O_2, high pH, low DIC, low N and P, etc.) .

Figure 4.6 shows that *Microcystis* contig coverage depth, which is a function of how many of the total reads from each dataset mapped to *Microcystis* versus other organisms, is an order of magnitude greater than any other organism from the non-bloom time points. This finding suggests that from a food web perspective, key zooplankton food sources for POD fishes may experience difficulty finding suitable phytoplankton to graze during bloom events. Cyanobacteria themselves are largely considered to be of poor nutritional quality due to their low lipid and fatty acid content, therefore their presence may indirectly influence zooplankton fitness. Feeding studies have demonstrated that *Microcystis* sp. is an unsuitable food source for the predominant calanoid copepods in the SFE, *Eurytemora affinis* and *Pseudodiaptomus forbesi*, with the former being the more sensitive of the two [Ger et al., 2010]. The presence of a large number of potential detrimental secondary metabolites produced by these genera furthers the possibility that *Microcystis* may also directly influence zooplankton fitness through these compounds. Another intriguing observation was that during *Microcystis* blooms the whole microbial community's richness was reduced (Fig. 4.7).

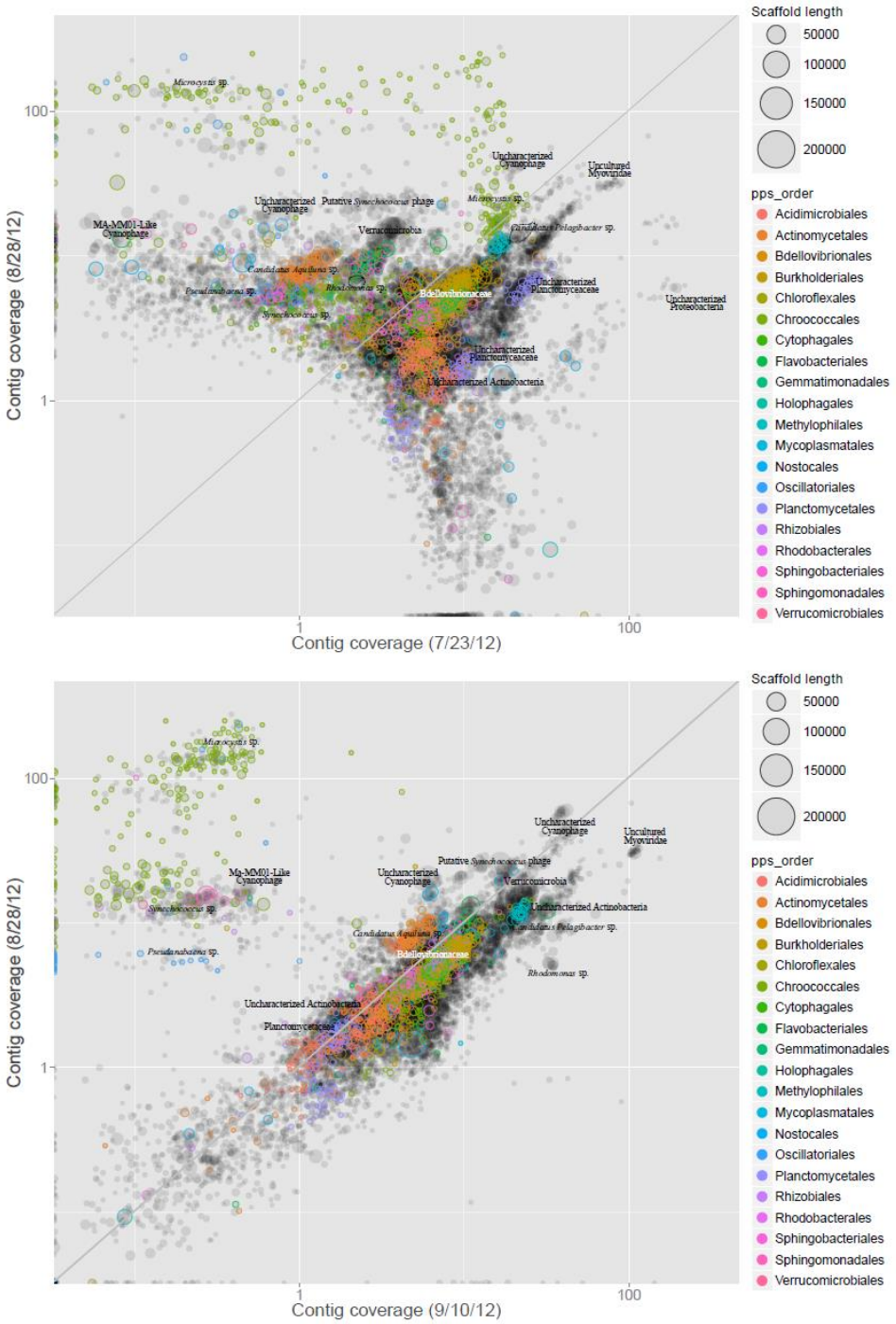
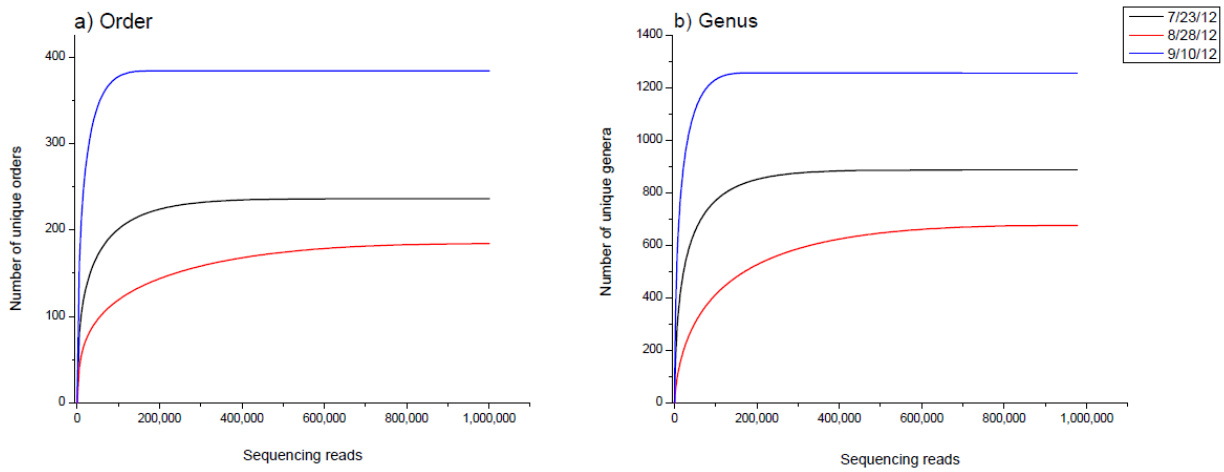


Figure 4.6: Assessment of community structure and relative abundance before (7/23/12), during (8/28/12) and after (9/10/12) a *Microcystis* spp. bloom at Mildred Island. Contig lengths and their average coverage depths are displayed. Contigs were categorized according to their homology to sequences in NCBI BLAST and on the basis of their essential genes, GC content and tetranucleotide frequencies using the software programs PhylopythiaS+ [Gregor et al., 2014] and mmgenome [Albertsen et al., 2013]. A normalized subset of reads (n = 34,560,000 sequences) from each of the three time points was mapped to the assembly contigs and visualized in R using the mmgenome package. Contigs along the gray line are uniformly represented between the compared time points. Only contigs longer than 3,000 bp are displayed.

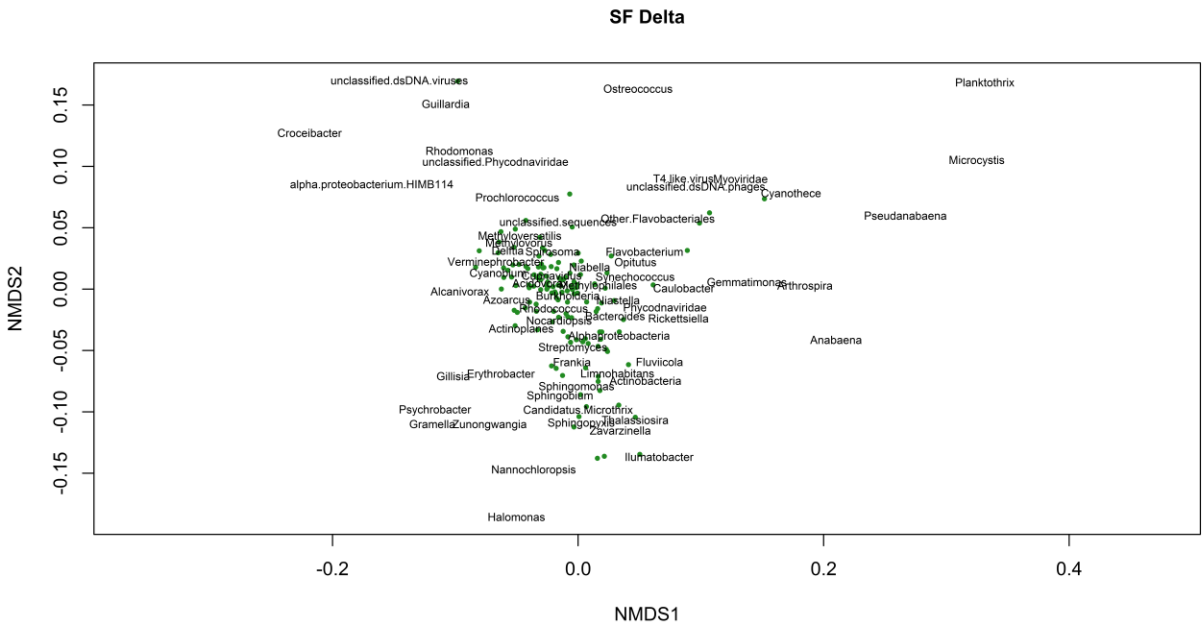
Figure 4.7: Three samples, comprising the early onset of a *Microcystis* spp. bloom (7/23/12), near peak *Microcystis* biomass (8/28/12), and subsequent bloom collapse (9/10/12) were shotgun sequenced from 0.2 m subsurface grab samples filtered onto 0.2 µm pore size membrane filters that were collected from Mildred Island in 2012. Sequencing reads (101 bp, paired-end) were translated into amino acids and proteins families were assigned using RAPSearch [Ye et al., 2011] and viewed in MEGAN [Huson et al., 2011]. Rarefaction curves were calculated using a normalized subset of predicted proteins (*n* = 1,029,684) assigned to order (a) and genus (b) taxonomic levels for each of the three time points.



This observation goes counter to the normal expectation that primary producers such as *Microcystis* will fix a large amount of new, labile carbon which should serve as a food source stimulating growth of the heterotrophic bacterial community. Instead, we observed that during bloom periods microbial diversity was halved and only after the bloom abated did it rebound. The rarefaction curves indicate that by sampling deeper we are unlikely to discover many new species and therefore this finding is not likely to be biased by the numerically abundant *Microcystis* cells sequestering a majority of the sequencing reads. Community analysis using nonmetric multidimensional scaling (NMDS) suggested that the loss in microbial diversity was not replaced with an increase in a few highly successful groups of heterotrophs, since these taxa would increase concomitantly with *Microcystis* (Fig. 4.8). Instead, we only observed other cyanobacterial taxa to increase and decrease in sync with the *Microcystis* population, suggesting a possible environmental driver of this pattern.

The observed reduction in microbial diversity has important ramifications for the food web; in pelagic environments energy is supplied to higher trophic levels by two main pathways, either directly from phytoplankton to larger zooplankton such as the calanoid copepods, or indirectly via the microbial food web The latter requires trophic intermediaries such as protists (i.e., ciliates) to feed on picoplankton and other bacteria, with them in turn being preyed upon by copepods, larger ciliates and dinoflagellates. This analysis suggests that due to the apparent lack of edible, non-cyanobacterial phytoplankton over a three-month period at Mildred Island, larger zooplankton such as the calanoid copepods integral to the diets of POD fishes were likely largely dependent upon the microbial food chain to sustain them. This analysis highlights the importance of examining all intermediaries within the food web and the role that cyanobacterial blooms may have on the microbial food web, protists and zooplankton alike. As such, *Microcystis* blooms could ostensibly be impacting copepod fitness via multiple stressors and this could be an important mechanism influencing the pelagic organism decline. This hypothesis goes outside the scope of the present study, but may be worthy of future exploration.

Figure 4.8: Nonmetric multidimensional scaling (NMDS) analysis of microbial community consortia and relative abundances from three samples collected before, during and after a large *Microcystis* bloom (Mildred Island, 2012). Low abundance taxa are displayed as green dots to reduce label crowding. The co-occurrence of *Microcystis* and other cyanobacterial genera, but not heterotrophic bacteria, suggests that *Microcystis* blooms may be detrimental to most microbial taxa.



TASK DELIVERABLES

Presentations

Otten, T. G, Paerl, H.W. 2014 The distribution, ecology and genetics of Microcystis blooms throughout the San Francisco Bay Delta. 8th Biennial Bay Delta Science Conference, Sacramento, CA. October 2014.

Otten, T. G, Paerl, H.W. 2012. DNA fingerprinting and quantitative assessments of toxigenic *Microcystis* assemblages and their environmental drivers in the San Francisco Estuary Delta. 7th Biennial Bay Delta Science Conference, Sacramento, CA. October 2012

Published papers

Paerl, H.W. and T.G. Otten. 2013. Harmful Cyanobacterial Blooms: Causes, Consequences and Controls. Microbial Ecology 65: 995-1010.

Paerl, H.W. 2013. Combating the global proliferation of harmful cyanobacterial blooms by integrating conceptual and technological advances in an accessible water management toolbox. Environmental Microbiology Reports 5:12-14.

Paerl, H.W. and T.G. Otten. 2013. Blooms bite the hand that feeds them. Science 342:433-434.

Otten, T.G., and H.W. Paerl. 2015. Health Effects of Toxic cyanobacteria in U.S. Drinking and Recreational Waters: Our Current Understanding and Proposed Direction. Current Environmental Health Reports. DOI 10.1007/s40572-014-0041-9.

Papers in prep

Otten, T. G. , H. W. Paerl, T. W. Dreher, W. Kimmerer, A.E. Parker. The Molecular Ecology of San Francisco Estuary *Microcystis* spp. Blooms and Their Putative Influence on the Pelagic Organism Decline. To be submitted to the journal Ecology Letters

Synergistic Activities

Paerl, H.W. (2014). Co-author on Waterborne and Foodborne Diseases Chapter: Interagency Special Report on the Impacts of Climate Change on Human Health in the United States: An Assessment of Observed and Projected Climate Change Impacts on Human Health in the U.S.. Report (Part of the President's Climate Action Plan and ongoing efforts within the US Global Change Research Program (USGCRP), the Interagency Crosscutting Group on Climate Change and Human Health (CCHHG) and a subset of the Interagency National Climate Assessment Working Group (INCA)). Washington D.C., November, 2014.

List of Students (Graduate/ Undergraduate) Supported

Timothy G. Otten, PhD, Environmental Science & Engineering at UNC Chapel Hill

TASK 5: FOOD WEB INTERACTIONS

PIs: Kimmerer and Ger

KEY FINDINGS

1. The microcystin-producing strain of *Microcystis* negatively affected copepod survival and reproduction when it was >50% of the total phytoplankton biomass, but this depended on the experimental conditions. *Microcystis* was less toxic to copepods when provided in water collected from the estuary rather than artificial experimental media. The reasons are uncertain but may involve microbial detoxification of toxins.

2. *Microcystis* ingestion by copepods in the field can be quantified via *Microcystis* DNA in copepod guts

3. Copepods collected from the field ingested more *Microcystis* during bloom conditions. The abundance of adult copepods was negatively related to the copy number of *Microcystis* genes found in their guts.

4. Taken together, there is some evidence for reduced reproductive success and reduced abundance of copepods at high levels of *Microcystis* in the field.

TASK NARRATIVE

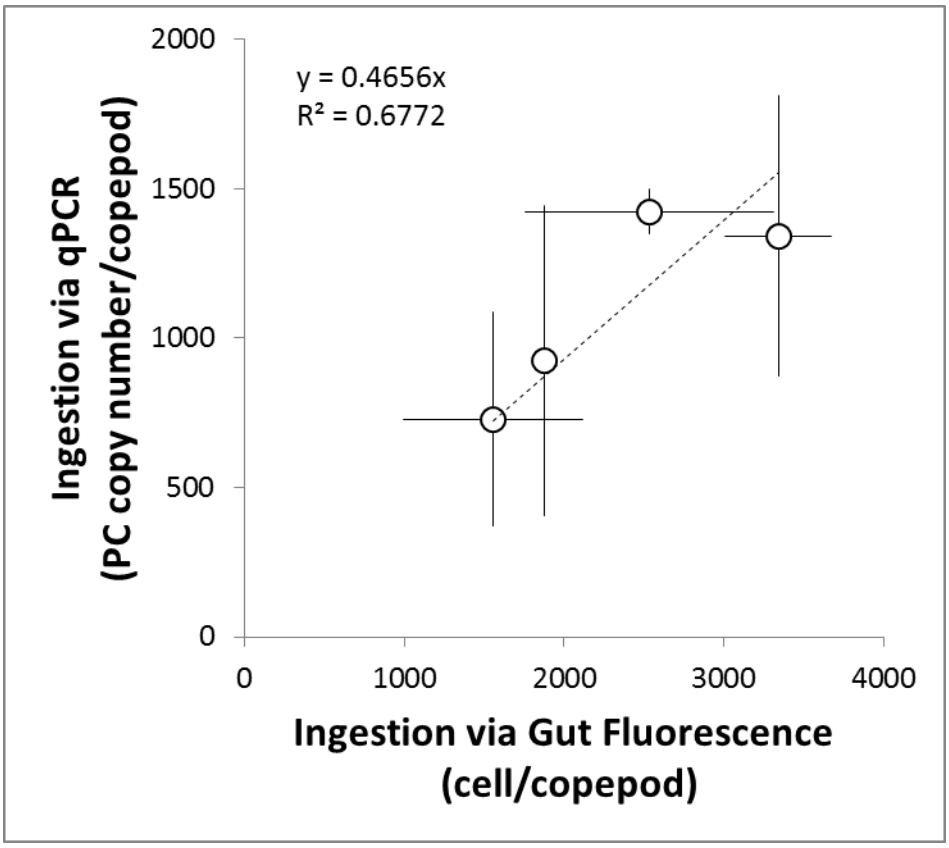
In previous works, the copepods of the SFE (*E. affinis* and *P. forbesi*, hereafter copepods) have ingested enough *Microcystis* cells to cause acute toxicity. While this confirmed the negative effects of *Microcystis* ingestion, these results were from highly controlled laboratory experiments that do not represent the ecological conditions of the SFE. Hence, the current project was designed to quantify *Microcystis* ingestion and its effects on copepods under the ecological conditions of the SFE.

Under this task R. duMais completed her Master's thesis and made significant progress toward a manuscript based on that thesis. The manuscript will describe the results of experimental work on reproductive rates of copepods as they are affected by *Microcystis*, and results of field work linking reproductive parameters to *Microcystis* abundance. These results have been described in previous reports. Work in this task continues with preparation of a manuscript based on R. duMais's thesis. Kimmerer is also serving as a graduate thesis committee member for Allison Johnson and has been working with Allison on statistical methods for analyzing her results and for understanding implications of zooplankton grazing in her experiments. Ger participated in the experimental design, thesis committee, and defense of DuMais.

Ger's experimental work has been completed, and is now in the data analysis and manuscript preparation phase. The manuscript will describe the use of the new qPCR method to evaluate the relationship between the gut contents and abundance of copepods during the Microcystis bloom. The goal was to develop a qPCR assay to quantify the ingestion of *Microcystis* by the copepod *Pseudodiaptomus* forbesi in the SFE and evaluate the relationship between copepod gut contents and copepod abundance in the estuary. Additionally, copepod gut fluorescence (GF), which is an effective indicator of how much phytoplankton is ingested by zooplankton, was measured in the laboratory and from SFE collected copepods during the two sampling seasons. The task has been completed successfully and the results, which have been detailed in the previous reports, are summarized below.

There was a linear relationship between *Microcystis* ingestion rates estimated from GF versus qPCR, which verified that the qPCR assay may be used to quantify the ingestion of Microcystis by copepods in the field (Fig. 5.1). Both methods worked well although the qPCR data indicated a faster degradation of Microcystis DNA than the chlorophyll. The copepods *Pseudodiaptomus* and *Eurytemora* both ingested *Microcystis* offered as the sole food source in controlled laboratory experiments, though the latter ingested about five times more *Microcystis* than the former. Both copepod species ingested similar amounts of the MC+ vs. the MC- strain, suggesting that microcystin had no effect as a grazer defense in this setup. The *Microcystis* strains used in this experiment contain various other metabolites toxic to zooplankton, including microviridin (Otten data), which could explain why copepods did not respond to microcystin.

Figure 5.1: Compares *Microcystis* ingestion [cells/copepod] by *Eurytemora affinis* estimated by gut fluorescence (GF) and the copy number of the PC gene amplified by qPCR from frozen copepod samples. The *Microcystis* specific PC gene occurs as a single copy in non- dividing cells, and thus, the PC copy number can be assumed to correspond directly to *Microcystis* cell numbers in copepod guts. Error bars indicate SD, n= 2-3. There were not enough replicates to compare the ingestion between GF and qPCR using frozen samples for the *mcy*E gene.



Analysis of the gut contents of copepods from the SFE confirmed that copepods ingested *Microcystis* during the bloom. Moreover, there was a positive relation between *Microcystis* abundance and the copy number of *Microcystis* genes in copepod guts (Figure 5. 2). The results also show that *Microcystis* abundance was unrelated to how much phytoplankton was ingested by copepods in the SFE. Finally, the abundance of adult copepods was negatively related to the copy number of *Microcystis* genes found in their guts, suggesting a potential negative effect in the SFE during bloom conditions (Figure 5. 3). Work on this task continues with analysis of results and manuscript preparation. The contribution of this project is two-fold. This is the first time that molecular tools have been used to quantify *Microcystis* ingestion by zooplankton. Moreover, we confirmed that copepods ingest *Microcystis* during bloom conditions in the SFE, with possible negative effects on copepod abundance depending on the intensity of the bloom.

Figure 5.2: The gut *Microcystis* content of adult *P. forbesi* increases in relation to the abundance of *Microcystis* in the SFE.

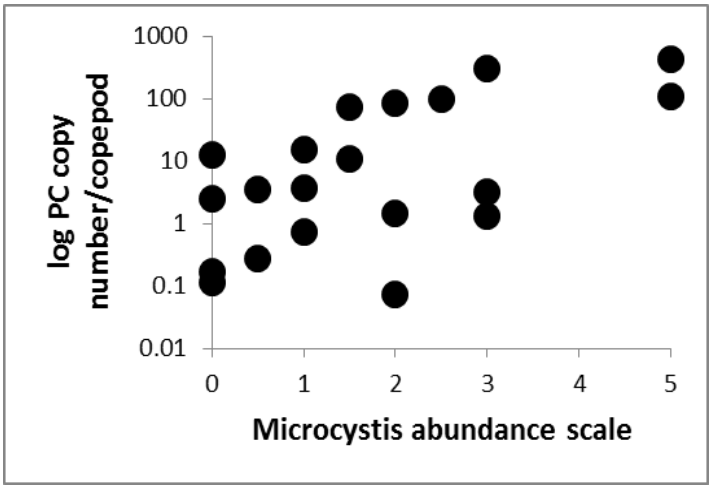
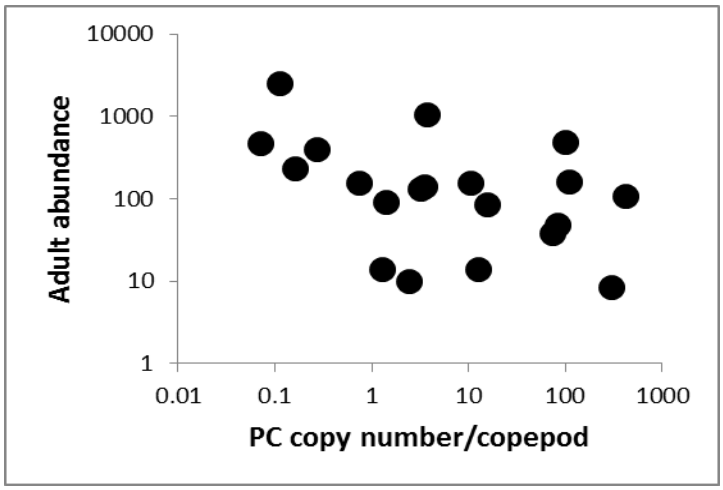


Figure 5.3: Shows the relationship between PC copy number in adult *Pseudodiaptomus forbesi* guts and the adult abundance of the same species across all sites.



Detailed findings have been reported before and are summarized here:

- Egg hatching success of the copepod *Pseudodiaptomus* forbesi was determined from field-collected samples and in the laboratory at various levels of *Microcystis* including both a microcystin-producing (MC+) and non-microcystin-producing (MC-) strain. Egg ratio (or clutch size) was also determined in the field, and female survival in the laboratory.
- Clutch size in field samples had a weak negative relationship to total chlorophyll. This is contrary to what we would expect if chlorophyll represented suitable food for the copepods. A positive relationship between total chlorophyll and chlorophyll in larger size fractions (i.e., >75 μm) indicates that much of the chlorophyll was in *Microcystis,* which may have depressed reproduction in the copepods.
- Egg-hatching success also had a negative relationship with chlorophyll. Both clutch size and egg-hatching success were positively related to temperature.
- Egg ratio (eggs per female) was also negatively related to chlorophyll, and to an index of *Microcystis* concentration based on the amount of *Microcystis* in net samples.
- In the laboratory, female survival and egg hatching success increased as the concentration of "good" food increased, saturating at around 500 μg C liter^{-1}. When the diets consisted of 50% *Microcystis* at a total phytoplankton concentration of 500 μg C liter^{-1}, egg-hatching success decreased in the MC+ treatment but not the MC- treatment. Female survival was lower at all levels in the MC+ treatment than the control. These results indicate that survival and egg-hatching success are reduced when *Microcystis* dominates (i.e., > 75%) the available phytoplankton,.
- The laboratory results contrast with those of Ger et al. (2010), who found impairment of copepods at much lower levels of *Microcystis.* We show that the differences are likely due to the media used. We initially used natural SFE water filtered at 35 μm, while Ger et al. used artificial media. We then conducted a follow-up experiment comparing survival and egg-hatching success in artificial media with that in natural SFE water filtered as above. With 10% *Microcystis* (MC+ or MC-) in 500 μg C liter^{-1} total phytoplankton, survival and egg-hatching success were at least 95% in the natural water, and 0 in the artificial media. Thus, a major, unexpectedfinding is the potential role of microbes in improving tolerance of copepods to *Microcystis*.
- Recovery and amplification of *Microcystis* genes from copepods differed between copepods that were frozen and those preserved in ethanol. Overall, the results suggest that frozen samples and the PC gene represented the most reliable method for the quantification of *Microcystis* cells in copepod guts using our protocol.
- *E. affinis* ingested about five times more *Microcystis* than *P. forbesi* in laboratory grazing experiments with pure *Microcystis* diets. Therefore E. affinis was used as the model copepod species for comparing the ingestion estimates between qPCR versus GF.
- The copy number of *Microcystis* genes underestimated ingestion relative to GF by a factor of 50%. However, the relationship between the two estimates was linear and indicated qPCR as a reliable method for quantifying *Microcystis* ingestion in the laboratory and field samples.
- Analysis of the gut contents of *Pseudodiaptomus* from the SFE confirmed that copepods ingested *Microcystis* during the bloom. Moreover, there was a positive relation between *Microcystis* abundance and the copy number of *Microcystis* genes in copepod guts.
- The abundance of adult *Pseudodiaptomus* was negatively related to the copy number of *Microcystis* genes found in their guts, suggesting a potential negative effect in the SFE during bloom conditions.

- The principal conclusion from these results is that *Microcystis* may cause some reduction in key population parameters (e.g., egg production rate and survival) of *Pseudodiaptomus* , but the effect is weak at current levels of *Microcystis*.
- Furthermore, conducting experiments in field-relevant conditions has led to
- Previous experiments (Ger et al. 2010) followed strict experimental design principles, under which all conditions are held constant except for those being investigated for their effect, in this case the proportion of *Microcystis* in the diets of copepods. However, in this study we violated those principles by varying the dietary proportions while allowing for unexamined variation among treatments due to the use of field-collected water with its microbial assemblage. This violation revealed a key, unexpected outcome which was that the microbial assemblage somehow altered the toxicity of *Microcystis* to the copepods. This outcome is an important topic for further investigation.

TASK DELIVERABLES

Presentations

duMais, R., Slaughter, A., Ignoffo, T., Kimmerer, W. (2013) Reproductive success of the calanoid copepod Pseudodiaptomus forbesi in the presence of sublethal levels of the toxic cyanobacteria Microcystis aeruginosa. ASLO Aquatic Sciences Meeting, New Orleans, LA. Also presented at Interagency Ecological Program Workshop in Folsom, CA.

duMais, R., Slaughter, A., Ignoffo, T., Kimmerer, W. (2013) Reproductive success of the calanoid copepod Pseudodiaptomus forbesi in the presence of sublethal levels of the toxic cyanobacteria Microcystis aeruginosa. Interagency Ecological Program Workshop in Folsom, CA.

duMais, R., Slaughter, A., Ignoffo, T., Kimmerer W. (2012). Reproductive Success of the Calanoid Copepod Pseudodiaptomus forbesi in the Presence of Sublethal Levels of Microcystis aeruginosa. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Papers in Review

Ger KA, Urrutia-Cordero P, Frost PC, Hansson L-A, Sarnelle O, Wilson AE, Lürling M. The interaction of cyanobacteria and zooplankton in a more eutrophic world. Harmful Algae (in revision)

Papers in preparation

Ger KA, duMais R, Otten T, Kimmerer W. The ingestion of *Microcystis* by copepods during bloom conditions in the San Francisco Estuary. In preparation.

duMais, R, KA Ger, and W Kimmerer. The effects of *Microcystis aeruginosa* on egg production and egg hatching success in the copepod *Pseudodiaptomus forbesi*. In preparation for *Harmful algae*.

List of Students (Graduate/ Undergraduate) Supported

Rita DuMais, SFSU MSc 2014.

Project Wide Deliverables

July 1, 2014 to February 1, 2015

Johnson, A. A. E. Parker, F.P. Wilkerson, T. Otten. 2015. Climate change effects on the San Francisco Estuary Delta: Phytoplankton community: the role of temperature and salinity tolerance on growth. ASLO Aquatic Science Conference, Granada, Spain February 2015.

Johnson, A., Parker, A. E., Wilkerson, F. P. 2014. The future of Microcystis spp. in the San Francisco Estuary Delta: Investigations into the role of temperature and salinity tolerance on growth. Biennial Bay Delta Science Conference, Sacramento, CA October 28-30.

Johnson, A., Parker. A.E., Wilkerson, F.P. 2014. Climate change effects on the San Francisco Estuary Delta phytoplankton community: investigations into the role of temperature and salinity tolerance on growth. CAERS, Bodega Marine Lab, CA. September 26-27.

Lee, J. 2014. Kinetics and inhibition of nitrogen uptake by Microcystis aeruginosa: results from culture and the San Francisco Delta. Master's Thesis defense, Tiburon, CA December 10, 2014

Lee, J., Parker, A.E., Wilkerson, F.P. 2014. Understanding nitrogen assimilation in Microcystis aeruginosa: implications for cyanobacterial bloom development in the San Francisco Delta. Bay-Delta Science Conference, Sacramento, CA. October 28-30.

Lee, J., Parker, A.E., Wilkerson, F.P. 2014. Investigating the nitrogen uptake in the cyanoHAB Microcystis aeruginosa: insight for bloom development in the San Francisco Delta. California Estuarine Research Society meeting. Bodega Marine Lab, CA. September 26-27.

Otten T.G, Paerl H.W. 2014. The distribution, ecology and genetics of Microcystis blooms throughout the SF Bay Delta. Bay Delta Science Conference, Sacramento, CA October 30, 2014.

Paerl, H.W. 2015. Controlling Harmful Cyanobacterial Blooms in a World Experiencing Human- and climatically-induced change. Plenary talk, University of Buffalo (SUNY) Dept. of Biology Annual Symposium, Buffalo, NY, Jan. 2015.

Paerl, H.W. 2015. Harmful algal blooms in a human and climatically impacted world: What's manageable and what's not? Invited talk, Grimes Symposium, Univ. of Southern Miss. Gulf Coast Research Lab. Ocean Springs, MS Jan., 2015.

Paerl, H.W., Gardner, W.S., McCarthy, M.J. Otten, T.G., Peierls, B.L., Rossignol, K.L. and Wilhelm, S.W. 2015. Nutrient controls of toxic cyanobacterial blooms in the context of global change: Moving beyond the "phosphorus only" paradigm. ASLO Aquatic Science Conference, Granada, Spain, February 2015.

February 2, 2014 to June 30, 2014

duMais, R., Slaughter, A., Ignoffo, T., Kimmerer, W. (2013) Reproductive success of the calanoid copepod Pseudodiaptomus forbesi in the presence of sublethal levels of the toxic cyanobacteria

Microcystis aeruginosa. ASLO Aquatic Sciences Meeting, New Orleans, LA. Also presented at Interagency Ecological Program Workshop in Folsom, CA.

Parker, A.E. 2014. Primary Production in the Delta: Nutrient Considerations to Guide Conservation. Delta Day of Science, Sacramento, CA May 16, 2014

Parker, A.E. 2014 Primary Production in the Delta: Nutrient Considerations to Guide Conservation: Lower Foodweb Dynamics in California's Bay-Delta Ecosystem: Current Understanding and Future Interactions with a Changing Landscape. The Delta Science Program and UC Davis Center for Aquatic Biology & Aquaculture. Davis, CA February 18, 2014

Mioni, C.E., Kudela, R.M., and J. Zehr. 2014. Understanding the environmental factors driving potentially harmful cyanobacteria growth and their toxins in Mt View Sanitary District marshes (McNabney, Moorhen marshes). Mt View Sanitary District (Grant#: 20130453), June 27, 2014.

Morrow, P.*, Kudela, R., Negrey, K., Zehr, J., Turbo-Turk, K., Davidson Chou, K., Corona, L., Detka, J., Los Huertos, M., and C.E. Mioni. 2014. What controls cyanobacteria blooms in Peyton Slough marshes? MBNMS: Annual Sanctuary Currents Symposium. Seaside, CA. April 26, 2014
July 1, 2013 to February 1, 2014

Johnson, A., Parker, A.E., Wilkerson, F. P. 2013. Climate change effects on the cyanobcterial blooms in the San Francisco Delta. Coastal and Estuarine Research Federation Meeting, San Diego, CA

Johnson, A., Parker, A.E., Wilkerson, F.P. 2013. Climate change effects on cyanobacteria blooms (Micorcystis aeruginosa) in the San Francisco Estuary Delta: evidence from experimental manipulations. State of the Estuary Conference, Oakland, CA

Lee, J., Parker, A.E., Wilkerons, F.P. 2013. Nitrogen uptake by the bloom-forming blue-green alga, Microcystis aeruginosa, in the San Francisoc Estuary Delta. State of the Estuary Conference, Oakland, CA

Lee, J. Parker, A.E., Wilkerson, F.P. 2013. Nitrogne uptake kinetics of Microcystis aueruginosa in the San Francisc Estuary Delta, CA. Coastal and Estuarine Research Federation Meeting, San Diego, CA

Kudela, R., Mioni, C., Peacock, M., Schraga, T. 2013. San Francisco Bay acts as a reservoir and mixing bowl for both marine and freshwater toxins. EPOC 2013 Annual Conference (Sept. 16-19, 2013 – Fallen Leaf Lake, CA).

Kurobe, Tomofumi, Baxa, V Dolores, Mioni, E Cécile, Kudela, M Raphael, Smythe, R Thomas, Waller Scott, Chapman, D Andrew, The, J Swee. 2013. Identification of harmful cyanobacteria in the Sacramento-San Joaquin Delta and Clear Lake, California by DNA barcoding. SpringerPlus, 2: 491 (http://www.springerplus.com/content/2/1/491)

Paerl, H.W. 2013. Invited Seminar: Managing Harmful Cyanobacterial Blooms in a World Experiencing Anthropogenic and Climatically-Indiced Change. Univ. of Mass, Amherst, Sept., 2013.

Paerl, H.W., 2013. Invited lecture: Rationale for dual nutrient control of cyanobacterial blooms: examples from hyoereutrophic systems wordwide. Nanjing Institute of Geography and Limnology, Taihu Lake Laboratory for Environmental Research, Wuxi, China. Oct. 2013

Paerl, H.W., , T. G. Otten, H. Xu, B. Qin, G. Zhu, N. S.Hall. 2013. Invited talk: Managing Harmful Cyanobacterial Bloooms Along the Freshwater-Marine Continuum in a World Experiencing Human and Climatically-Mediate Change. 7th US Harmful Algal Bloom Conference, Sarasota, FL, Oct. 2013.

Paerl, H.W. 2014. Invited Lecture: Algal Blooms and Red Tides: What's Manageable and What's Not? Moonlight in the Marsh Lectures, Kapnick Center, Florida Gulf Coast University, Naples, FL. January, 2014.

Parker, A.E. Dugdale, R.C. Wilkerson, F.P. 2014 Consideration of forms of DIN when evaluating the impact of nutrients in the Urban Ocean: Results from the Delaware Bay and the San Francisco Estuary. ASLO Ocean Science Meeting, Honolulu, HI

February 1, 2013 to June 30, 2013

duMais, R., Slaughter, A., Ignoffo, T., Kimmerer, W. (2013) Reproductive success of the calanoid copepod Pseudodiaptomus forbesi in the presence of sublethal levels of the toxic cyanobacteria Microcystis aeruginosa. Interagency Ecological Program Workshop in Folsom, CA.

Johnson, A., Parker, A.E., Wilkerson, F.P. (2013). Do increased temperature and salinity influence the success of cyanobacteria in the San Francisco Bay Delta? Interagency Ecological Program Workshop in Folsom, CA.

Lee, J., Parker, A.E., Wilkerson, F.P. (2013) Ammonium vs. Nitrate Uptake by the cyanoHAB Microcystis aeruginosa in the San Francisco Estuary Delta Presented at: Interagency Ecological Program Workshop in Folsom, CA.

July 1, 2012 to January 31, 2013

Baxa, D., Kurobe, T., Mioni, C.E. Lehman, P., Teh, S. (2012) Occurrence and abundance of other toxin-producing cyanobacteria in the San Francisco Bay-Delta. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

duMais, R., Slaughter, A., Ignoffo, T., Kimmerer W. (2012). Reproductive Success of the Calanoid Copepod Pseudodiaptomus forbesi in the Presence of Sublethal Levels of Microcystis aeruginosa. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Johnson, A., Parker. A.E., Wilkerson, F.P. 2012. Climate change effects on cyanobacteria blooms in the San Francisco Estuary Delta. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Kurobe, T., Baxa, D., Mioni, C.E., Kudela, R., Smythe, T., Waller, S., Teh, S. (2012) Monitoring toxin-producing cyanobacteria in Clear Lake and Sacramento-San Joaquin Delta by DNA barcoding and development of quantitative PCR assays. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Kudela, R., Mioni, C.E., Gibble, C., Hayashi, K., Worcester, K., Schraga, T. (2012) Cyanobacterial toxins, environmental drivers, and watershed connectivity: How serious is the threat? 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Lee, J., Parker, A.E., Wilkerson F.P. (2012). Nitrogen uptake kinetics of Microcystis aeruginosa in the San Francisco Estuary Delta. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Mioni, C.E., Baxa, D., Grimaldo, L., Kimmerer, W., Kudela, R., Kurobe, T., Lucas, L., Paerl, H., Parker, A.E., Paytan, A, Thompson, J., Waller, S., Wilkerson, F. (2012). Determining Environmental controls and ecological impacts of CyanoHABs in the San Joaquin-Sacramento Delta – A multidisciplinary approach) 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Otten, T.G., Paerl, H.W. (2012) DNA fingerprinting and quantitative assessments of toxigenic Microcystis assemblages and environmental drivers in the San Francisco Estuary Delta. 7th Biennial Bay-Delta Science Conference, Sacramento, CA.

Parker, A.E., Johnson, A., Lee, J., Mioni, C.E., Pimenta, A., Wilkerson, F.P. (2012). Carbon and nitrogen uptake rates associated with cyanobacterial blooms in the San Francisco Delta. 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

Parker, A.E., Johnson, A., Lee, J., Pimenta, A., Mioni, C., Wilkerson, F.P. (2012). Carbon and nitrogen uptake rates associated with cyanobacterial blooms in the San Francisco Estuary Delta. California Estuarine Research Society, Long Beach, CA.

Pimenta, A.R. Mioni, C.E., Parker, A.E., Wilkerson, F.P. (2012). Are nutrients a driver of cyanobacterial abundance in the SFE Delta? 7Th Biennial Bay-Delta Science Conference, Sacramento, CA.

February 1, 2012 to June 30, 2012

Mioni, C.E., Kudela, R., Baxa, D., Kimmerer, W., Kurobe, T., Paerl, H., Parker.A.E., Wilkerson, F. 2012 Determining Environmental controls and ecological impacts of CyanoHABs in the San Joaquin-Sacramento Delta – A multidisciplinary approach. Interagency Ecological Program Conference, Folsom, CA.

CA Sea Grant. 2012. What Controls Harmful Algal Blooms and Toxicity in the Sacramento-San Joaquin Delta? http://wwwcsgc.ucsd.edu/NEWSROOM/NEWSRELEASES/2012/AlgalBloomDeltaresearch.html

Mioni C. E., Baxa, D., Kudela, R., Kimmerer, W., Kurobe, T., Paerl, H., Parker, A.E. 2012. Determining Environmental controls and ecological impacts of CyanoHABs in the San Joaquin-Sacramento Delta – A multidisciplinary approach. American Fisheries Society Meeting, San Diego, CA.

DuBose, M., Johnson, A., Parker, A.E., Wilkerson, F.P. 2012. Temperature and light availability as drivers of cyanobacteria blooms in the San Francisco Estuary Delta: evidence from experimental enclosures. Interagency Ecological Program Conference, Folsom, CA.

July 1, 2011 to January 31, 2012

Parker, A.E., Dugdale, R.C., Ger, A., Kimmerer, W., Kudela, R., Mioni, C., Otten, T., Paerl, H., Wilkerson, F.P. (2011). The role of Microcystis blooms in the Sacramento-San Joaquin Delta foodweb: a functional approach. 10th Biennial State of the Estuary Conference, Oakland, CA

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