Harmful cyanobacteria blooms and their toxins in Clear Lake and the Sacramento-San Joaquin Delta (California)

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Surface Water Ambient Monitoring Program (SWAMP)

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Abstract

Harmful cyanobacteria and their toxins are growing contaminants of concern. Noxious toxins produced by HC, collectively referred as cyanotoxins, reduce the water quality and may impact the supply of clean water for drinking as well as the water quality which directly impacts the livelihood of other species including several endangered species. USEPA recently (May 29, 2008) made the decision to add microcystin toxins as an additional cause of impairment for the Klamath River, CA. However, harmful cyanobacteria are some of the less studied causes of impairment in California water bodies and their distribution, abundance and dynamics, as well as the conditions promoting their proliferation and toxin production are not well characterized.

The goal of the work presented here was to monitor the distribution of *Microcystis aeruginosa* as well as other harmful cyanobacteria of concern and their associated toxins in the surface waters of two California water bodies listed in the 303(d) and that have been impacted by recurrent harmful cyanobacterial blooms (cyanoHABs): the Sacramento-San Joaquin Delta and Clear Lake. We used a multidisciplinary approach combining traditional microscopy and toxicology analyses with molecular analyses. Using this approach, we successfully identified key cyanobacteria species that could not be identified using traditional microscopy-based techniques alone. This combined approach allowed us to accurately determine the cyanoHABs composition and toxicity as well as to gather critical information from the literature to interpret the correlations between individual cyanoHABs taxa and their environmental controls. Results from this study indicate that several environmental drivers rather than one single stressor act in conjunction to control cyanoHABs and toxicity in Clear Lake and the Delta. Surface water temperature, nitrogen and phosphorus concentrations appear to be key drivers of cyanoHABs composition and toxicity but additional environmental stressors specific to each system and to each individual taxa may also play a significant role. Based on our findings, suggestions for the implementation of future research programs as well as future management plans are also provided in this report.

List of abbreviations and acronyms

Abbreviation	Definition	Comments
ALG-sp.	*Algoriphagus* sp.	Either *Algoriphagus yeomjeoni* or *A. ratkowskyi*
ANA-LM	*Anabaena lemmermannii*	Taxonomic synonym of *Dolichospermum lemmermannii*
ANA-sp	*Anabaena* spp.	
APHA-FL	*Aphanizomenon flos-aquae*	
aPR	*Alpha proteobacterium*	Sequences of alpha proteobacterium in the data base are limited precluding identification at species level
BCL-PM	*Bacillus pumilus*	
CyanoHABS	Harmful Cyanobacterial Blooms	
DIN	Dissolved inorganic nitrogen	
DO	Dissolved oxygen	
DOC	Dissolved Organic Carbon	
DWR	Department of Water Resources	
EC	Electrical conductivity	
EMP	Environmental Monitoring Program	
FLV-TF	*Fluviicola taffensis*	
IEP	Interagency Ecological Program	
LYNG-HIE	*Lyngbya hieronymusii* f. *robusta*	*Planktothrix cryptovaginata* has identical sequences in GenBank database but microscopic observations validated *L. hieronymusii* as the taxonomic ID
LYNG-sp	*Lyngbya* spp.	
MSAE	*Microcystis aeruginosa*	
NH4	Ammonium	
NO3	Nitrate	
NTU	Nephelometric Turbidity Unit	
PNB-AL	*Paenibacillus alvei*	
PNB-sp.	*Paenibacillus* sp.	
PO4	Phosphate	
POD	Pelagic Organism Decline	
QA	Quality Assurance	
QAPP	Quality Assurance Project Plan	
QC	Quality Control	
RHD-SPH	*Rhodobacter sphaeroides*	
RHD-sp.	*Rhodobacter* sp.	
SOP	Standard Operating Procedures	
SYN	*Synechococcus* sp.	
WHO	World Health Organization	

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Introduction

Report purpose

The purpose of this report is to document the methods and summarize the results of the harmful cyanobacteria and toxicology study in Clear Lake, CA and the Sacramento-San Joaquin Delta, CA (also referred as the "Delta" thereafter in the text) from June to October of 2011. The report will describe the methods used for the field and laboratory analyses, as well as describe the performance of the field and analytical crews and evaluate the quality of the data set as defined in the Quality Assurance Project Plan (QAPP, Mioni 2011).

Study Goal and Objectives

The main objective of the work presented here was to monitor the distribution of *Microcystis aeruginosa* and other harmful cyanobacteria of concern (*Aphanizomenon spp., Anabaena spp., Gloeotrichia spp.. Lyngbya spp.*) and their toxins in the surface waters of two Californian water bodies listed in the 303(d) that have been affected by recurrent cyanoHABs: the Delta and Clear Lake. The work described therein builds upon previous work on cyanoHABs in these tow waterbodies (CDHS 1991, Richerson 1994; IEP-POD 2007; Lehman 2000; Lehman et al. 2005, 2008, 2010; Moisander et al. 2009; Spier et al. 2010; Winder et al. 2010; Mioni and Kudela 2011). We worked closely with preexisting monitoring programs such as the Department of Water Resources (DWR) Environmental Monitoring Program in the Delta (http://www.baydelta.water.ca.gov/emp). In Clear Lake, we collaborated with Lake County Department of Water Resources and the Department of Health Services.

The specific objectives of this study were to:

1) Monitor monthly discrete and continuous sampling stations located in critical habitats of the Sacramento-San Joaquin Delta and in each arm of Clear Lake in order to:
    a. Perform an assessment of the toxicity of the cyanobacteria growing in Clear Lake and the Sacramento-San Joaquin Delta.
    b. Identify and enumerate harmful cyanobacteria in these two systems using traditional microscopy and molecular methods.
2) Provide a better understanding of the mechanisms underlying the source, occurrence and toxicity levels of harmful cyanobacteria in these systems,
3) Investigate possible algae-related symptoms by Lake County residents, domestic animals and wildlife (Dr. Tait, Lake County Department of Health).
4) Serve as a source of information that will direct and promote actions to improve water quality and enhance other monitoring programs.

Background

Over the past decades, freshwater and brackish harmful (toxic and food-web disrupting) cyanobacteria blooms (CyanoHABs) have increased in extent and frequency throughout the world (Paerl 1988, Carmichael 2001, Smetacek and Cloern 2008, Paerl and Huisman 2008, 2009). The potential adverse impacts of CyanoHABs are large and negatively affect domestic, industrial and recreational uses of large freshwater ecosystems such as Clear Lake, CA and the Sacramento-San Joaquin Delta, CA. Harmful cyanobacteria affect both water quality (e.g. dissolved oxygen sags, taste and odor problems in drinking water, toxins) and the efficiency of water diversion and treatment operations (e.g. clogging filters in water treatment plants, fish screens or channels). Noxious toxins produced by harmful cyanobacteria, collectively referred as cyanotoxins (Table 1), reduce the water quality and may impact the supply of clean water for drinking as well as the livelihood of wildlife, including endangered species (Carmichael 2001, EPIRAB-OEHHA-CAEPA 2009, Lehman et al. 2010, Miller et al. 2010). The current global water crisis infers that it is crucial to gain a better understanding on the factors affecting water quality that can impact current and future management options and develop sustainable solutions to minimize impacts to drinking water and ecosystem health. The Southwestern United States ranks among the most vulnerable regions to the current global water crisis (Barnett et al. 2008). Indeed, models predict that water supply shortage will increase due to global warming and to overpopulation, while the contamination of water supplies is rising due to anthropogenic pollution and to the emergence of harmful and noxious toxin-producing algae blooms. A central question faced by environmental researchers and managers is "*what environmental drivers control CyanoHABs occurrence and toxigenicity in impacted systems*"?. The answer to this question is of primordial ecological importance as it dictates the strategies and costs involved in mitigating serious water quality problems associated with toxic blooms (Paerl et al. 2011).

The literature suggests that the success of cyanobacteria is a result of complex and synergistic environmental factors rather than a single dominant variable. More than a dozen hypotheses have been suggested and are summarized in Table 2 (reviewed for example by Dokulil and Teubner 2000, Paerl 2008). However, many of these factors are influenced directly and indirectly by temperature (Paerl and Huisman 2008, 2009; Wagner and Adrian 2009). Rising temperatures would give cyanobacteria a direct competitive advantage as they generally grow better at higher temperature than do other phytoplankton taxa such as diatoms and green algae (Paerl 1988, Paerl and Huisman 2008, 2009). The indirect positive effects of temperature are manifold. Many cyanobacteria, including toxigenic species such as *Microcystis aeruginosa*, produce intracellular gas vesicles that make cells buoyant. Buoyant cyanobacterial species exploit vertical stratification that coincides with warming of surface waters with high atmospheric temperatures (Paerl 1988). These conditions are likely to become more prolonged and more intense under future climate scenarios, which would lengthen optimal growth periods (Paerl and Huisman 2008, Wagner and Adrian 2009). During stable thermal conditions, buoyant cyanobacteria can accumulate at the surface, forming a dense surface scum that shades underlying non-buoyant phytoplankton. Such scum- or mat- forming blooms usually results in a decrease in the diversity of the phytoplankton assemblage through light competition, ultimately leading to the dominance of cyanobacteria (Paerl 1988, Paerl and Huisman 2008). Warmer temperatures also stimulate nutrient release at the sediment-water interface as well as the

mineralization of organic matter which contributes to the accumulation of dissolved nutrients under the surface.

Nitrogen (N) and phosphorus (P) are the key nutrients of concerns related to cyanoHABs (Dokulil and Teubner 2000, Ferber et al. 2004, Paerl 2008, Paerl 2009, Paerl et al. 2011). Some cyanobacteria gain dominance by fixing atmospheric nitrogen (N_2) when the dissolved inorganic N (DIN) is depleted in the water column and eukaryotic algae (which cannot fix N_2) are severely N-limited (Schindler 1977). Because the atmospheric supply of N_2 is vast, substrate limitation of nitrogen fixation is not expected. Low DIN and excess P loads in the water column can therefore alter the phytoplankton assemblage by favoring nitrogen-fixing cyanobacteria over eukaryotic algae and non-nitrogen fixing cyanobacteria. In P-rich, DIN-depleted waters (low N:P ratios), diazotrophic (nitrogen-fixing) cyanobacteria (e.g. *Aphanizomenon* and *Anabaena*) may gain a competitive advantage over non-diazotrophic (non nitrogen-fixing) cyanobacteria (e.g. *Microcystis*, *Lyngbya*, *Oscillatoria;*Paerl 2009). However, the presence of diazotrophic cyanobacteria does not always indicate that N_2 fixation is a significant N source; regenerated DIN can be a key N source for sustaining blooms, even among diazotrophs (Ferber et al. 2004, Paerl et al. 2011). Non-diazotrophic cyanobacteria are also effective competitors for reduced N forms, especially ammonium (Blomqvist et al. 1994), which can be quickly regenerated in the water column and sediments (Ferber et al. 2004, Paerl et al. 2011). Recent research also suggest that dissolved organic nitrogen (DON) such as urea favors cyanobacterial species over other algal groups (Glibert et al. 2004). When a benthic ammonium source is available, buoyant, non-diazotrophic cyanobacteria, such as *Microcystis* and *Lyngbya*, can migrate vertically with low energy expenditure which allows these species to have access to nutrient pools at depth (both N and P) that are otherwise low or depleted in the surface (Paerl 1988). Furthermore, many diazotrophic cyanobacteria are also vacuolated and colonial and may be as likely to migrate as non-diazotrophic cyanobacteria, and thus may dominate both when there is a benthic N source and when fixation is required to obtain adequate N supply and vertical migration is necessary to compensate limiting nutrient supplies in surface (Ferber et al. 2004, Paerl et al. 2011). Additionally, other factors than nutrient availability and N:P ratios control the composition and abundance of cyanobacterial assemblage. For example, diazotrophic cyanobacteria have higher requirement in iron than non-diazotrophs because this trace metal serves as a cofactor to the enzymes involved in N_2 fixation (Wursbaugh and Horne 1983). Hence, nutrient reduction strategies to mitigate cyanoHABs appear system-specific.

The conditions that favor cyanotoxin production are not well understood. Many cyanobacteria species produce a group of toxins known as microcystins that may cause serious damage to the liver. Microcystins are the most numerous of the cyanotoxins, comprising at least 89 analogs (Welker and Von Döhren 2006). They are all synthesized non-ribosomally by the same enzymatic machinery. This large enzymatic complex can incorporate a variety of different amino acids in the microcystin molecule, thus producing variants with different carbon-nutrient stoichiometry and toxicity (Table 3, Van de Waal et al. 2009). The species most commonly associated with microcystin production is *M. aeruginosa* (WHO 1999, EPIRAB-OEHHA-CAEPA 2009). Culture experiments suggest that microcystin production is the highest at intermediate water temperature and light intensity (van der Westhuisen and Eloff 1985). In the field, the highest toxicity occurs at low water temperature, water transparency and salinity (Lehman et al 2005). The production of microcystin variants differs between strains and also

depends on the intracellular availability of different amino acids that can occupy the variable positions (Tonk et al. 2008). Some common variants of microcystin include microcystin-LR, microcystin-RR, microcystin-LA and microcystin-YR, all of which have been detected in the surface waters of the Sacramento-San Joaquin Delta (Lehman et al. 2008). The amount of nitrogen in these amino acids differs and thus, the molar N:C ratio between analogs varies (Van de Waal et al. 2009). Recent lab experiments showed that an increase in nitrogen availability can shift microcystin composition toward nitrogen-richest variant and that toxin composition is especially affected by rising CO_2 concentrations in nitrogen rich waters (Welker and Von Döhren 2006, Van de Waal et al. 2009), which may present a likely future scenario in California water reservoirs. This would have important implications considering that toxicity varies between microcystin variants (Table 3, Rinehart et al. 1994). Microcystins are extremely stable and resist common chemical breakdown such as hydrolysis or oxidation under conditions found in most natural water bodies. These toxins break down slowly even at high temperature (40°C or 104°F) and at extremely acidic (<1) or alkaline (>9) pH (EPIRAB-OEHHA-CAEPA 2009). The half-life (the time it takes for one-half of the toxin to degrade) at pH 1 and 40°C is 3 weeks; at typical ambient conditions half-life is 10 weeks. Sunlight also breaks down microcystins by photoxidation (although slowly, from 2 to 6 weeks for 90% breakdown) especially when water-soluble pigments are present (Sinoven and Jones 1999, EPIRAB-OEHHA-CAEPA 2009). Although microcystins can be broken down by some bacterial proteases, in many circumstances these bacteria are not present so the toxin persists for months or even years once released into cooler, dark, natural water bodies (EPIRAB-OEHHA-CAEPA 2009).

To test hypotheses that could explain cyanobacterial abundance in these systems, we monitored monthly harmful cyanobacteria abundance (cell or filament counts) and toxin (microcystins, anatoxin-a, cylindrospermopsin, lyngbyatoxin-a, saxitoxin) concentrations as well as other environmental variables (temperature, electrical conductivity, pH, chlorophyll *a* (chl-a), dissolved oxygen, dissolved inorganic nutrients, dissolved organic carbon) at four discrete stations in the Sacramento-San Joaquin Delta (Figure 1) and seven discrete stations in Clear Lake (Figure 2) from June, 2011 to October, 2011. The study period was centered during the cyanoHAB season (CDHS 1991, Richerson 1994; IEP-POD 2007; Lehman 2000; Lehman et al. 2005, 2008, 2010; Moisander et al. 2009; Spier et al. 2010; Winder et al. 2010; Mioni and Kudela 2011). Because toxins concentrations vary greatly on a spatiotemporal scale, we also used the SPATT (Solid Phase Adsorption Toxins Tracking) methodology which is a new technology designed for continuous toxin tracking by passively absorbing dissolved toxins from the water column (Miller et al. 2010, Kudela 2011). SPATT devices were deployed each month for continuous monitoring between monthly sampling events at three coastal stations in Clear Lake and at four of the CA-DWR Environmental Monitoring Program (EMP) real-time continuous monitoring stations[1] located in the Delta. All the ancillary data collected by the EMP/DWR group at these real-time monitoring stations were available to this project at no extra cost.

[1] Information regarding these EMP/DWR continuous stations are available at the following website: http://www.water.ca.gov/bdma/meta/continuous.cfm

Description of Study Areas

Sacramento-San Joaquin Delta –

The relative abundance of different phytoplankton groups has changed since the start of time series monitoring in 1970's. Total cyanobacteria and dinoflagellate biomass have increased between 1975 and 1993 throughout the Sacramento-San Joaquin Delta coincident with a decline in diatom biomass (Lehman 2000). *Microcystis* single-celled form is currently a common cyanobacterium in the Delta but was not identified as a dominant genus in the phytoplankton community between 1975 and 1982 (Lehman and Smith 1991). The colonial form of *M. aeruginosa* is also the first known introduced phytoplankton species with adverse impact to the estuary (Lehman et al. 2005). Blooms of the hepatotoxin-producing cyanobacterium *M. aeruginosa* have been recorded in the Sacramento-San Joaquin Delta since 1999 (Lehman 2000, Lehman et al. 2005, 2008, 2010, Spier et al. 2010). Since 1999 the distribution, biomass and toxicity of this toxic cyanobacterium have been documented and indicate that the colonial form of *M. aeruginosa* is present throughout 180 km of waterways from freshwater to brackish water environments (IEP-POD 2007) and might have affected indigenous invertebrates and fishes (IEP-POD 2007, Lehman et al. 2010). The presence of other potentially harmful cyanobacteria has been documented. The potentially harmful genera *Aphanizomenon, Anabaena* and *Oscillatoria* are also frequently observed in the Sacramento-San Joaquin Delta although to a lesser extent than *Microcystis* (Cloern and Dufford 2005, Lehman et al. 2010, Spier et al. 2010). The toxin-producing cyanobacterium *Cylindrospermopsis raciborskii* has been observed also recently in the Delta (Mueller-Solger, *pers. com.*). This cyanobacterium was originally thought to be a tropical or subtropical taxa but has been recorded as rapidly expanding in some temperate regions and is regarded as an invasive species (Briand et al. 2004, Paerl and Huisman 2008). It is adapted to the low-light conditions that characterize eutrophic waters, prefers water temperatures above 20°C, and survives adverse conditions through the use of specialized resting cells (Paerl and Huisman 2008). It is thought that increased occurrence, rather than being just a recent invasion, is a combination of several factors such as improved water quality monitoring, availability of suitable habitat through climate warming and eutrophication (Paerl and Huisman 2008, Wagner and Adrian 2009).

The potential adverse impact of harmful cyanobacteria on the Delta is significant. Water from the Sacramento-San Joaquin Delta is used directly for drinking water and irrigation and the region is an important recreational area for sport fishing and water contact sports. Approximately two-thirds of all Californians – *ca*. 23 million people - obtain at least some of their water from the Delta. The estuary is habitat for many anadromous, commercial and recreational fish including striped bass (*Morone saxatilis*) and Chinook salmon and is a feeding ground for marine mammals (harbor seals). The estuary also contains many threatened or endangered aquatic organisms and many of these species are declining (Bennett and Moyle 1996, CABDA 2000). The coincident appearance of *Microcystis* and the decline of various pelagic organisms including the delta smelt (*Hypomesus transpacificus*) striped bass and threadfin shad (*Doromosa petenense*) and their copepod preys (*Eurytemora affinis* and *Pseudiaptomus forbesii*) in the freshwater sections of the Delta suggest that the presence of *Microcystis* in the Sacramento-San Joaquin Delta may be one of many factors responsible for the fishery decline since 2000 (Lehman et al. 2010, IEP-POD 2007). The increase of cyanobacteria in the Sacramento-San Joaquin Delta since 1980's coincide with several changes that might favor their growth:

increased water transparency (secchi depth) in the Delta, increased water temperature, increased specific conductance, residence time, wind velocity changes and rainfall changes (Lehman and Smith 1991, Lehman 2000, IEP-POD 2007, Lehman et al. 2008). Some of these environmental changes (water temperature, specific conductance, water transparency) appear to correlate also with the decline of pelagic fish species in the Sacramento-San Joaquin Delta (IEP-POD 2007). Some of these declines may be linked to the quantity and quality of the phytoplankton carbon available at the base of the food web (Ger et al. 2010). Indeed, *M. aeruginosa* blooms can reduce the growth of other phytoplankton impacting food quality and availability (Lehman et al. 2005, 2008). Previous research also confirmed the presence of microcystins in phytoplankton, zooplankton and fish in the Sacramento-San Joaquin delta (Lehman et al. 2010). Interestingly, histopathology of fish liver tissue suggested that tumor promoting toxins produced by *Microcystis* was impacting the health of two common fish in the SFE: striped bass and Mississipi silversides (*Menidia audens*) (Lehman et al. 2010).

The literature suggests that rising temperatures would give cyanobacteria a direct competitive advantage as they generally grow better at higher temperature than do other phytoplankton species such as diatoms and green algae (Paerl and Huisman 2008, Wagner and Adrian 2009). Over the 100-years simulations ran as part of the CASCaDE (Computational Assessments of Scenarios of Change for the Delta Ecosystem) effort, all scenarios showed warming of 3-5°C during the summer months (*i.e. Microcystis* bloom season) in the San Francisco Estuary, with slightly less warming during the winter (James Cloern, *pers. com.*). Preliminary data collected during the bloom of summer 2009 suggest that water temperature is directly correlated with *Microcystis* cells abundance and toxicity in the San Joaquin Delta (Mioni et al. *in prep.*). Our preliminary data further suggest that increased temperature correlated with higher concentrations of the most toxic microcystin variants (MC-LR and MC-LA). Furthermore, summer droughts, rising sea levels, increased withdrawal of underground freshwater to meet the increasing demand of water supply for agricultural, domestic and industrial use are predicted to lead to rising conductivity/salinity in the Delta (Jim Cloern, *pers. com*). Several common cyanobacteria are more halotolerant than freshwater phytoplankton species as suggested by the increasing reports of toxic cyanobacterial blooms in brackish waters (Paerl and Huisman 2008). Strain-specific variability exists in salinity tolerance between *Microcystis* ecotypes. Several *Microcystis* morphological types (Spier et al. 2010) and phylogenetic types (Moisander et al. 2009) have been observed in the Sacramento-San Joaquin Delta. Previous studies in the San Francisco estuary suggest that the presence of *Microcystis* cells at a fairly broad range of tolerance in salinity (0.1 to 18 ppt, Lehman 2000, Lehman et al. 2005, 2008, IEP-POD 2007) might reflect the distribution of different *Microcystis* strains, as some of these strains could be more halotolerant than the others (Moisander et al. 2009).

Clear Lake –

Clear Lake is the largest natural lake in California, located in Central Coast range approximately 80 miles north of San Francisco. It is a shallow, warm, nutrient-rich system with three distinct arms, which behave like separate lakes in some respect (Figure 2, Richerson et al. 1994). The maximum depth in the main basin (Upper Arm) is ca. 12 m, and in the other basin ca. 18 m, depending on the water level (Richerson et al. 1994). Due to its relatively modest depth

and the influence of strong west/northwest winds, Clear Lake is a polymictic lake which can episodically stratify during the day in summer due to the solar heating of the top few meters of the water column (Richerson et al. 1994). The diatom sequence from sediment cores indicates that Clear Lake has been a shallow, productive system, essentially similar to the modern lake, since the end of the Pleistocene (ice age) epoch (Bradburry 1988).

Clear Lake has been severely impacted by human activities. Since 1914, water levels have been regulated by Cache Creek dam and development in the watershed, especially since 1930, has led to the eutrophication of the lake (Suchanek et al. 2008). Furthermore, due to nearly a century (1873-1957) of mining along the shoreline of Clear Lake and subsequent erosion, mercury concentrations in sediment and water are some of the highest of any lentic system reported to date (Suchanek et al. 2008). These mining activities may have also affected the iron cycle and the internal phosphorus loading in the lake (Richerson et al. 1994). Previous studies indicate that the fine sediments entering the lake during winter runoff is the predominant source of phosphorus and trace metals (Richerson et al. 1994).

Clear Lake has been subject to recurrent seasonal blooms of noxious scum-forming cyanobacteria, which greatly impair water quality and recreational activities thereby causing substantial economic losses (Richerson et al. 1994, Winder et al. 2010, Mioni et al. 2011, 2012). These blooms typically occur in Clear Lake from April to November (CDHS 1991, Richerson 1994). The potential adverse effects of these cyanoHABs in Clear Lake are significant. Clear Lake serves as a drinking water reservoir for the communities living around the lake and supports diverse recreational activities (e.g. boating during summer and sport fishing all year-round). Furthermore, the Wetland regions of the lake are a source of fish, tules (plant family Cyperaceae, native to freshwater marshes of North America) and other resources used by Pomo Indian Tribes (Suchanek et al. 2008). Clear Lake has also a considerable value a wildlife habitat, as it is heavily used by fish-eating birds and mammals as well as overwintering waterfowl (Elbert 1993). Clear Lake has been on the 303d list of impaired water bodies in 1986, with a TMDL (total maximum daily loads) to reduce nutrients adopted by the State in 2006. This TMDL was based on reducing phosphorus loading to Clear Lake. However, despite the efforts to reduce phosphorus loading and accumulation in the lake sediments, cyanoHABs increased significantly in 2009-2011 (Mioni and Kudela 2011).

The first description of algal blooms in Clear Lake was detailed by Livingston Stone in 1873 in a report of operation to the commissioner of fish and fisheries (Livingston 1873). It has been suggested that the blooms have become more of a problem in the second half of the 20th century (Richerson 1994, Winder et al. 2010). Historical records suggest that the cyanobacterial assemblage reached its highest densities from the mid 1970's to 1990 (Horne 1975, Richerson 1994, Winder et al. 2010). During this period, the cyanobacterial assemblage was dominated by diazotrophic (aka. nitrogen-fixing) cyanobacteria, such as *Anabaena* and *Aphanizomenon,* as well as the non-nitrogen fixing cyanobacterium *Microcystis spp.* (Horne 1975, CDHS 1991, Richerson 1994). One main assumption for nitrogen-fixing cyanobacteria dominance was that *Aphanizomenon* and *Anabaena* had a competitive advantage in the phosphorus rich and nitrogen depleted waters of Clear lake because of their abilities to fix nitrogen from the atmosphere (Richerson et al. 1994, Tetratech 2004, Winder 2010). *Aphanizomenon, Anabaena* and *Microcystis* are all toxin producers (Table 1). Fish kills and neurological disorders in domestic

cats were reported in the Clear Lake area in 1989 following an exceptionally dense cyanobacterial bloom (*Microcystis, Anabaena and Aphanizomenon*) observed during summer 1990 (CDHS 1991, Richerson 1994). These events prompted a special toxicological and epidemiological studies program by the California Department of Health services (CDHS 1991). Very low levels of microcystin toxins were detected over the course of this special study (CDHS 1991).

Monitoring of the water quality by Caliornia Department of Water Resources (DWR) indicates that Clear Lake's ecology underwent significant changes which might have contributed in changes in the cyanobacteria assemblage composition since the 1990's. Water clarity increased to all arms since 1991 (Tetra Tech 2004). Submerged aquatic macrophytes, which require relatively transparent water to thrive, had been absent in most years from the late 1930's to the 1990's but have become more abundant since the mid-1990's (Winder et al. 2010). The nutrient chemistry in Clear Lake has also substantially changed over the past decades. Although phosphorus did not change significantly relative to levels measured in the 1970's and 1980's (Winder et al. 2010), changes in nitrogen chemistry were observed over the past two decades: (1) ammonia and total nitrogen have remained relatively constant while nitrate+nitrite decreased since 1970's (Winder et al. 2010) suggesting a possible switch from "new" (external inputs of nitrogen such as nitrogen fixation or groundwater inflow) to a "regenerated" (due to zooplankton excretion and microbial mineralization) production; and (2) although nitrate + nitrite were the dominant nitrogen sources (ca. 90%) in the 1970's (Winder et al. 2010), ammonia is now the dominant nitrogen sources while nitrate+nitrate are now below the detection limits or close to the detection limit levels. These changes in N speciation and water clarity correlate with changes of cyanobacteria assemblage composition, such as the decline of the diazotrophic cyanobacterium *Aphanizomenon* (Winder et al. 2010). Other factors such as zooplankton grazing may have also contributed to the changes in cyanobacterial assemblage composition (Winder et al. 2010). Since 2009, the predominant bloom-forming cyanobacteria are characterized by diazotrophs (*Lyngbya* in July/August) and non-diazotrophs (*Microcystis* in September/October). *Lyngbya* had been present in Clear Lake in the past but was not a nuisance prior to 2009. It has been suggested that the increase in clarity since the 1990's may have led to the predominance of *Lyngbya* since 2009. Efforts aiming to reduce phosphorus alone did not appear to reach Clear Lake water quality objectives with regard to cyanoHABs. The fact that both diazotroph and non-diazotroph cyanoHABs taxa are now coexisting in eutrophic Clear Lake is also raising questions as to whether P, N, or both N and P inputs should be controlled to mitigate cyanoHABs.

Materials and Methods

Quality Assurance and Quality Control

The data presented in this report were handled and evaluated following the Quality Assurance/Quality Control (QA/QC) requirements as outlined in our QAPP. The QAPP provided both a QA process and QC requirements for production of accurate and precise water quality analyses from the field and laboratory in support of the project objectives. These requirements included established procedures for data collection and processing by the field crew and the laboratory analysts, oversight by the QA/QC manager, and review by data analysts.

All field work and laboratory analyses were carried out according to the Standard Operating Procedures (SOPs) included in the QAPP or according to manufacturer's instructions when kits were used. The SOPs insured consistency in the analytical procedures, data reporting and QA/QC requirements. The SOPs were kept in the analytical laboratories.

Data produced by analysts were recorded electronically and in a laboratory notebook. Electronic spreadsheets were used for entering data and compute results for the environmental samples and standards. Data quality was completed to confirm that all the standards and quality control samples met the QA/QC requirements. Computations were verified and corrected when required. If the QA/QC requirements were not met, the problem was identified and the samples were then re-analyzed after remediation of the problem with respect to the QAPP procedures. Data that passed the QC guidelines were then entered into the master spreadsheet.

Data in the master spreadsheet were subject to further review by identifying outliers or by comparing the data to those obtained by companion programs (e.g. all nutrients data were compared to the nutrients data collected by the DWR/EMP program, toxins samples were sent to independent labs for validation, the microscopy counts were compared to the molecular –DNA- results). This procedure was used to check data entry or calculation errors. If problems were discovered during this process, the analyst was asked to recheck the data entry and quality of the sample analysis and, if necessary rerun the sample analysis.

Quality control procedures for each laboratory analysis and field sampling events included calibration of instruments with certified standards according to the QAPP. The Measurement quality objectives and specifications for laboratory measurements are summarized in the QAPP Table 7-3. Quality control standards were run in parallel to the environmental samples, and depending on the analysis, could include all or some of the following: calibration check standards, laboratory control samples, sampling and analytical duplicates, matrix spikes, field/travel blanks and analytical blanks.

Summary of QC samples

Measurements of QC samples were used to evaluate the performance of the field crew and analytical labs. The summary of the QC samples run in conjunction with the environmental samples does address the actual values or trends in the samples collected. The QC data collected addressed the precision, accuracy and the overall confidence in the produced data set.

Pass rates for the QC samples of each individual analysis are presented in Table 5. The present study has an overall QC sample pass rate of 99.63%. This included all the required QC samples: calibration checks, analytical and field duplicates, matrix spikes, and blanks ran in conjunction with the environmental samples.

Pass rates for the field QC samples are included in Table 6. The overall pass rate for the field was 95.41%. Due to extreme field conditions (exceptionally strong winds) in Clear Lake at the beginning of our study, several SPATT devices were lost or damaged and therefore could not be processed. Despite repeated redeployments and our attempts to use additional precautions to secure these devices to the buoys, the strong wind conditions resulted in either the total loss of the device or in damaging the device (e.g. SPATT were recovered but not enough resin was recovered from the device to perform the toxicology analysis). The Lake County YSI used to measure dissolved oxygen generated suspicious readings (abnormally low) in August/September. Multiple field calibration attempts and replacement of the piece suspected defective did not resolve the issues. As such, the data were not included in the master spreadsheet.

Sampling and Field Water Quality measurements

Field sampling at discrete stations consisted of collecting grab surface water samples, measuring water quality variables with a sonde, and recording field conditions at sites within the study areas (Figure 1, Figure 2, Table 4). Surface water samples and SPATTs were collected in the Sacramento-San Joaquin Delta with the assistance of the Environmental Monitoring Program of the California Department of Water Resource (Field officer: Scott Waller, field assistance: Brianne Noble) on board of the *R/V San Carlos*. Surface water samples were collected in Clear Lake with the assistance of the Lake County Water Resources Department (Field officer: Tom Smythe, field assistance on Sept. 8, 2011: Christ White) on board of the Lake County boat.

Sample collection was carried out according to our QAPP and following the protocols outlined in "SWAMP Bioassessment procedure 2009 - Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California" (June 2009) and in SWAMP Quality Assurance Program Plan (November 19, 2008). Field equipment was calibrated by field officers prior to sampling and trip blanks were collected parallel to the environmental samples for each field trip. Field data and additional relevant observations (e.g. odor, bloom conditions) were recorded into the field sheet the day of sampling. When relevant, field pictures were taken in support of our field observations and saved electronically on the P.I. computer. Toxin grab samples, SPATTs, nutrients, chl-a and water samples for molecular (DNA) analysis as well as associated field blanks were stored on dry ice and in the dark upon collection and transported in this condition (dry ice/dark) to the lab for analysis the same day. These samples were stored in a freezer (-20°C) until processing. Samples for dissolved organic carbon (DOC) and for taxonomy (microscopy) as well as their associated field blanks were stored in an ice cooler and in the dark and transported to the lab within the same day. They were stored in a cold room until processing.

The YSI sondes were calibrated and tested each day prior to start of field measurements according to manufacturer's instructions by the field officers. The summary of the QC samples for field analysis are presented in Table 6. Each of the probes was calibrated independently. The

field parameters measured by the sonde at each site included date and time, field crew, GPS coordinates, field conditions (e.g. weather, bloom observation), temperature (°C), specific conductance (mS/cm), secchi depth (cm), dissolved oxygen (mg/L and % saturation), depth (m), pH, turbidity (NTU, Delta only), and fluorescence (RFU, Delta only). All these field data were recorded on the appropriate field sheet the day of collection.

Field equipment, instruments, and sampling materials were cleaned prior to the field trip according to the procedures listed in the QAPP. Bottles and sample storage containers were labeled with the appropriate sample number, site name and code, sampling date, sampling crew names. Sample collection time was noted on the container at the time of collection. When filtration was required (e.g. molecular samples, and chl-a), the volume of filtered water was recorded on the appropriate field sheet at the time of collection.

While the field officer operated the instruments to log the water quality data, surface water samples were collected according to the procedures listed in the QAPP. Grab water samples were collected in surface using a bucket and processed immediately on site in accordance with requirements for different lab analysis and volume requirements. Samples that have the highest sensitivity to sunlight (toxins, chl-a, DOC) were processed first and in the shade when possible. All containers were rinsed with sample water prior to collection of the sample. Cyanotoxin samples (*ca*. 60 mL) were collected in certified pre-cleaned wide mouth amber jars with Teflon liner (Environmental Sampling Supplies, cat #: 0125-0655-QC). Chl-a samples were filtered immediately at the sampling site using a clean filtration apparatus operated with a hand pump and collected on GF/F filters (Whatman, Fisher cat#: 1825 047). The GF/F filters were stored in sterile plastic tubes (50 mL, BD falcon, Fisher cat# 352098). All the Chl-a samples were collected in duplicates. DOC samples were filtered using a clean 60-mL syringe and a sterile PES 0.2-µm syringe filter (Nalgene, Fisher cat #194-2520). To minimize DOC contamination from the filtering device, a flow through volume of ca. 50 mL was used prior to start collection. DOC samples were stored in acid washed and combusted (450°C for at least 4 hours) glass vials with Teflon liner (22mL, National Scientific, cat# B7990-5) and acidified (HCl) immediately. Samples for dissolved inorganic nutrients were filtered on site using a clean filtration device (hand pump) and 0.45-µm membrane filters (Millipore, Fisher cat #: HATF04700). The filtrates were collected in certified pre-cleaned HDPE flasks (500mL, Fisher cat# 05-719-145). Samples for taxonomy were collected in sterile plastic tubes (50 mL, BD falcon, Fisher cat# 352098) and preserved in the field with glutaraldehyde (2.5% v/v) immediately upon collection. Molecular samples were collected using a clean filtration apparatus (hand pump) and sterile membrane filters and placed on ice or dry ice. Post field activities included cleaning and storing all field equipment in accordance to the QAPP and manufacturer's instructions.

Sample preparation and processing

All samples were filtered (nutrients, chl-a, DOC, molecular samples) and preserved (taxonomy/microscopy) in the field, at the sampling site immediately after collection. Samples were transported to the laboratory (UC Santa Cruz) the same day they were sampled, logged in, inspected for damage and stored in the appropriate conditions until analysis in accordance to the QAPP. Samples were analyzed in a timely fashion in accordance to the holding times listed in

the QAPP. Archived samples were stored from all sites for any needed re-analysis or additional analysis that may be determined necessary.

In order to disrupt the cells and release intracellular toxins, grab samples for cyanotoxin analysis were submitted to a freeze-thawing cycle and ultra-sonicated for 2 minutes. Samples for LC/MS analysis were immediately filtered (0.2-µm) using sterile PES filter to remove cell debris and other particulate materials.

Microscopy preparation and analysis

Epifluorescence microscopy was used to identify and enumerate the harmful cyanobacteria present in environmental samples in accordance to the QAPP SOP (SOP UCSCCM-002). Samples were fixed in the field with 2.5% (v/v) glutaraldehyde and were filtered through 1-µm pore size, 25-mm diameter, black polycarbonate filters (GE Osmonics). The abundance of autofluorescing phycoerythrin containing cells (aka. cyanobacteria) was determined on a Zeiss Axioplan epifluorescence microscope with a magnification of 400x using green excitation (Zeiss filter set 20, excitation 546-nm bandpass, and emission 575–640-nm bandpass filters). The volume filtered depended from the algal assemblage density (from 1 mL to 50 mL). Before aliquoting, the sample was gently mixed by inverting the collection tube approximately 10 times.

The densities of cells (*Microcystis, Anabaena*, and *Woronichinia*) or filamentous (*Aphanizomenon, Lyngbya,* Oscillatoriales, *Gloeotrichia*) cyanobacteria were estimated by counting the number of units within one large grid square using a 400x magnification. Twenty grids per filter were counted for each sample. Cells per colonies (*Microcystis, Woronichinia*) or per filaments (*Anabaena*) were recorded. Presence (or absence) of cells specialized in nitrogen-fixation (heterocysts) were noted for *Aphanizomenon, Anabaena* and *Gloeotrichia*. The physiological status of the cells (as determined based on cell shape, change in autofluorescence or cell organization for example) were recorded on the data sheet.

Samples were also sent to independent labs (EcoAnalysts, Inc. or Greenwater Lab) using an inverted microscope in an attempt to gain further qualitative information regarding the taxonomic identification of strains of interest.

Molecular analysis for species identification

Algal samples were processed for genomic DNA extraction following a phenol extraction method previously used for cyanobacteria from the San Francisco Bay delta (Baxa et al. 2010). Algal cells were aseptically scraped from each filter membrane with forceps and transferred into a 100 µl of lysis buffer (10 mM Tris HCl pH 8.0, 1 mM EDTA, 100 mM NaCl, 0.2 % sodium dodecyl sulfate). The filter was rinsed with the lysis buffer to recover all algal cells remaining on the filter. After suspension of the algal cells by vortex mixer, proteinase K was added to a final concentration of 50 µg/ml. The samples were placed in a shaking incubator at 50ºC until algal cells were completely digested (4872 hrs). At the end of the extraction procedure, TE

buffer was added to suspend the genomic DNA, and concentration was measured by Nanodrop spectrophotometer (BioPhotometer plus, Eppendorf, NY).

The 16S ribosomal RNA gene (rDNA) and its adjacent internal transcribed spacer (ITS), a region of bacterial genomes commonly used for species identification, was amplified by PCR using the generic primer sets, pA (Edwards et al. 1989) and B23S (Lepere et al. 2000) as described in Rajaniemi et al. (2005). In addition, another set of primers (CYA 108F and CYA16S, Table 8) amplifying the partial fragment of the 16S rDNA but not ITS was used for one of the algal samples from the San Francisco Delta as we observed inhibition of PCR amplification with the primer set described above. The volume of the PCR cocktail was 50 µl containing 200 µM each of dNTP, 1.5 mM of $MgCl_2$, 40 pmol of each primer, 2 units of Taq DNA polymerase (High Fidelity Platinum, Invitrogen Corp, Carlsbad, CA) and 10x buffer at 1/10 the volume of the reaction. Bovine serum albumin (final concentration of 0.1 mg/ml) was added to the reaction cocktail for the algal samples from San Francisco Delta to resolve the inhibition of the PCR. The PCR cycling condition was performed as follows: initial denaturation step of 95°C for 5 min, 40 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 2 min 30 sec, followed by a final extension step at 72°C for 5 min and then held at 4°C. The PCR product was separated on 1% agarose gels and observed by a trans-illuminator after staining with 0.5 µg/ml ethidium bromide for 20 min. The DNA bands at the expected size (1.5–2 kb) were excised from the gel and extracted using QIAquick II gel extraction kit (Qiagen). The eluted DNA was then ligated into pGEM-T Easy vector (Promega BioSciences, San Luis Obispo, CA) that was used to transform *Escherichia coli* DH5αcompetent cells (Invitrogen). The length of the inserted DNA fragment was verified by running a PCR on colonies carrying the plasmid. The PCR cocktail (50 µl) contained 200 µM each of dNTP, 1.5 mM of $MgCl_2$, 40 pmol of M13 forward and reverse primers (Table 8), 0.5 unit Platinum Taq DNA polymerase (Invitrogen) and 10x buffer at 1/10 the volume of the reaction. The PCR cycling condition included an initial denaturation step of 95°C for 5 min, 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 2 min 30 sec, followed by a final extension step at 72°C for 5 min and then held at 4°C. Clones (n=20) carrying the inserted fragment size of 1.5 to 2 kb with variable length were chosen for plasmid extraction and sequencing. The plasmid was extracted using QIAprep Spin Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions.

The sequence of the inserted DNA fragment was determined from both ends using M13 forward and reverse primers in addition to primers that we designed (AlgaeIDSqF1 and AlgaeIDSqF3) for sequencing the middle fragments (Table 8). The samples were submitted to Davis Sequencing for sequencing (http://www.davissequencing.com/) and reaction was performed using an ABI 377 automated DNA sequencer (Applied Biosciences, Foster City, CA). The obtained sequences were processed using Geneious software ver. 5.0.3 (Drummond et al. 2010). Following manual removal of vector regions and correction of ambiguous sequences, batch BLASTN search was performed by Personal BLAST Navigator program (PLAN, http://bioinfo.noble.org/plan/index.php). The clones showing sequence similarity to HC (Harmful Cyanobacteria) were subjected to regular BLASTN search available at NCBI website (http://www.ncbi.nlm.nih.gov/) to verify the results such as query coverage, maximum identity, and alignments. Although similarity search by BLASTN program is commonly used for identification of different sources of species of unknown sequences, it is not always practical especially for distinguishing taxa or genera that share high degree of similarity in 16S rDNA and

ITS regions. Phylogenetic trees were therefore constructed for species that share very similar gene sequences such as those observed for *Anabaena* and *Aphanizomenon*, and between *Lyngbya* and other closely related taxa. Multiple alignments were generated by Clustal W program version 1.83 (Thompson et al. 1994) with unknown sequences as well as representative sequences. A phylogenetic tree was generated based on the alignment of 16S rDNA-ITS sequences by the Neighbor Joining method using PAUP program ver. 4.0 in Genious package (Swofford 2003, Drummond et al. 2010). Jukes-Cantor model was used to analyze the sequences using jModeltest ver 0.1.1 (Jukes and Cantor 1969, Posada 2008). The reliability and robustness of the phylogram depicted by PAUP program was tested by bootstrap analysis with 1,000 replications (Felsenstein 1985, Hedges 1992).

Analytical chemistry procedures

Nutrient concentrations, including nitrate, nitrate, ammonium, and orthophosphate were determined colorimetrically using Hach water quality kits following the manufacturer's instructions as outlined in the QAPP. The kits have low detection limits, permitting small quantities of nutrients to be detected. Because we determined at the beginning of the study that the cadmium reduction step of the Hach procedure for the nitrate+nitrite measurements was source of variations (aka. precision and/or accuracy were not meeting our QAPP requirements), we used a more reliable method (flow injection analysis on Lachat) to analyze the nitrate+nitrite samples. All calibration standards, QCs, and samples were analyzed with 2 or 3 replications. The QCs standards were prepared using Hach certified reference materials (nitrate: Hach #307-49, nitrite: UCSC reference materials, ammonia: Hach 153-49, phosphorus: Hach 14204-16, and wastewater effluent certified reference material: Hach #28332-49). The range of nutrient concentrations that can be accurately detected differs for each method (see QAPP). If a sample presented concentrations above the calibration range, it was diluted and reanalyzed. For validation and as part of our QA/QC checks, our data were compared to the dissolved nutrient data obtained by companion program (EMP/DWR). When differences were identified between our dataset and the EMP/DWR dataset, computations were verified to troubleshoot potential errors (e.g. offset not accounted for) in which case, samples were re-analyzed if necessary.

Dissolved organic carbon (DOC) was determined via high temperature catalytic combustion using a Shimadzu TOC Analyzer according to the QAPP (SOP UCSCLM-011). Each sample run included a certified reference material (SPEX Certiprep, #669), at least 1 field blank and field duplicate, and at least 1 analytical duplicate. All calibration standards, QCs, and environmental samples were analyzed with 3-5 replications. If a sample contained DOC concentration above the calibration range, it was diluted (or the injection volume was modified) and reanalyzed.

Chl-a was extracted and analyzed using the acidification method in accordance to the QAPP (SOP UCSCLM-002). Chl-a samples were analyzed in duplicates and corrected for phaeophytins using the calculations outlined by standard methods. QC standards were prepared using a certified reference material (Sigma: *Anacystis nidulans* algae free of chlorophyll b, cat# C6144-1MG). Five QC standards of known concentrations were used for calibration. Calibration standards were prepared fresh prior to start the analysis of each analytical batch and were ran in duplicates at the beginning and at the end of each analytical batch.

Toxicology analyses

Cyanotoxin analyses were performed according to protocols established through State of California Water Resources Control Board contract 07-120-250 according to our QAPP (SOP UCSCCM-002). Calibration standards of certified reference materials were included with each run, and the environmental sample concentrations were determined by a real integration of the peaks corresponding to known toxins (LC/MS) or by colorimetry using a plate reader (ELISA). Concentrations of the standards correspond to the expected range of concentrations found in the samples analyzed. Calibration standards are prepared by utilizing secondary dilution standards and/or stock solutions. The LC/MS instrument used in this study performs auto-tuning and sensitivity tests before each run.

Microcystins, anatoxin-a, lyngbyatoxin-a analyses were conducted at UC Santa Cruz (UCSC) using an Agilent 6130 liquid chromatography/mass spectrometry (LC/MS) system with an Agilent Zorbax Rapid Resolution column and Selected Ion Monitoring (SIM) of microcystin-LA, -LR, -RR, and -YR generally following the method of Mekebri et al. (2009) but adapted from an LC–MS-MS system to LC–MS as described in Miller et al. (2010). Sample concentrations were determined by calibration with certified standards obtained from various sources (Mekebri et al., 2009; Miller et al., 2010). The estimated method detection limits and reporting limits are 0.2 µg/L on-column for all toxin congeners. For a subset of samples, toxin extracts were split and run using both LC–MS and commercially available ELISA kits from Abraxis LLC (PN 520011) and Envirologix (PN EP 022) following the manufacturer's guidelines but with additional dilution using the manufacturer-supplied buffer solutions. Grab sample cyanotoxins were reported as total cyanotoxin (dissolved plus particulate) analyzed from whole water with sonication to disrupt cell membranes. Saxitoxins and cylindrospermopsins were measured from sonicated whole water (grab) samples using ELISA kits (Abraxis) following manufacturer's instructions because they are not being measured routinely on UCSC LC/MS at this time. Solid Phase Adsorption Toxin Tracking devices for continuous monitoring of cyanotoxins (microcystins) were prepared and processed following the method of Kudela (2011).

In order to validate our toxicology bioassessments,some field replicate samples (grab samples) were sent to three independent labs for analysis using different methods as follows:

(1) Thirteen field replicates were collected during September and October sampling events at selected SWAMP sampling stations in Clear Lake in accordance with our QAPP. These field replicates were analyzed within 48 hours by Tom Smythe (Field Officer, Water Resources Engineer, LCWRD) using Envirologix QuantiTube kit for microcystin (cat #: ET 039). This is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA). In this test, microcystin toxin in the sample competes with horseradish peroxidase-labeled microcystin for a limited number of antibody binding siteson the inside surface of the tubes. Envirologix Quantitube kit is designed for quantitative analysis of microcystin toxin in water samples. The kit is supplied with QC standards at 0, 0.4, 1.0 and 2.5 µg/L (ppb). The limit of detection (LOD) of the kit for microcystin is 0.18 µg/L. The limit of quantification (LOQ) of this kit is 0.7 µg/L. The mean recovery was 78.9 ±17.6%. Samples were kept frozen and in the dark until

analysis. Samples were not sonicated prior to analysis. The cost for this analysis was supported by Lake County.

(2) One field replicate collected at Horseshoe Bend station (513LAK003, Clear Lake) was collected by Tom Smythe (Lake County) during our August sampling event and shipped within 48 hours for processing to a private laboratory specialized in the identification and quantification of algae and algal toxins: Greenwater Laboratories/CyanoLAB (GW) (http://greenwaterlab.com/). The cost for this analysis was supported by Lake County. The sample was ultra-sonicated to lyse cells and release toxins prior to analysis. Solid phase extraction was used for anatoxin-a, lyngbyatoxin-a, microcystins, and cynlindrospermopsin for clean up and preconcentration (100x). For saxitoxins (aka. PSP: Paralytic Shellfish Poisoning toxins), 100 mL of sample was frozen, lyophilized and reconstituted in 75% acidified Methanol, blown to dryness and reconstituted in 1mL of dilute hydrochloridric acid. Liquid Chromatography/mass spectrometry, mass spectrometry (LC/MS/MS) was used tfor the determination of anatoxin-a, cylindrospermopsin, and lyngbyatoxin-a. The methodologies established a LOD of 0.05 µg/L and a LOQ of 0.1 µg/L for both cylidrospermopsin and anatoxin-a and a LOD of 0.5 µg/L and a LOQ of 1.0 µg/L for lyngbyatoxin-a. LC/MS was used for the determination of four of the most common microcystin variants. The LOD and LOQ for each variant are 0.5 µg/L and 1.0 µg/L respectively. The Association of Official Analytical Chemists (AOAC) Official Methods 2005.6 was used for saxitoxins analysis. The LOD for saxitoxin is 0.2 µg/L. Detailed methodology information can be found in the appendix.

(3) Nine field replicate samples collected in Clear Lake and the Delta were sent to Fish and Game, Rancho Cordova (FGRC) analytical lab (Dave Crane's lab). The cost for these analyses was supported by Regional Water Quality Control Board. Archived frozen samples were shipped to FGRC lab at the end of the study (November 2011). All but one sample were archived sonicated samples, the remaining sample was an archived non-sonicated replicate samples. Samples were cleaned up and preconcentrated using solid phase extraction and analyzed for the eight most common microcystin variants, anatoxin-a, cylindrospermopsin and saxitoxins using LC/MS/MS as described in Mekebri et al. (2009).

Results from these comparative analyses are presented in Appendix 1. Cylindrospermopsin, saxitoxin and lyngbyatoxin-a were not detected in all samples analyzed by the four independent labs (UCSC, GW, LCWRD, FGRC) and therefore are not presented in the summary table (appendix 1). Our LC/MS results (UCSC) globally agreed with ELISA results (LCWRD) for microcystin quantifications. Two samples did not have detection with both methods and two samples had low-level detectable microcystin by LC/MS only although below the method detection limit (0.2µg/L). Detection of microcystins only by LC/MS likely was due to lower analytical detection limit relative to the ELISA (0.4µg/L). Two samples with high levels of microcystin were quantifiable by LC/MS (UCSC) but exceeded the detection window of the ELISA (LCWRD) method. Microcystin concentrations in samples with detections by both LC/MS (UCSC) and ELISA (LCWRD) were comparable. LC/MS concentrations were lower than (71% of the samples processed) or higher than (29% of the samples processed) ELISA.

Differences in microcystin concentrations between these two methods may be due to variations between field replicates due to the colonial nature of the cyanobacteria, matrix interferences in either method or non-specific binding during ELISA analysis (Graham et al. 2010). LC/MS concentrations lower than ELISA concentrations may have been due to matrix interference or the presence of microcystin variants without commercial standards (Graham et al. 2010). Indeed, there are over 80 known microcystin variants and the availability of commercially available analytical standards at the time of this study limited quantitation to four variants. Our results were comparable to Greenwater Lab (GW) results with regard to microcystin. All microcystin variants were non detect in the sample processed by Greenwater Lab, low microcystin –LR level (0.07 µg/L) were detectable in our sample but below our method detection limit. Anatoxin-a was detected in our grab sample but was non detect in Greenwater Lab grab sample. This difference might be explained by variations between field replicates (the samples were not collected from the same homogenized bucket and at slightly different times) or by degradation of anatoxin-a during transport. Indeed, anatoxin-a is not as large a molecule as microcystin and has a shorter half life than microcystin (days versus months, Smith and Sutton 1993). Bacteria associated with cyanobacteria filaments can accelerate its degradation (Smith and Sutton 1993). The UCSC detection limit for this toxin is also lower than that of GWL. Except for one sample, cyanotoxin concentrations determined by LC/MS (UCSC) were globally higher than that determined by LC/MS/MS (DFGRC) but followed the same trend. These differences might be due to the aging of the archived samples resulting in toxin degradation. Samples were processed within days after collection at UCSC while samples were not shipped until several months after collection to DFGRC lab. Previous work also suggest that sonication can result in loss of toxin due to oxidation (Keith Loftin, *pers. com.*). Comparison via LC/MS/MS of the cyanotoxin concentrations in one sample archived after sonication with a duplicate of the same samples archived without sonication suggests that sonicated samples do not preserve as well as non sonicated samples. For example, microcystin-LR was ca. four times higher in non-sonicated replicates compared to the sonicated replicate (513LAK006, 9/8/11). Differences between methods might also account for the variations between LC/MS (UCSC) and LC/MS/MS (DFGRC) results. DFGRC use solid phase extraction prior to analysis of the sample which might result in loss of toxin, while UCSC uses direct injection. The sample with higher LC/MS/MS concentration than LC/MS could be explained by the heterogeneity of the grab samples due to the colonial nature of the cyanobacteria, as well as the difference between methods. DFGRC processed larger volumes (ca. 50 mL versus ca. 2 mL) and used an initial preconcentration step which might limit the variations due to sample heterogeneity. UCSC reran this sample and obtained the same level of toxin after re-analysis.

Statistics

Relationships between environmental variables and biological variables (chl-a, taxon abundance) or toxin concentration were determined using the JMP (SAS) statistical software. Data were log transformed ($Log_{10}(X)$ or $Log_{10}(X+k)$ (when some of the data entries were equal to zero because below the detection limit) if necessary to meet data assumptions (normal distribution) (Quinn and Keough 2002). The constant k was equal to 1 for species abundance and one tenth of the mean for the environmental variables. Some data deviated from normality and homoscedasticity even after transformation (e.g. EC Clear Lake) and therefore were not considered in the statistical analysis. One assumption of our multiple regression analyses is the

statistical independence of the observations on a given variable, however some environmental effects (e.g. wind driven surface current) might result in the violation of this asumption. For the Delta, ANOVA with Least Square Difference (LSD) pairwise comparison were used to test the effects of environmental variables on each biological variable of interest (chl-a, *Aphanizomenon* abundance, *Microcystis* abundance). In Clear Lake, in order to include a categorical variable (arm location), ANCOVA with LSD pairwise comparison were chosen to test the effects of environmental variables on biological variables of interest. Two levels of significance were set for all tests: (1) probabilities of $p < 0.05$ were considered significant, (2) probabilities of $p < 0.01$ were considered very significant.

Predictive Modeling

The main goals of this modeling effort were to: (1) develop bloom models (in term of chl-a and individual key cyanoHABs taxa) that are straightforward and useful in their application toward bloom monitoring and environmental management; (2) to further identify environmental variables that are significant predictor of bloom incidence and determine threshold values for each of these variables.

Based on ANOVA (Delta) and ANCOVA (Clear Lake) results, environmental covariates significantly correlated with biological variables of interest (chl-a, individual taxa abundance) were selected to develop binary logistic regression models using Minitab 15 statistical software. Only significant environmental covariates ($p < 0.05$) were included in the final models. When environmental variables exhibited severe covariation (as determined by multiple regression analyses), only one of the two was used in order to avoid colinearity in the model.

To develop these predictive logistic models, we coded the chl-a concentration and the abundance of key cyanoHAB taxa into new dichotomous variables (e.g. present = 1/absence = 0, or bloom=1/non bloom=0). For the models developed to predict bloom vs. non bloom conditions, we used defined thresholds. The choice of these thresholds was based on TMDL values when available (e.g. for Clear Lake, chl *a* = 73 µg/L was used as the threshold to define bloom versus non bloom conditions, Tetra Tech 2004). When TMDL thresholds were not available, we used the World Health Organization (WHO) risk guideline categories, established for recreational and drinking waters (WHO 2003). These categories are summarized in table 9. All the models in this report are presented as the probability (LOGIT (p) = 0 to 1) of a bloom occurrence in function of the relevant environmental covariate.

Results and discussion

1. Sacramento-San Joaquin Delta

Physico-chemical conditions

The limnological data collected for the period of June 2011 – October 2011 are shown in Table 10 and Figures 3 and 4. Surface waters were the coolest in June (16.8 ± 0.67°C) and October (18.7 ± 0.46°C), but relatively warm surface water temperatures (21.6 – 24.2°C) favorable for cyanobacteria growth occurred from July to September (figure 3A). Indeed, optimal temperatures for cyanobacteria occur between 20 and 30°C, which are temperatures higher than those preferred by green algae and diatoms (Msagati et al. 2006). Previous research in the delta indicate that *Microcystis* bloom initiation requires water temperature exceeding 20°C but accumulation of high biomass requires long residence time for this slow growing species (Lehman et al. 2008).

Surface waters were circumneutral (pH 7.0 – 7.8) during most of the study period but were slightly alkaline (pH 8.0 – 8.3) at some stations in August. EC varied spatiotemporally over the course of the study (Figure 3B). EC was globally low and homogeneous between stations at the beginning of the study period (June-July) suggesting a strong influence of riverine freshwater inputs. The study period was indeed preceded by an abnormally wet Winter/Spring with high snow fall in elevations associated with the El-Niño/La-Niña Southern Oscillation. Increased EC variability was observed between stations during the remaining of the study period (August-October). The highest EC was recorded at the Antioch Ship Channel station (544CCC001) in August (702 mS/cm) indicating marine intrusion. Nevertheless, EC remained within a range favorable for cyanobacteria growth. Previous research in the Delta suggest that *Microcystis* accumulation is correlated with low salinities (< 5‰, Lehman et al. 2005). Turbidity and secchi depths were also highly variable spatiotemporally and were globally negatively correlated with each other (Figures 3C and 3D). Secchi depths were lower in average at station 544CCC001 (Antioch Ship Channel) relative to the other stations because of higher turbidity and higher algal biomass (in term of chl-a).

According to the molar [DIN]/[PO_4^{3-}] ratio (averaging 4.5 mol:mol throughout the study), all sites remained potentially N-limited (based on the presumption that [DIN]/[PO_4^{3-}] <15 indicates N limitation, Downing et al. 2001). The literature suggests that freshwater systems harboring low N to P (<15) are most likely to experience cyanobacterial dominance over eukaryotic algal taxa (Downing et al. 2001). Furthermore, evidences from eutrophic Canadian lakes suggest that low [DIN]/[PO_4^{3-}] ratios (1 - 6) can trigger nitrogen-fixing cyanobacteria blooms (Barica 1994). Ammonia concentrations were globally low although they increased slightly after July (Figure 4A). Nitrite levels were near or below the detection limit throughout the study period (Table 10). Nitrate was the dominant DIN source (average N-NO3: 0.18 ± 0.05mg/L, Figure 4B). Dissolved inorganic phosphate concentrations remained relatively constant throughout the study period but increased slightly in October (Figure 4C). The highest dissolved organic carbon levels were observed in July (mean ca. 600 µM) but remained generally constant (ca. 100 – 200 µM) throughout the rest of the study period (Figure 4D).

Biological conditions

Chl-a concentrations were relatively low risk (<10 µg/L, Table 9, Graham et al. 2009, WHO 2003) from June (1.81 ± 0.67 µg/L) to October (4.71 ± 1.3 µg/L) but rose to moderate risk levels (10 - 32 µg/L, Table 9) in August – September (Figure 5A). The highest chl-a level was observed in September at station 544CCC001 (Antioch Ship Channel, 31.80 µg/L). Chl-a levels dropped in October to concentrations similar to those in June/July.

Results from microscopy and toxicology analyses for grab samples are summarized in Table 11 and Figure 5. The cyanobacterial assemblage during summer 2011 differed from past observations. Although seasonal (June-October), *Microcystis* blooms were observed every year from 1999 to 2008 (Baxa et al. 2010; Lehman 2000, Lehman et al. 2005, 2008, 2010; Spier et al. 2010). However, during this study, the presence of *Microcystis* was very sporadic and varied spatiotemporally during the study period (Figure 5B). The higher cell densities observed in September/October 2011 reflect the presence of colonial *Microcystis* in the samples which might have skewed the counts but field observations indicated that the abundance of *Microcystis* remained low throughout the study period as compared to the summers of 2008 and 2009. Indeed, *Microcystis* colonies were observed at some stations from August to October but at density levels several orders of magnitude lower than in previous years (Baxa et al. 2010, Lehman et al. 2005, 2008, 2010, Mioni et al. *in prep.*). Summer 2010 was also an atypical year for *Microcystis* as no bloom was observed during the expected bloom season (Mioni et al. *in prep*). These observations might reflect the influence of El Niño/La Niña-Southern Oscillation or longer-lasting environmental changes. *Anabaena* and *Oscillatoria* were also observed at low abundance at some stations.

Aphanizomenon was the most common cyanobacteria genus during this study and was observed at all stations from August to October. *Aphanizomenon* abundance varied spatiotemporally over the course of the study period (Figure 5C). The highest abundance was observed in September at station 544CCC001 (Antioch Ship Channel, 82,818 filaments/mL). Microscopic observations also indicate a very low abundance of heterocystous *Aphanizomenon spp.* filaments. Heterocysts are differentiated cells that are specialized for the fixation of atmospheric dinitrogen (N_2) into ammonium (NH_4^+). Heterocysts provide anoxic conditions which are necessary for the enzyme nitrogenase to convert N_2 to NH_4^+. The formation of the heterocysts is induced by the lack of combined ("fixed") nitrogen in the extracellular environment, the number of heterocysts usually correlates with N_2 fixation rates (Laamanen and Kuosa 2005). Consequently, the number of heterocysts can be considered as an indicator of the N_2 fixation capacity of a cyanobacterial population (Laamanen and Kuosa 2005). Heterocyst frequencies of up to 24% have been reported for freshwater systems with vigorous nitrogen fixation (Levine & Lewis 1987; Findlay et al. 1994): heterocyst frequency was ca. an order of magnitude lower in our study. These low heterocyst frequencies suggest that *Aphanizomenon* was not actively fixing nitrogen and that DIN levels (or other sources of N not considered in this study such DON) might have been sufficient to support its growth. Molecular analyses identified this taxa as *Aphanizomenon flos-aquae* (see below).

To the best of our knowledge, there is no previous report of *Aphanizomenon spp.* bloom in the San Francisco Bay-Delta in the literature at such densities. *Aphanizomenon flos-aquae*

(Cloern and Dufford 2005) and *Aphanizomenon spp*. (Lehman et al. 2010) have previously been observed in the San Francisco Bay-Delta. Compiled data from previous monitoring studies suggest that optimal temperatures and salinity for *Aphanizomenon flos-aquae* in the San Francisco Estuary are from *ca*. 13 - 19°C and within the freshwater range of salinity (Cloern and Dufford 2005). During their study in summer (August-September) of 2005, Peggy Lehman and colleagues (2010) also observed *Aphanizomenon spp.* at 3 of the 10 monitored stations (Old River at Rancho del Rio, Sacramento River at Cache Slough, and San Joaquin River at Turner Cut) and estimated that this strain contributed ca. 5 to 20% abundance of the phytoplankton assemblage at these stations.

Molecular analyses

Results from molecular analyses are summarized in Table 12. The *Aphanizomenon* strain was identified as *Aphanizomenon flos-aquae* (Figure 6). The dominant species found in the samples examined from the Sacramento-San Joaquin Delta were *Synechococcus* sp., *A. flos-aquae*. and *Anabaena* spp. Although *Microcystis aeruginosa* was microscopically detected at different sampling sites and times in the Delta, this species was not detected using molecular techniques. It is possible that *M. aeruginosa* was lacking in the molecular samples examined due to its colonial nature (subsample heterogeneity) or that the large background of other cyanobacterial DNA may have prevented the detection of the small amounts of *M. aeruginosa* DNA. Although the field samples that we chose for molecular analyses were based on morphologic microscopic analysis (qualitative and quantitative), the DNA fragments indicating the expected algal species were not obtained from all of the samples. One potential explanation may be due to the small number of samples used for analysis. Due to time and budget limitations, we have analyzed only 20 clones from each sample, which is a very small proportion that may not represent the wide variety of cyanobacterial species present in the blooms. We have proven that the primers used in this study effectively amplify sequences of potentially harmful cyanobacteria. Analyzing more clones from appropriate field samples would have probably yielded a greater number of toxin producing cyanobacterial sequences (Table 12). An alternative approach would have been to use parallel algal samples for morphologic taxonomic identification and molecular analyses to better understand the cyanobacterial composition and distribution in the ecosystem of interest.

Toxicology analyses

Microcystin toxins were near or below the detection limit in the grab samples of surface water collected over the course of the study (Table 11). The other cyanotoxins (saxitoxin, anatoxin-a, cylindrospermopsin) monitored during this study were not detected at all stations throughout the study period (Table 11). However, continuous toxicology measurements (SPATT) suggest an increase of dissolved microcystin levels in August/September when *Aphanizomenon flos-aquae* was the most abundant (Table 13). This observation suggests that *A. flos-aquae* or *Microcystis aeruginosa* may have produced low levels of microcystin toxins. *Aphanizomenon spp*. strains, including *A. flos-aquae*, are known producers of a variety of cyanotoxins, including microcystins, anatoxin-a, cylindrospermopsin and saxitoxin (Sivonen and Jones 1999, Castle and Rodgers 2009).

Correlations

Environmental variables that may favor cyanoHABs were examined by analysis of variance (ANOVA). Chl-a concentrations and individual taxa abundances (*Aphanizomenon flos-aquae, Microcystis aeruginosa*) were compared to limnological variables (temperature, EC, turbidity, Secchi depth, NH4, NO3, PO4, N:P ratio). Temperature was the only significant covariate explaining chl-a concentrations (ANOVA, $p = 0.0326$, Table 14). In agreement with the findings of Cloern and Dufford (2005), our results show that *A. flos-aquae* abundance was significantly explained by EC (ANOVA, $p = 0.0034$, Table 14). Additionally, we found that ammonium concentrations also explained a significant amount of the variation in *A. flos-aquae* abundance (ANOVA, $p = 0.0188$, Table 15). Our results suggest that the relationship between *A. flos-aquae* abundance and $N\text{-}NH_4$ concentrations did not depend on EC (ANOVA, p (log(NH4)*log(EC) = 0.4473) which validates the homogeneity of slope asumption. Although Lehman et al. (2010) observed that *Aphanizomenon spp*. abundance covaries with *Microcystis* abundance, this correlation wasn't significant based on our current dataset (ANOVA, p = 0.2275, Table 15). None of the environmental variables considered in this study were significantly correlated with *Microcystis* abundance (Table 16). Our preliminary results for the period of 2008-2010 suggest that surface water temperature is the best predicting variable for *Microcystis* occurrence (presence/absence) and that the probability of *Microcystis* occurrence in the Delta reaches ca. 50% when water surface temperature exceeds 25°C (Mioni et al. *in prep*.). Surface water temperatures during Summer 2011 never reached this temperature threshold. Surface water temperatures averaged 22.69± 0.92°C during the warmest months (July – September 2011). It is possible that these relatively cool conditions as compared to the 1999-2009 period, as well as other environmental variables not considered in this study, may have limited the recruitment of *Microcystis* in the Sacramento-San Joaquin Delta over the past two summers.

Modeling

The logistic model generated for bloom (chl a > 10µg/L) prediction show that surface water temperature had a marginally significant effect on bloom prediction (p = 0.094, Figure 7, Table 17). This result indicates a surprisingly good relationship given the small number of bloom events (chl-a > 10 µg/L) within the dataset. Indeed, chl-a levels exceeded this threshold concentration at only 6 out of 31 events over the course of the study period and suggests that this modeling approach is promising for larger dataset. Although the small sample size might have altered the outcome of the modeling, the *Aphanizomenon* "bloom" was indeed modest as compared to blooms around in other systems around the world (e.g. Ferber et al. 2004, Laamanen and Kuosa 2005).

The logistic model generated for *Aphanizomenon flos-aquae* occurrence (presence/absence) demonstrated a good fit (p = 0, Figure 8, Table 17). The logistic binary regression indicates that ammonium concentration was the best predictive environmental variable of the bloom during summer 2011 although the p value for this environmental variable was slightly above our threshold p-value for significance (p = 0.051). By contrast to the ANOVA

results, this predictive model indicates that EC was not a significant environmental driver. The disparities between ANOVA and logistic regression tests suggest that other variables not considered in this study might have regulated the accumulation of *Aphanizomenon flos-aquae* (e.g. flow) and/or that the sample size was too small to observe significant correlations using logistic modeling.

Previous work suggests that nitrogen speciation influences the cyanobacteria structure and abundance (Paerl 1988). Ammonium and other reduced N forms (e.g. dissolved free amino acids) are more bioavailable than oxidized N forms (e.g. nitrate and nitrite) to cyanobacteria because less energy is required for their assimilation (Gardner et al. 2004, Flores and Herrero 2005). Moreover, previous studies have also shown that diazotrophic cyanobacteria such as *A. flos-aquae* preferentially assimilate ammonium when it is available (Ferber et al. 2004).Although *Aphanizomenon flos-aquae* did not appear N-deficient based on the low heterocyst frequencies, it is possible that the bioavailability of various N forms might have influenced its growth. $N\text{-}NO_3$ levels and other sources of N not considered in this study (e.g. organic nitrogen such as urea) might have been sufficient to support *A. flos-aquae* growth and to repress heterocyst formation. Further work is needed to investigate the effect of N speciation on the Sacramento-San Joaquin Delta cyanobacteria assemblage, and more specifically on *A. flos-aquae* growth and distribution. We will combine our dataset with the data gathered by a companion program (Delta Stewardship Council funded, lead PI: Alex Parker, co-PI: Cécile Mioni & Raphael Kudela) to examine in more detail the roles of various N forms as well as the ratio of oxidized N forms over reduced N forms on the cyanobacteria assemblage abundance and composition. This companion program included amendment incubation experiments as well as surface water monitoring. We anticipate that this collaborative approach will enhance these companion programs by expanding their respective dataset and will help provide a better understanding of the environmental controls of the cyanobacteria community structure and abundance.

2. Clear Lake

Physico-chemical conditions

The limnological data collected in Clear Lake from June to October 2011 are summarized in Table 18 as well as Figures 9, 10 and 11. Surface water temperatures increased between June and August time period in all arms. Surface water temperatures the Upper Arm peaked as early as July (Figure 9). Surface water temperatures dropped in October (mean: 19.77±0.59°C). Data collected from Hobos deployed at our continuous monitoring stations suggest that the Fall cooling of surface water temperatures began a couple of days after our September 8, 2011 sampling event (Figure 10) which coincided with decreasing air temperatures and gusty winds (September 10-11, 2011). The first rain event occurred on September 25, 2011. The Upper Arm (including Horseshoe Bend) was the warmest arm of the lake, with water temperatures as high as 29°C in August. The lowest surface water temperatures were generally observed at the Lower Arm stations throughout the study period but remained within the favorable temperature window for cyanobacterial growth (Msagati et al. 2006). Maximum growth rates of cyanobacteria often occur at or above 25°C (Kardinaal and Visser, 2005). This temperature optimum is, usually, higher than the optimum temperature found for green algae and diatoms (Kaardinal and Visser

2005, Msagati et al. 2006, Paerl and Huisman 2008). This optimal temperature threshold was met or exceeded at most station from July to September.

Concomitant with to the warming of surface waters and the accumulation of algal biomass, water clarity decreased in all arms between June and July, (Figure 9, Table 18). The horseshoe bend station was located at the margin of the scum-forming bloom that developed in this area of the lake over the study period. The lowest secchi depths were recorded during the warmest months (July-September) when noxious algal scums were observed in the lake. Secchi depths were shallowest at Horseshoe Bend (station 513LAK003: 20 cm on July 14, 2011) and Oaks Arm (station 513LAK006: 0 cm on September 8, 2011) where scum forming cyanoHABs were observed. Water clarity was generally highest at the Upper Arm stations where cyanobacteria biomass was usually lower as compared to other monitored stations.

DIN was very low throughout the study period in Clear Lake but increased at most stations in October (Figure 11, Table 18). This finding is consistent with previous observations of nitrogen-limiting conditions during the summer months in Clear Lake (Winder et al. 2010, Tetra Tech 2004, Richerson et al. 1994). Nitrogen is chronically low in Clear Lake during summer, especially during wet years such as 2011 (Winder et al. 2010). Ammonium was the dominant source of inorganic nitrogen. Dissolved nitrate and nitrite in surface water was near or below detection levels at all stations throughout the study period. This finding is consistent with the long-trend observations of a general decrease in Nitrate + Nitrite in the lake between 1970 and 2005 (Winder et al. 2010). The highest ammonium concentrations were observed in the Lower arm. Ammonium concentrations were about an order of magnitude higher at the Lower Arm coastal station (Clearlake city, 513LAK005) as compared to the other stations, reaching a maximum of 0.40 mg/L (as N-NH4) on September 8, 2011. These conditions are generally favorable for cyanobacterial dominance (Blomquist et al. 1994).

Dissolved inorganic phosphorus levels were globally low (average P-PO4 0.02 ± 0.01 mg/L) in June, especially at the upper arm and horseshoe bend stations where orthophosphate levels were near detection limit (average: 0.011 mg/L ± 0.002, Figure 11, Table 18). Orthophosphate concentrations increased steadily in all arms from July to October. The range and trend of orthophosphate concentrations observed during this study period are consistent with the monthly-averaged orthophosphate concentrations observed between 1969-2008 (Winder et al. 2010). The highest orthophosphate levels were observed at the Upper Arm and Horseshoe bend stations in October 2011 (average: 0.49 ± 0.07 mg/L). Orthophosphate concentrations over summer were globally lower in Oaks Arm and Lower Arm compared to the Upper Arm. The lowest phosphate concentrations were observed in the Lower Arm, with levels roughly twice lower than in the other arms. This trend is consistent with previous observations (Winder et al. 2010). The increase in dissolved inorganic phosphorus concentrations over summer and fall are typically a result of internal phosphorus loading from the sediment while external phosphorus load usually arrives with the winter flood (Winder et al. 2010). The DIN:DIP molar ratio were generally extremely low at all stations (<1), suggesting nitrogen limiting conditions (Table 18). Cyanobacteria are generally better competitor for nitrogen than phosphorus and are thus favored in lakes with low ratios of the N to P source (Schidler 1977, Smith 1983, Ferber et al. 2004).

Biological conditions

Chl-a concentrations varied spatially and temporally over the course of the study period (Table 18 and Figure 12). The temporal fluctuations in chl-a reflected a succession in the cyanobacteria assemblage from a *Lyngbya* dominated community in July/August to a *Microcystis* dominated community in September/October. Chl-a levels ranged from low to moderate risk (Table 9, WHO 2003, Graham et al. 2009) in June (average: 12.97 ± 8.97 µg/L) and generally increased by an order of magnitude from June to July to exceed high risk threshold level (WHO 2003) at all stations (average: 352.1 ± 294.7 µg/L). The highest chl-a levels were observed at the Oaks Arm stations. The TMDL target for compliant year (chl-a = 73 µg/L, Tetra Tech 2004) was exceeded at all stations in July. This increase in chl-a coincided with a primary cyanobacterial bloom dominated by *Lyngbya*. Chl-a concentrations dropped in August to moderate risk levels at all stations except at the Horseshoe bend (82.45µg/L) and the coastal Oaks Arm stations (>2000 µg/L at station 513LAK006) stations which exceeded the high risk threshold levels (WHO 2003, Graham et al. 2009). *Lyngbya* mat-forming blooms were also observed in the Upper Arm during early summer (July) but the sampling stations were located outside of this cyanoHAB. Most stations (except Upper arm stations) exceeded again the chl *a* TMDL in September-October and displayed chl-a concentrations exceeding the high risk threshold level (WHO 2003, Graham et al. 2009).

Microscopic observations indicate that the cyanobacterial assemblage was composed of four potentially toxic filamentous cyanobacteria (*Aphanizomenon spp.*, *Anabaena spp.*, *Lyngbya spp.* and *Gloeotrichia echinulata*) as well as two other potentially toxic colonial cyanobacteria (*Microcystis aeruginosa* and *Woronichinia naegeliana*) (Table 19). The taxonomic composition of the cyanobacterial assemblage is consistent with historical records (Richerson et al. 1994). *Gloeotrichia echinulata* was mostly present in surface water samples at the beginning of the study (June-August) but was not detected in any of the samples collected in September/October. This species is a known microcystin producer (Carey et al. 2007). *Aphanizomenon spp*. abundance varied spatially and temporally (Table 19). The highest densities of *Aphanizomenon spp*. were observed in June and October. *Anabaena spp.* was relatively ubiquitous throughout the study period (Table 19). The highest *Lyngbya* densities were observed at the Horseshoe Bend station in July and at the coastal Oaks Arm in August and September (Figure 12, Table 19). These elevated *Lyngbya* abundance at these stations coincided with the development of mat-forming blooms associated with foul odors at these locations. *Microcystis aeruginosa* was not detected at the upper arm stations and at the horseshoe station at the beginning of the study period (June/July) but was ubiquitous for the rest of the study period (Figure 12, Table 19). When present, *Microcystis* cell densities exceeded the high risk threshold levels (WHO 2003, Graham et al. 2009). An additional sample was collected on October 6, 2011 near Redbud Park launching dock. This sample was collected within a scum-forming bloom dominated by *Microcystis aeruginosa*, *Anabaena spp*. and *Lyngbya spp*. Cyanobacteria abundance exceeded the very high risk threshold level at this location (*Microcystis*: 65,802,625 cells/mL, *Anabaena spp*: 1,0568,063 cells/mL, *Lyngbya spp*: 49,063 filaments/mL). *Woronichinia naegeliana* was sporadically spotted at some stations but wasn't a dominant taxa.

Molecular analyses

Molecular analyses results (see summary in Table 12) and microscopic analyses (Greenwater lab, Florida) identified the *Lyngbya* strain as *Lyngbya hieronymusii* f. *robusta* (synonym: *Lyngbya robusta*). Information is scarce with regard to *Lyngbya hieronymussii* f. *robusta* characteristics and ecology in the literature. *Lyngbya hieronymussii* was recently identified as the dominant cyanoHABs species blooming in lake Atitlan, Guatemala (Rejmanková et al. 2011). The first bloom was observed in December 2008 and a much larger bloom that spread to ca. 40% of lake Atitlan occurred in October 2009. This bloom was associated with very trace amounts of saxitoxin and cylindrospermopsin (Rejmanková et al. 2011). Results from the same study suggest that this species is a non-heterocystous diazotroph fixing atmospheric nitrogen at night (Rejmanková et al. 2011). In contrast to other *Lyngbya* species, *L. robusta* contains reversible gas vesicles, concentrated in fasciculated aerotopes, which can be irregularly distributed over the whole cell volume and giving buoyancy to the filament (Rejmanková et al. 2011). The P content of this strain is higher than the typical cyanobacterial P content of 0.6%, which suggests that increased phosphorus availability in Lake Atitlan provided favorable conditions for this strain to thrive (Rejmanková et al. 2011).

The taxonomic identity of the other potentially harmful cyanobacteria present in Clear Lake was confirmed by molecular analyses. *Microcystis spp.* was identified as *M. aeruginosa*, a known producer of microcystin toxins (Carmichael 2001). *Aphanizomenon* was identified as *A. flos-aquae*. *Aphanizomenon spp*. strains, including *A. flos-aquae*, are known producers of a variety of cyanotoxins, including microcystins, anatoxin-a, cylindrospermopsin and saxitoxin (Sivonen and Jones 1999, Castle and Rodgers 2009). *Anabaena* was identified as *Anabaena lemmermanii*. This species is capable of producing hepatotoxins (microcystins) and different types of neurotoxins (anatoxin-a, anatoxin-a(S) and putatively PSP-toxins; Lepistö et al. 2005). Furthermore, molecular analyses indicated that *Synechococcus*, a small unicellular cyanobacterium, became abundant toward the end of the study period. Compiled literature evidences suggest that some *Synechococcus* strains are capable of producing diverse cyanotoxins with neurotoxic and hepatotoxic effect, including microcystins (Martins et al. 2005, Carmichael and Li 2006). Several diazotrophic bacterial strains were also detected: *Paenibacillus sp*., *Panibacilluus alvei, Rhodobacter sphaeroides*, *Rhodobacter sp*.

Toxicology analyses

Despite the high accumulation of cyanobacteria biomass, cyanotoxins were not detected or fell within the low risk level in the grab samples throughout the study period (< 10µg/L, Table 19). Cyanotoxins concentrations were usually higher at near-shore stations with scums as compared to those without surface scums. Cylindrospermopsin and saxitoxins were not detected in all grab samples throughout the study period. Low risk levels of anatoxin-a were only detected in August in two grab samples collected in the Lower Arm (station 513LAK004, 0.43 µg/L) and Oaks Arm (station 513LAK007, 2.25 µg/L). Lyngbyatoxin-a was only detected at one station located in the Oaks Arm (station 513LAK006, 0.5-2.5µg/L) where the abundance of *Lyngbya hieronymussii* f. *robusta* was high (463,675 filaments/mL) but no scum or mat were observed during collection. Moderate risk level of microcystins was detected in the grab sample collected at the coastal Oaks Arm station on September 8, 2011. This sample was collected within a mat

forming bloom dominated by *Lyngbya hieronymusii* f. *robusta* (1,408,094 filaments/mL), *Microcystis aeruginosa* (19,507,250 cells/mL), *Anabaena lemmermanii*.(706,500 cells/mL) and *Aphanizomenon flos-aquae* (44,156 filaments/mL). High risk level of cyanotoxins was detected in the additional sample collected near Redbud park launch in October. This sample was collected within a scum forming bloom dominated by *Microcystis aeruginosa* (65,802,625 cells/mL), *Anabaena lemmermanii* (10,568,063 cells/mL) and *Lyngbya hieronymusii* f. *robusta* (49,063 filaments/mL).

Continuous toxin tracking devices (SPATTs) deployed in June and July were lost due to high winds and wave traction. The SPATTs deployed in August-October indicate an increase in dissolved microcystin toxins at all stations (Table 20). Microcystin-LR was the most abundant variant at all stations. Microcystin-YR and microcystin-LA were not detected in August/September but were detected at low levels in September/October. Anatoxin-a were not detected in any of the SPATT deployed during that period.

Correlations

Environmental variables that may favor cyanoHABs were examined by analysis of variance (ANCOVA) using arm location as a categorical variable. Chl-a concentrations and individual taxa abundances (*Aphanizomenon flos-aquae, Microcystis aeruginosa, Anabaena lemmermanii, Gloeotrichia echinulata,*and *Lyngbya hieronymusii* f. *robusta*) were compared to limnological variables (temperature, NH4, NO3, PO4).

Temperature (p = 0.0001), nitrate concentration (p = 0.0011) and arm location (p = 0.0129) were significant covariates explaining chl-a concentrations (Table 21). The relationships between chl-a and temperature or chla and nitrate did not depend significantly on the arm location suggesting that another environmental driver not considered in this study must explain the differences between arms.

ANCOVA analyses for *Lyngbya hieronymusii* f. *robusta* suggest that temperature (p < 0.0001) and nitrate+nitrite (p = 0.0103) were the most significant environmental variables explaining *L. hieronymusii* f. *robusta* abundance (Table 22). Based on the scarce information available in the scientific literature, this rare strain occurrence has only been reported in subtropical systems such as Brazil (Werner 2010) and Guatemala (Rejmanková et al. 2011). It is therefore not surprising that warmer surface water temperatures correlate with this strain accumulation. However, the surface water temperatures observed during the study period were comparable to that observed historically during summer in Clear Lake when this strain was not yet dominant (Winder 2010). The direct correlation of *L. hieronymusii* f. *robusta* abundance with nitrate+nitrite was also somewhat surprising as nitrate+nitrite were low (i.e. at or below detection limit) during the climax of the bloom. The literature also suggests that this *Lyngba* strain is diazotrophic (Rejmanková et al. 2011) and therefore should have a competitive advantage in N-depleted conditions as compared to non nitrogen-fixing cyanobacteria such as *M. aeruginosa*. As such, other environmental factors not considered in this study may have controlled *L. hieronymusii* f. *robusta* recruitment and dominance in Clear Lake. For example, water clarity has dramatically increased in Clear Lake since the 1990's (Winder et al. 2010). The literature suggests that clearer waters where light reaches the bottom may favor benthic cyanobacteria such

as *Lyngbya* spp. (Paerl 2008). Water clarity were not considered in our model as variations in water clarity were rather the consequences of algal bioaccumulation in surface rather than a predicting variable of bloom formation. Further work (e.g. incubation experiments) is therefore needed to investigate the role of environmental variables such as water clarity that can control the recruitment and growth of *L. hieronymusii* f. *robusta* in Clear Lake. Further research is needed to investigate the nitrogen preference of Clear Lake *Lyngbya* strain (i.e. verify its ability to fix atmospheric nitrogen, investigation of DON sources).

ANCOVA results suggest that orthophosphate levels ($p = 0.0037$) and arm location ($p = 0.0002$) were highly significant covariates explaining *Microcystis aeruginosa* abundance (Table 23). The relationship between *M. aeruginosa* abundance and orthophosphate concentration depended significantly on the arm location ($p = 0.0011$) indicating that dissolved inorganic phosphorus was the environmental driver explaining differences in *M. aeruginosa* abundances between arms. The lack of significant correlation between *M. aeruginosa* abundance and N concentration was somewhat surprising. *M. aeruginosa* cannot fix nitrogen from the atmosphere and therefore the availability of nitrogen sources was suspected to control the abundance and distribution of this species. Previous works in eutrophic lake have reported persistence and success of *Microcystis* spp. during chronic N-limiting conditions (Paerl et al. 2011). Possible explanations for *M. aeruginosa* success under N-limitations include: superior ability of *Microcystis spp*. to compete for ammonium and phosphorus from sediments and mutually beneficial bacteria-cyanobacterial interactions in the phycosphere of *Microcystis spp*. colonies which can enhance nutrient cycling and growth of host *Microcystis spp.* populations (Paerl et al. 2011). Additionally, *Microcystis spp.* ability to vertically migrate throughout the water column by buoyancy compensation may enable exploitation of the entire water column, taking advantage of regenerated N sources (Ferber et al. 2004, Paerl et al. 2011). A recent study undertaken in a eutrophic lake highlighted the importance of N regeneration to support cyanobacterial bloom, even when the assemblage was dominated by diazotropic cyanobacteria (Ferber et al. 2004). Very little is known with regard to internal N cycling and regeneration in Clear Lake although these processes are important for the maintenance and species succession of cyanobacteria blooms in lakes exhibiting chronic N-limititation conditions (Ferber et al. 2004, Paerl et al. 2011). Further work would be needed to investigate these processes in Clear Lake, especially as it pertains to key species such as *M. aeruginosa*.

ANCOVA results suggest that orthophosphate concentration ($p < 0.0001$) was the most significant predicting environmental variable explaining *Gloeotrichia echinulata* abundance (Table 24). None of the other environmental variables considered in this study significantly correlated with *G. echinulata* abundance. *G. echinulata* is a diazotrophic cyanobacterium and is therefore favored under N-limiting conditions. The literature suggests that *G. echinulata* has a unique phosphorus uptake and life strategy (Istvanovics et al. 1993). *G. echinulata* abundance decline in Clear Lake coincided with the increase of orthophosphate levels in surface waters. Previous works suggest that *G. echinulata* depends on absorbing P on the lake sediment to meet its nutritional requirements and can have a considerable impact on P translocation from the sediments to the water column (Carey et al. 2008) possibly triggering other cyanobacteria blooms (Noges et al. 2004). It has been estimated that *G. echinulata* can contribute as much as 66% of the total lake yearly internal P load (Istvanovics et al. 1993, Barbiero and Welch 1992). Furthermore, previous research in eutrophic lake suggests pelagic *G. echinulata* abundance is

usually linked to the presence of recruiting colonies. It generally sinks out of eutrophic lake water column during the late summer and produce akinetes that overwinter on the lake sediment (Carey et al. 2008). This is consistent with our observations: *G. echinulata* abundance declined in August and was not detected in surface samples collected in September and October. The decline of *G. echinulata* in late summer 2011 is however not likely to have been the result of grazing pressure. Indeed, *G. echinulata* also produces large (~2 mm) and buoyant colonies and is thought to be inedible to most zooplankton (Carey et al. 2008). The unique behavior of *G. echinulata* may have implications for lake management strategies. Indeed, because this species may have limited pelagic P uptake (Istvanovics et al. 1993), attempts to limit blooms by reducing in lake-nutrient concentrations could have little effect (Carey et al. 2008). It has been suggested that management strategies should focus instead on preventing or reducing recruitment of *G. echinulata* to prevent blooms (Carey et al. 2008).

ANCOVA results suggest that temperature (p = 0.0245) was the most significant predicting environmental variable explaining *Aphanizomenon flos-aquae* abundance (Table 25). *A. flos-aquae* abundance was lower during the warmest months and higher during the beginning and at the end of the study period when the Lake surface water temperatures were cooler. No significant correlation were found between *Anabaena and lemmermanii* and the environmental variables investigated in this study (Table 26). The abundance of *A. lemmermanii* appeared to be marginally driven by temperature (p = 0.0629) and orthophosphate concentration (p = 0.0855). The weak coefficients of correlation of these two models however suggest that environmental drivers not considered in this study might have played a role in *A. flos-aquae* and *A. lemmermanii* growth and distribution. For example, previous work suggests that iron availability limits diazotrophic cyanobacteria growth and nitrogen fixation in Clear Lake (Wurtsbaugh and Horne 1983). Further work is therefore needed to investigate the influence of other factors such as iron on Clear Lake cyaobacterial assemblage abundance and composition.

Correlations between microcystin toxin concentrations, environmental variables and individual cyanobacteria species abundances were investigated. Ammonium concentration was the only physico-chemical environmental variable correlating marginally with the microcystin toxin concentrations (ANOVA, p = 0.0695). *Gloeotrichia echinulata* (p =0.0194) abundance correlated significantly (indirectly) with microcystin toxin concentrations. All the other cyanobacteria taxa abundance did not correlate significantly with microcystin abundance (p > 0.05). *Microcystis spp.*, *Anabaena spp*. and *G. echinulata* are the only known microcystin producers among the cyanoHAB species observed in Clear Lake (Table 1). Based on the literature, *Lyngbya spp.* and *Aphanizomenon spp*. are not known producers of this hepatotoxin. Although *G. echinulata* is a known producer of Microcystin-LR, the level of hepatotoxin produced by this strain is usually low (Carey et al. 2007). Recent research suggests that microcystin production is regulated by multiple factors and that a well-distinguishable control of microcystin production by one environmental factor should be excluded (Janichen et al. 2011). Although our results indicate that ammonia concentration was the best environmental covariate explaining microcystin levels in Clear Lake, it is possible that other factors might have contributed to regulate microcystin production. High irradiance and excess phosphorus have been shown to reduce microcystin production (Janichen et al. 2011). As such, excess phosphorus in Clear Lake might explain the generally low levels of microcystin toxin observed in Clear Lake throughout the summer. Sulphur availability is also another factor known to influence both *M.*

aeruginosa growth and microcystin production (Janichen et al. 2011). Further work is needed to investigate the role of sulphur availability of microcystin production in Clear Lake. Further work should also investigate the contribution of small, nondescript and potentially toxigenic species, such as *Synechococcus spp.,* which cannot be assessed by traditional morphological methods. Indeed, our molecular analyses indicate that this potential microcystin producer was a dominant species in late summer/early fall.

Modeling

The logistic model generated for bloom occurence (chl-a > 73µg/L) displays a marginal significance (p = 0.067, Figure 13, Table 28). This result may suggest that other factors not included in the model might play a role on bloom occurrence. Our study period also took place during the warmer months (aka bloom season). A better fit may have been obtained with a larger dataset including pre- and post-bloom conditions.

The logistic model generated for *Lyngbya hieronymusii* f. *robusta* bloom occurence (> 10,000 filaments/mL) demonstrates a very good fit accuracy (p = 0.000, Figure 14, Table 28). This model indicates that surface water temperature is a good predictor of *Lyngbya hieronymusii* f. *robusta* bloom occurrence in agreement with ANCOVA analyses. The difference in temperature thresholds between the arms of the lake suggest that other environmental variables not included in the model may control *L. hieronymusii* f. *robusta* abundance in surface rather than indicating differences in surface water temperature threshold for this strain to bloom. Winds for example may have influenced the accumulation of this strain in the surface waters. Dominant W/NW winds tend to contribute to the biomass accumulation of buoyant cyanobacteria in the eastern section of the lake (Richerson et al. 1994). As such, increased accumulation of *L. hieronymusii* f. *robusta* in the Oaks Arm and Lower arms at lower temperature thresholds as compared to the Upper Arm may reflect the influence of dominant winds.

The logistic model generated for microcystin detection (> 0 µg/L) did not demonstrate a good fit accuracy (p = 0.075, Figure 15, Table 28). This result may suggest that other factors not included in the model might play a role on microcystin production.

The logistic model generated for *Gloeotrichia echinulata o*ccurence (> 0 filaments/mL) demonstrates a very good fit accuracy (p = 0.000, Figure 16, Table 28). This model indicates that *G. echinulata* occurrence is significantly negatively correlated with surface water orthophosphate levels in agreement with ANCOVA analyses. The model predicts a *G. echinulata* occurrence probability of 100% when P-PO4 concentration falls below ca. 10^{-2} mg/L. This finding are in agreement with previous studies that indicate that *G. echinulata* may have limited pelagic P uptake and may rely instead on intracellular P reserves stored while the recruiting colonies were in the P-replete sediments (Istvanovics et al. 1993).

Conclusions

Delta –

Although *Microcystis* has been the main cyanoHAB species of concern from 1999 up to the present, observations from this study indicate that *Aphanizomenon* blooms can also co-occur in the Delta. Cyanotoxins remained below detection limit in the grab surface water samples and microcystin was only detectable in the SPATT samples. These results may suggest that toxins were transported from sites not monitored during this study. Further work is also needed to determine if the Sacramento-San Joaquin Delta is undergoing long-term changes (*Microcystis* decline) or if the observed changes are related to temporary changes linked to the El-Niño/La-Niña Southern Oscillation.

Our results suggest surface water temperature was key environmental drivers of algal abudance (in term of chl-a). Based on our dataset, ammonia and EC were the best predicting environmental variables explaining *A. flos-aquae* abundance and distribution in the Sacramento-San Joaquin Delta. However, other variables not considered in this study (e.g. DON, stratification, residence time, grazing) may also control cyanobacterial dominance and toxicity (Lehman et al. 2008. Glibert et al. 2011) and should be investigated. Further work is needed to investigate the role of nitrogen sources on cyanobacterial success in the Sacramento-San Joaquin Delta. We are currently collaborating with Dr. Alex Parker on a companion study focused on investigating the role of zooplankton grazing as well as nitrogen availability and speciation on cyanoHABs in the Delta. This collaborative approach should shed some light on the environmental controls of cyanobacteria growth and toxicity in this system.

Clear Lake –

Our results suggest that the ability to mitigate cyanoHABs in Clear lake relies on more than one single nutrient. Indeed, although our results suggest that P is a key variable affecting the abundance of key cyanoHABs species (*Microcystis aeruginosa, Gloeotrichia echinulata*) occurrence and distribution. Our results also highlight the importance of other environmental variables such as temperature and nitrogen (nitrate+nitrite) for *Lyngbya hieronymusii* f. *robusta*.

Although phosphate load reductions are an important component of cyanoHABs mitigation, management strategies should take into account the biological influence on the translocation of this nutrient (as well as other nutrients) between the sediment and the water column as well as the modes of recruitment of cyanoHAB species. For example, due to its unique behavior, *Gloeotrichia echinulata* may have limited pelagic phosphorus uptake (Istvanovics et al. 1993) and may contribute to phosphorus translocation from sediments to the water column (Carey et al. 2008), possibly triggering other cyanobacteria blooms (Noges et al. 2004). As such, attempts to limit cyanoHABs by reducing in lake (pelagic)-nutrient concentrations could have little effect (Carey et al. 2008). It has been suggested that management strategies should focus instead on preventing or reducing recruitment of *G. echinulata* at the sediment level rather than at the water column level to prevent blooms (Carey et al. 2008). Management strategies focused at the sediment level rather than the pelagic level may also be more efficient in controlling blooms from benthic species such as *Lyngbya* spp.

The success of non-nitrogen fixers such as *M. aeruginosa* during summer N-limiting, P-replete conditions suggest that internal N-cycling might be an important environmental driver to consider. Previous studies indicate that *M. aeruginosa* thrives on both newly supplied and previously loaded N sources to maintain its dominance in eutrophic lake (Paerl et al. 2011). This observation highlights the need to examine internal nutrient cycling of both N and P. Indeed, although the emphasis has been placed on P load reduction, failure to control N input may result in continued eutrophication issues caused by not only non nitrogen-fixing but also nitrogen-fixing cyanobacterial blooms.

Other variables (light, DON, trace metals such as iron and sulfates) that may influence cyanoHABs occurrence and severity should be examined in future research programs. Previous research indicates that Clear Lake has experienced major anthropogenic stressors and changes over the past century that have resulted in increased inorganic sedimentation and may have contributed to chronic eutrophication and cyanoHABs issues in this system (Richerson et al. 2008). Strip mining at the Sulphur Bank Mercury Mine (1927-1957) has increased sulfate loading to Clear Lake water columns and sediments. Furthermore, increased sulfate in sediments has resulted in increased sulfate reducing bacteria activity which may have altered nutrient cycling via regeneration processes (Richerson et al. 2008). Water clarity has also increased in recent years (Winder et al. 2010) which may have favored the recruitment of benthic species such as *Lyngbya spp.* A recent study indicates that sulphur as well as irradiance are key environmental controls of both *M. aeruginosa* growth and microcystin production (Janichen et al. 2011). As such a better understanding of the processes occurring at the sediment-pelagic interface is critical for the implementation of management plans aiming at controlling cyanoHABs in Clear Lake. We will dedicate our future efforts (June 2012) to investigate the effect of these variables on Clear Lake cyanoHABs species.

Relevance of a combined approach using molecular/microscopy/chemical analyses

Combining complementary methodologies such as morphological taxonomy (microscopy), molecular analyses and toxicology analyses has been critical to gain a better understanding of cyanoHABs species composition and toxicity that can both be altered by various factors unique in the San Francisco Bay/Delta and in Clear Lake. Molecular analyses were powerful tools for this study enabling us to accurately identify some key cyanobacterial species that could not be identified using microscopy alone (e.g. *Lyngbya hieronymusii* f. *robusta* and *Aphanizomenon flos-aquae*). Indeed, the accurate identification of key harmful cyanobacteria species is important for gathering critical information from the literature and for the interpretation of environmental variables that regulate cyanoHABs structure and toxicity.

Analyzing more samples from different habitats and various stages of the bloom season in the future will help to generate a greater number of sequences representing a wide variety of cyanobacterial species present in the blooms. As DNA barcoding initially relies on taxonomic information, using parallel algal samples for morphologic taxonomic identification and molecular analyses is an ideal approach that can used in the future to better understand the cyanobacterial composition in Clear Lake and in the San Francisco Bay/Delta. Similarly, parallel environmental samples for cyanobacterial identification and toxicology analysis can provide cues

with regard to the toxigenicity of individual taxa. In addition, successful DNA barcoding depends on the availability of sequences from the database. Most of the cyanobacterial sequences deposited in the NCBI-GenBank database originate from geographically distant locations such as Portugal, Japan, India, and Nordic countries (Lopes et al. 2012, Robertson et al. 2001, Ezhilarasi et al. 2009, Rajaniemi et al. 2005). Because local cyanobacteria such as *Microcystis aeruginosa* have been shown to be unique in the San Francisco Delta (Moisander et al. 2009), initial analysis using morphologically identified cyanobacteria inhabiting local habitats are critical for DNA barcoding of toxin producing cyanobacteria that may impact economically relevant habitats in the current study. The ribosomal DNA sequences of the key cyanobacterial species from Clear Lake and the San Francisco Bay/Delta can be used in the future for developing specific real-time quantitative PCR (qPCR) assays to identify and simultaneously quantify targeted cyanobacteria species and toxin genes. Developing specific qPCR assays will provide specific quantification of target DNA in an unknown sample by comparing with a standard curve with known samples of serially diluted DNA fragment.

In conclusion, results from this study highlight the importance of combining complementary techniques to enhance our understanding of the environmental drivers controlling cyanoHABs structure and toxicity. As such, combining molecular-based, microscopy-based and chemistry-based techniques can be used successfully in the future to investigate cyanobacterial bloom in Clear Lake and San Francisco Bay Delta and other habitats impacted by recurring algal blooms enabling us to:

1) Assess cyanobacterial species composition and abundance rapidly and precisely,

2) Determine the potential relationship between cyanobacterial composition, toxigicity and toxin concentrations across critical habitats (e.g. identification and quantification of *Aphanizomenon flos-aquae* and relevance to ambient toxin levels) and,

3) Enhance the current cyanobacterial monitoring efforts by identifying and quantifying small nondescript and potentially harmful cyanobacterial species (e.g. *Synechococcus*) that cannot be assessed by traditional morphological methods.

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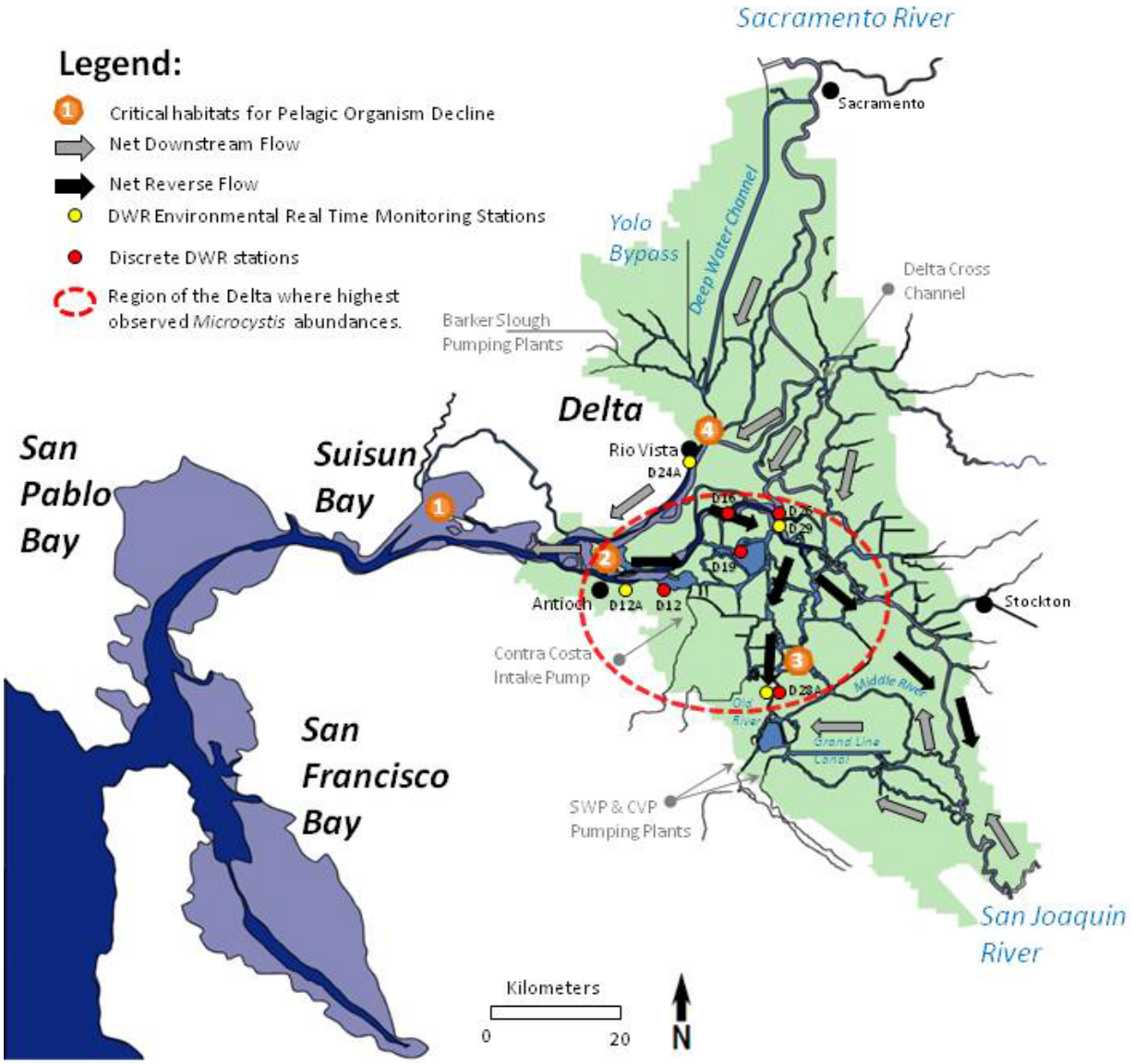

Figure 1- Map of the Sacramento-San Joaquin Delta and sampling sites locations. Due to space limitation, stations are identified by their DWR codes. Continuous stations: D24A/510SOL005 (Rio Vista), D12A/544CCC002 (Antioch), D28A/544CCC004 (Old River at Racho del Rio), D29/544SJC011 (Prisoners point). Discrete stations: D12/544CCC001 (Antioch ship channel), D16/544SAC004 (Twitchell Island), D19/544CCC003 (Frank's tract, flooded island), D26/544SJC010 (Potato point), D28A/544CCC004 (Old river at Rancho del Rio). Stations were selected that reflect different critical habitats within the delta: 1. Suisun Bay – habitat of Delta Smelt, Longfin smelt, Striped Bass, threadfin shad and Splittail; 2. Confluence of Sacramento and San Joaquin rivers near Sherman island where the highest abundance of Delta smelt has been observed; 3. San Joaquin Delta – seasonal habitat of Delta and habitat of Threadfin shad; 4. Sacramento river/Yolo bypass – habitat of Splittail. *Microcystis* blooms in the core summer habitat of the Threadfin shad and the Striped bass and might have shifted the distribution of the Delta smelt to higher salinity during late summer 2007.

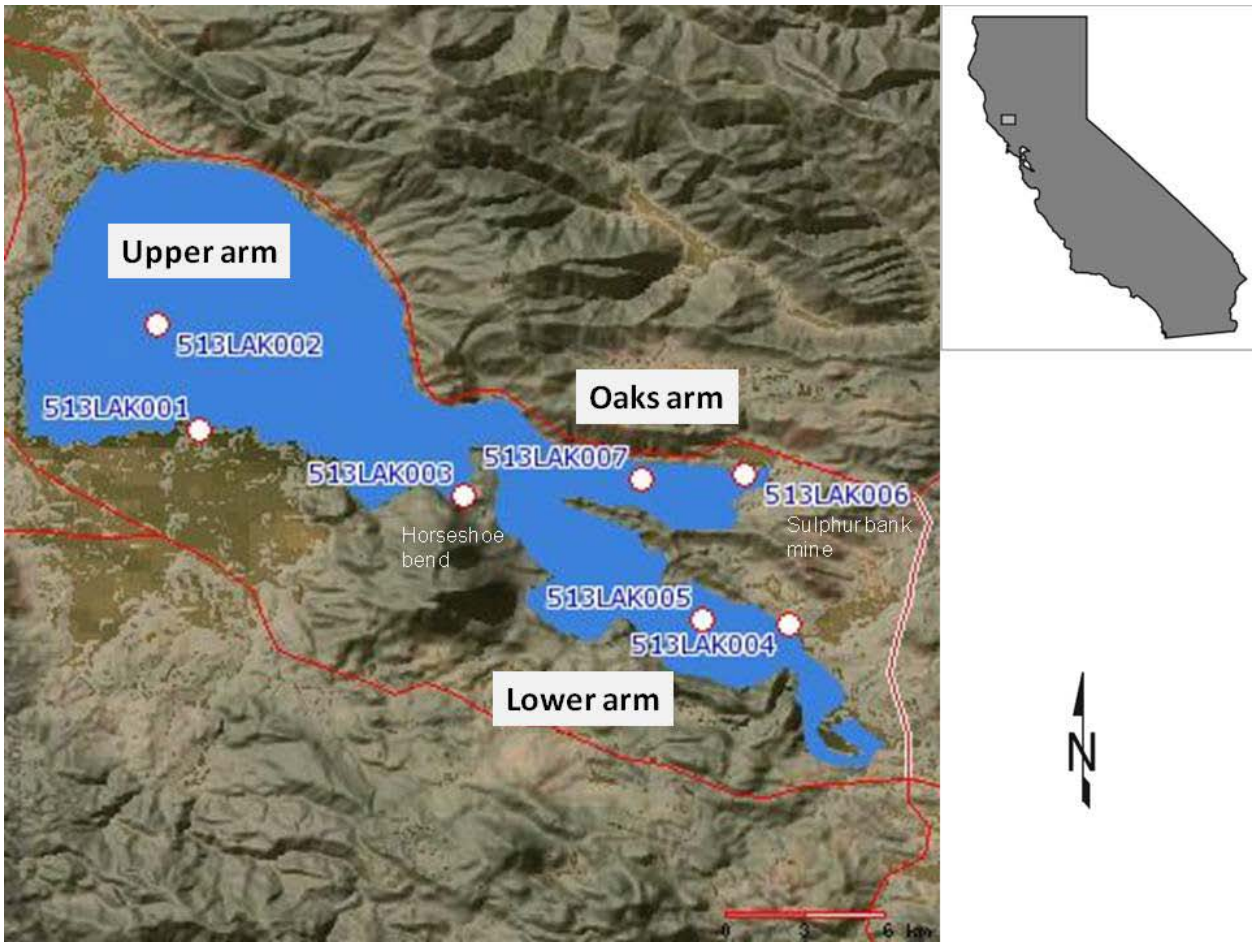

Figure 2 – Map of Clear Lake, CA and sampling site locations. Stations 513LAK002 (CL-1), 513LAK005 (CL-3) and 513LAK007 (CL-4) are offshore discrete stations located within each of the three arms of Clear Lake. These stations were selected because they have been monitored by various monitoring programs (e.g. DWR, CDHS, UC Davis) since the 1960's and therefore historical data are available at these locations for comparison (CDHS 1991, Richerson et al. 1994, Winder et al. 2010). The stations 513LAK001 (1), 513LAK003 (2), 513LAK004 (3) and 513LAK006 (4) are located near coastal buoys (county owned). These coastal stations were used as discrete and continuous (except 513LAK001, because the buoy disappeared between summer 2010 and summer 2011). SPATTs and Hobos were deployed at the coastal continuous stations to monitor toxins and temperature over time in between monthly sampling events.

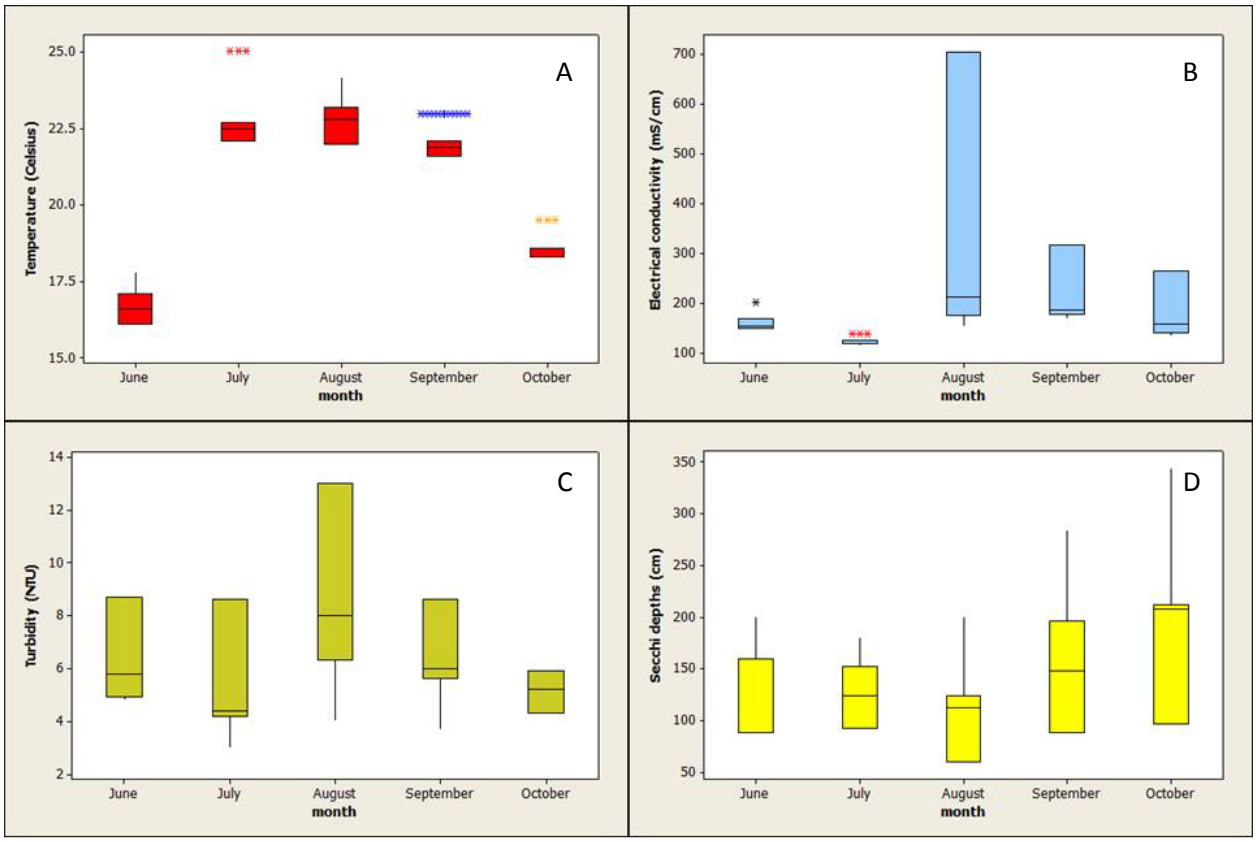

Figure 3: Box-whisker plots of environmental variables in the Sacramento-San Joaquin Delta during the study period (June – October 2011). A: Temperature, B: Electrical conductance, C: Turbidity, D: Secchi Depths.

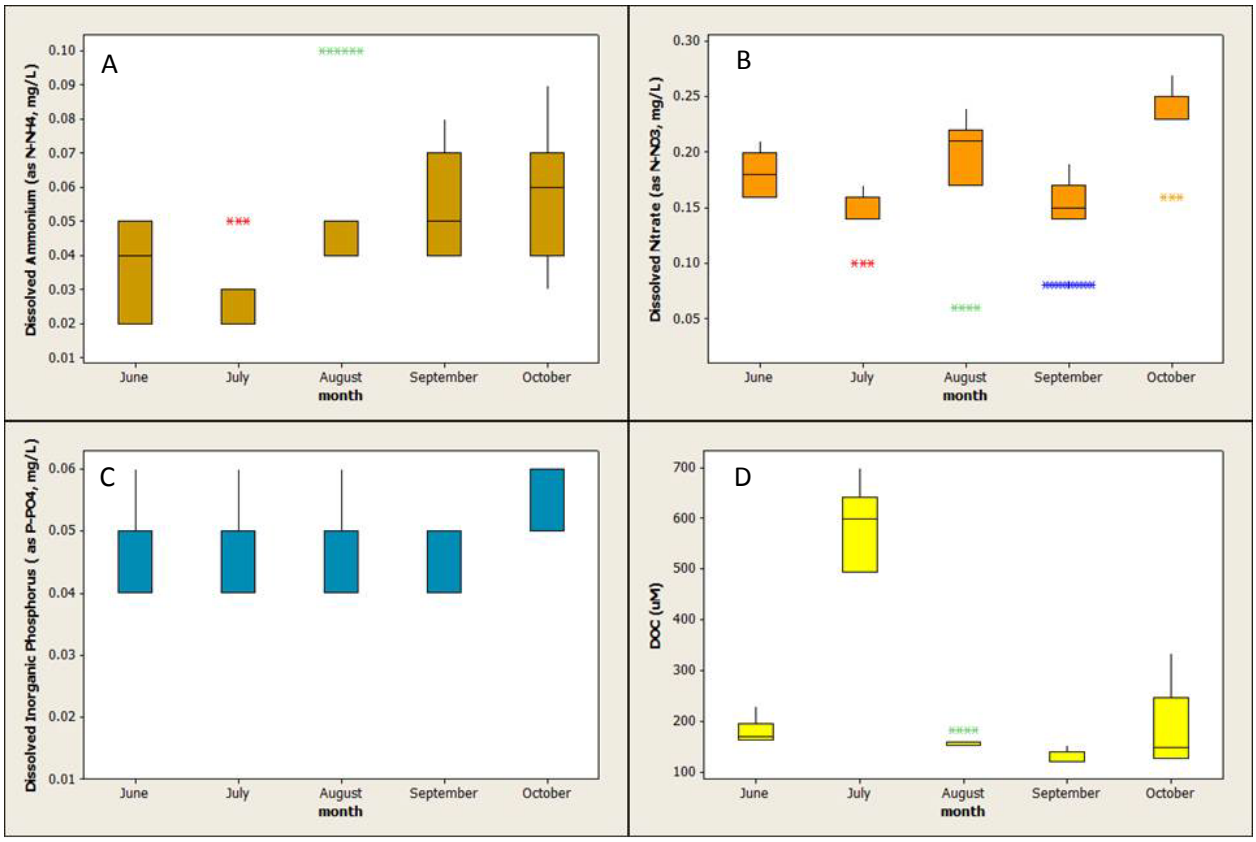

Figure 4: Box-whisker plot of dissolved inorganic nutrients and dissolved organic carbon (DOC) in the Sacramento-San Joaquin Delta during the study period (June – October 2011). A: N-NH4, B: N-NO3, C: P-PO4, D: DOC.

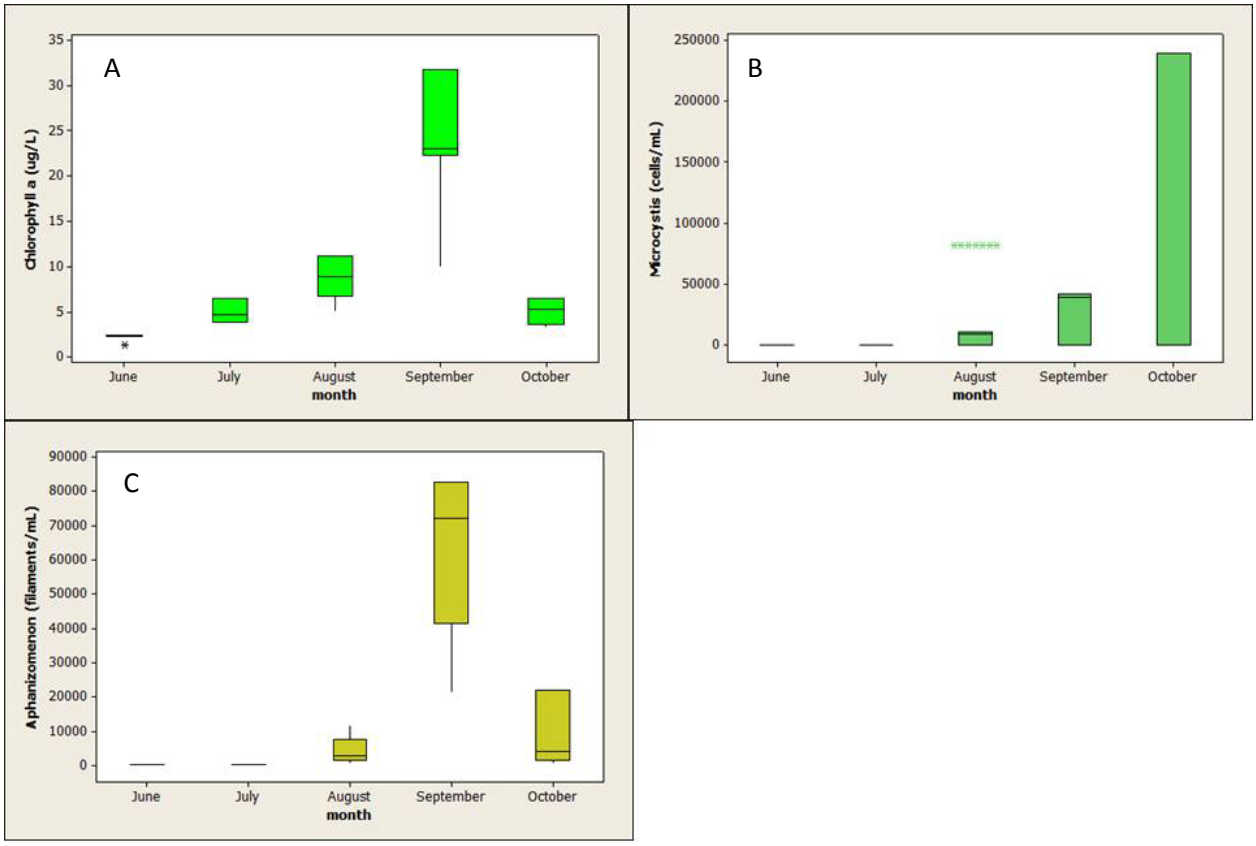

Figure 5: Box-whisker plot of biological variables in the Sacramento-San Joaquin Delta during the study period (June – October 2011). A: chlorophyll *a*, B: *Microcystis* abundance, C: *Aphanizomenon* abundance.

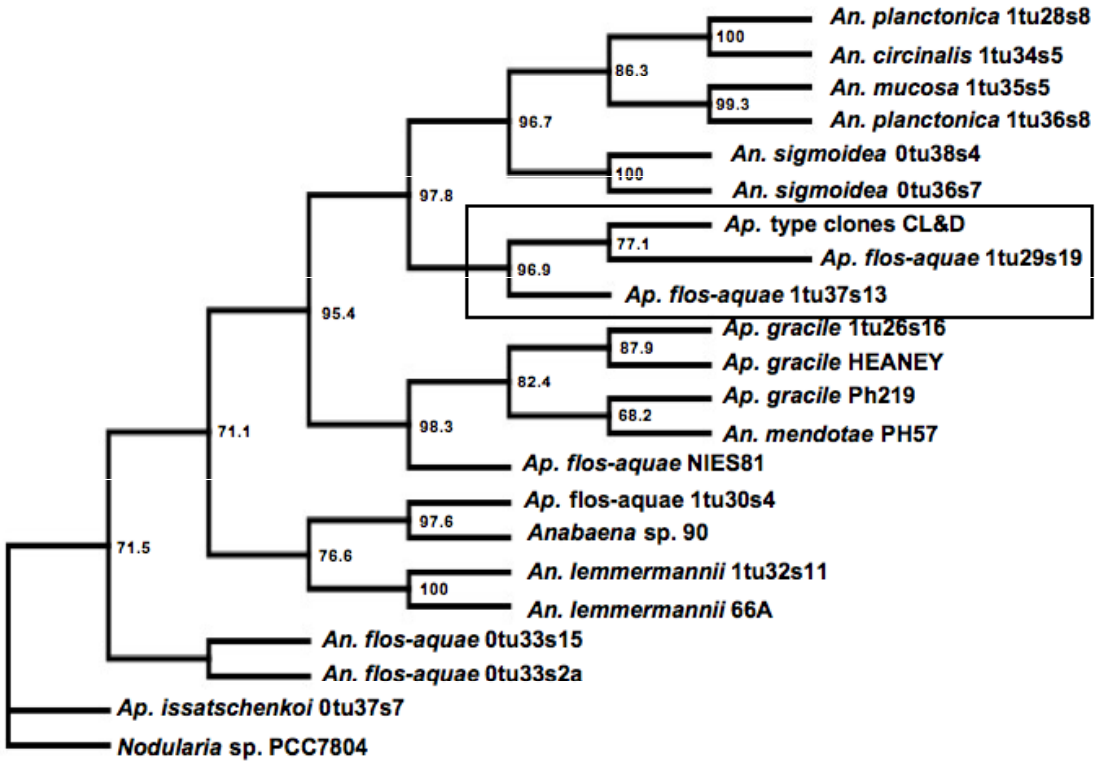

Figure 6: Phylogenetic tree for *Aphanizomenon* type clones obtained from Clear Lake and the Sacramento-San Joaquin Delta algal samples (*Ap*. type clones CL&D) with representative *Aphanizomenon* (*Ap.*) and *Anabaena* (*An.*) species described in Rajaniemi et al. (2005). A clade of *Aphanizomenon flos-aquae* including a type clone from the SWAMP project is indicated by box. Numbers at each node represent bootstrapping values of the neighbor joining analysis. Sequences from *Nodularia* sp. (strain PCC7804) and *Ap. issatschenkoi* were used as outgroups.

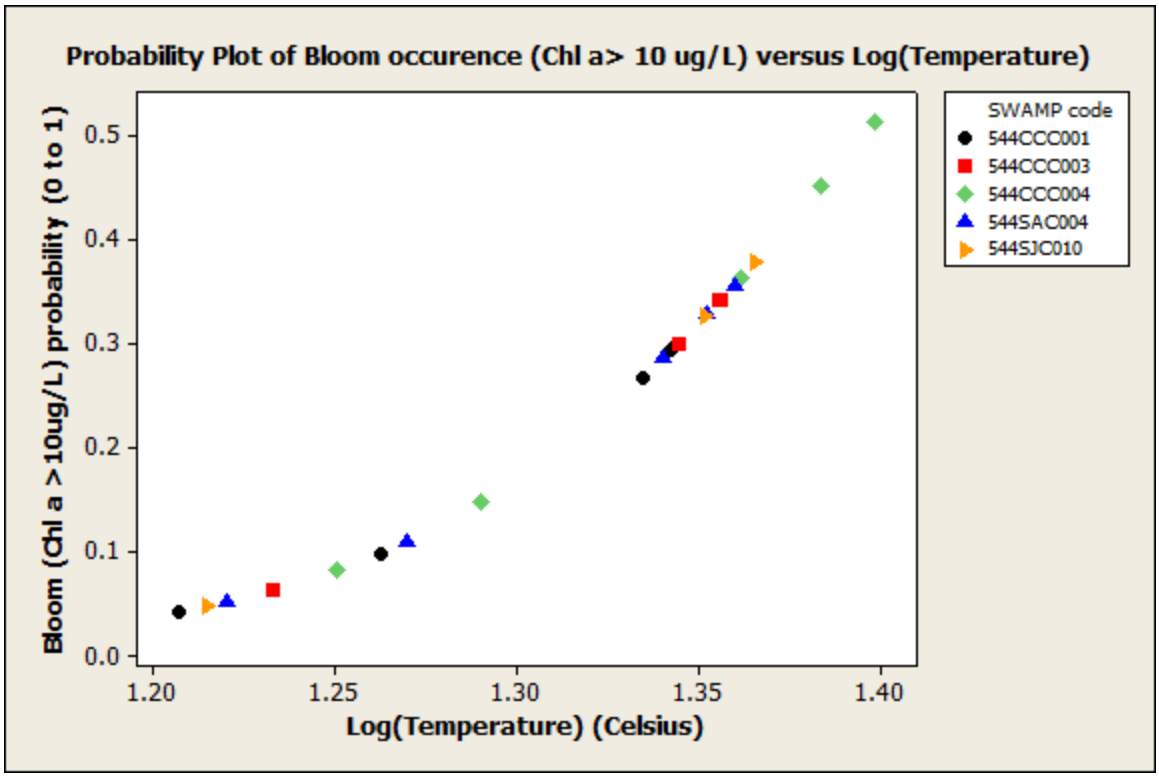

Figure 7: Binary logistic regression model testing the effect of temperature on bloom (in term of chlorophyll *a*) incidence in the Sacramento-San Joaquin Delta.

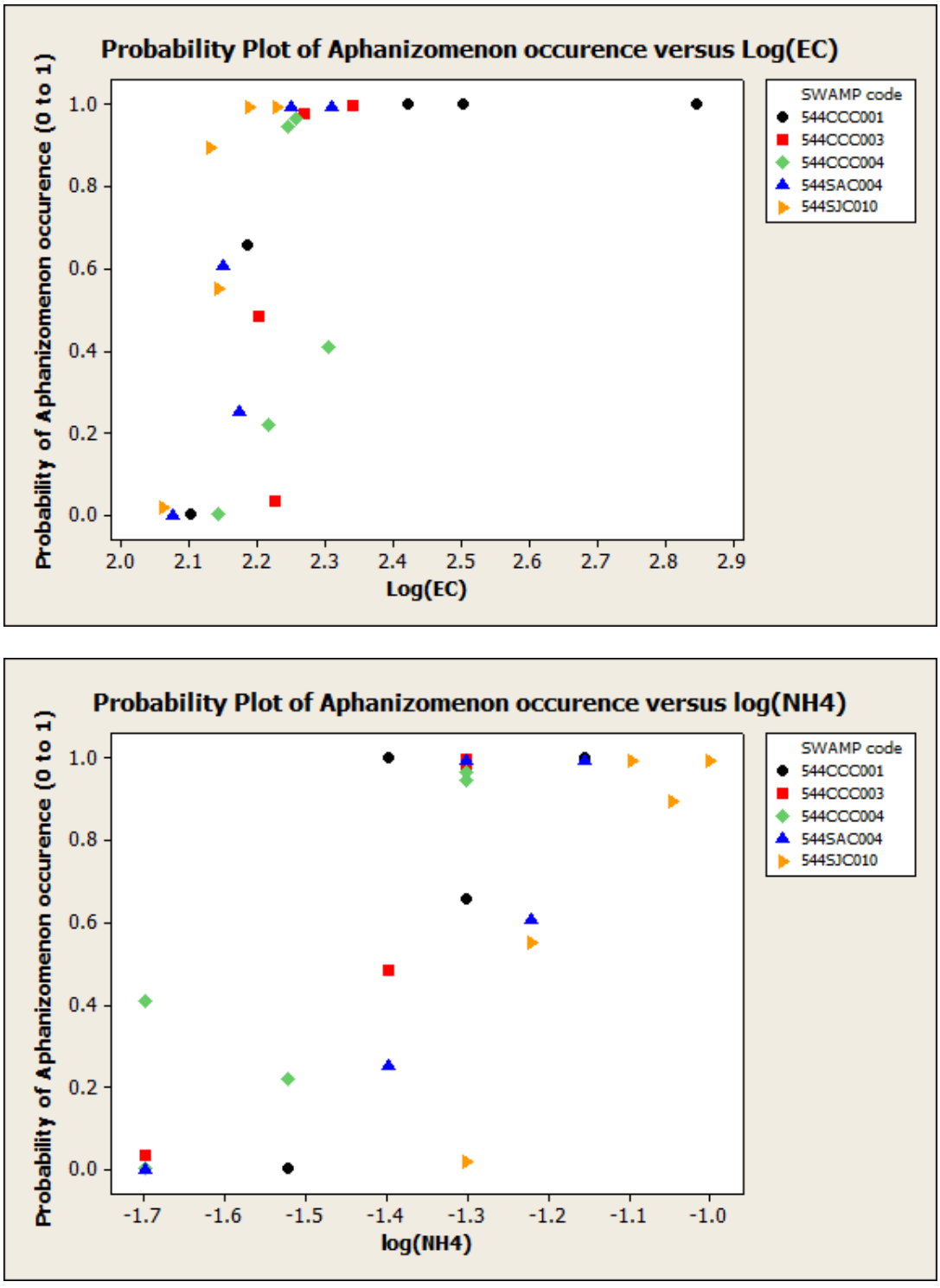

Figure 8: Binary logistic regression model testing the effect of electrical conductance (EC, top panel) and ammonium concentrations (NH4, bottom panel) on *Aphanizomon flos-aquae* incidence (presence/absence) in the Sacramento-San Joaquin Delta.

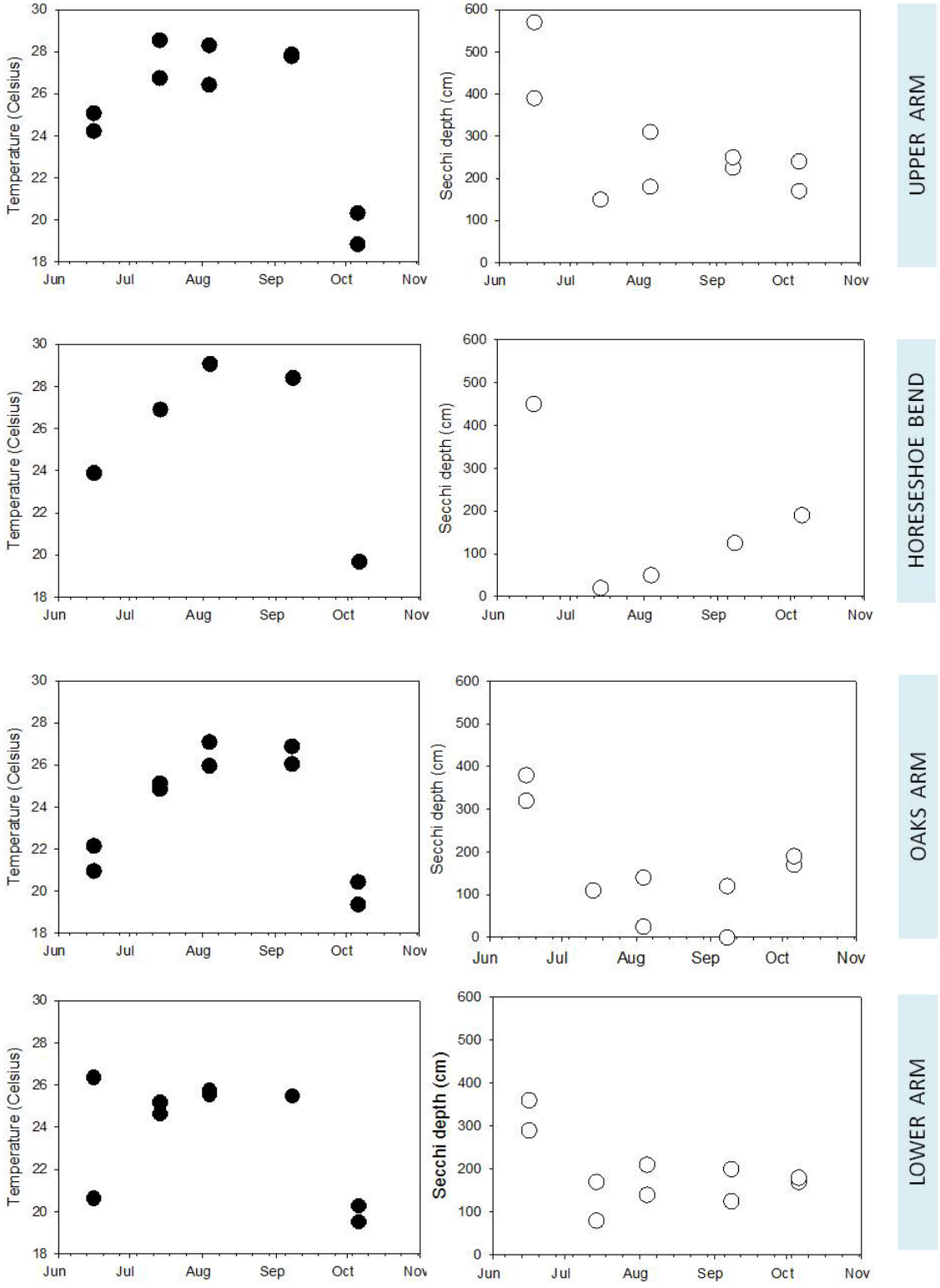

Figure 9: Surface water temperatures and Secchi depths time series in Clear Lake during June – October 2011.

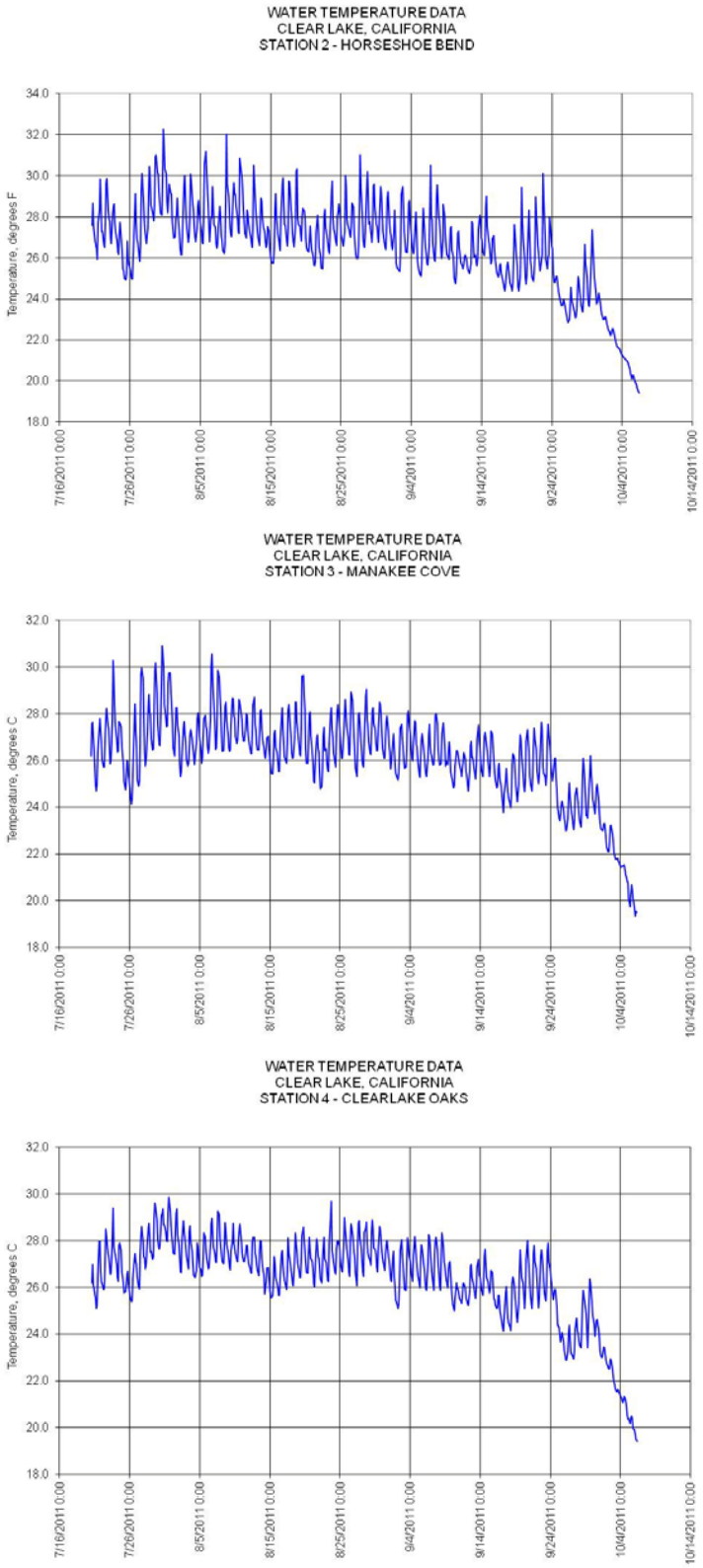

Figure 10: Continuous monitoring of water surface temperatures (Hobos) in Clear Lake between June and October 2011

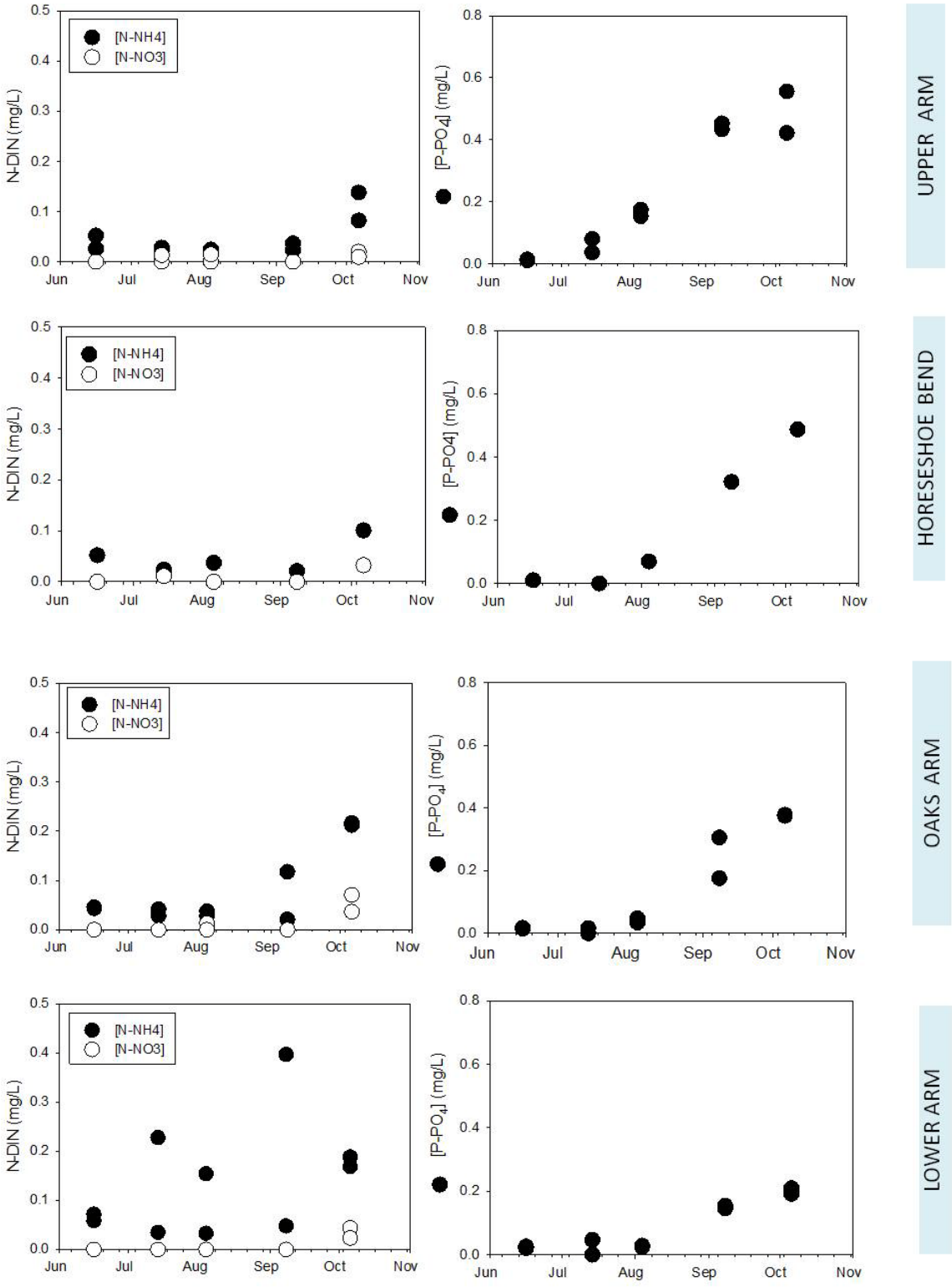

Figure 11: Dissolved inorganic nutrients (ammonium, nitrate and phosphate) time series in Clear Lake during June – October 2011.

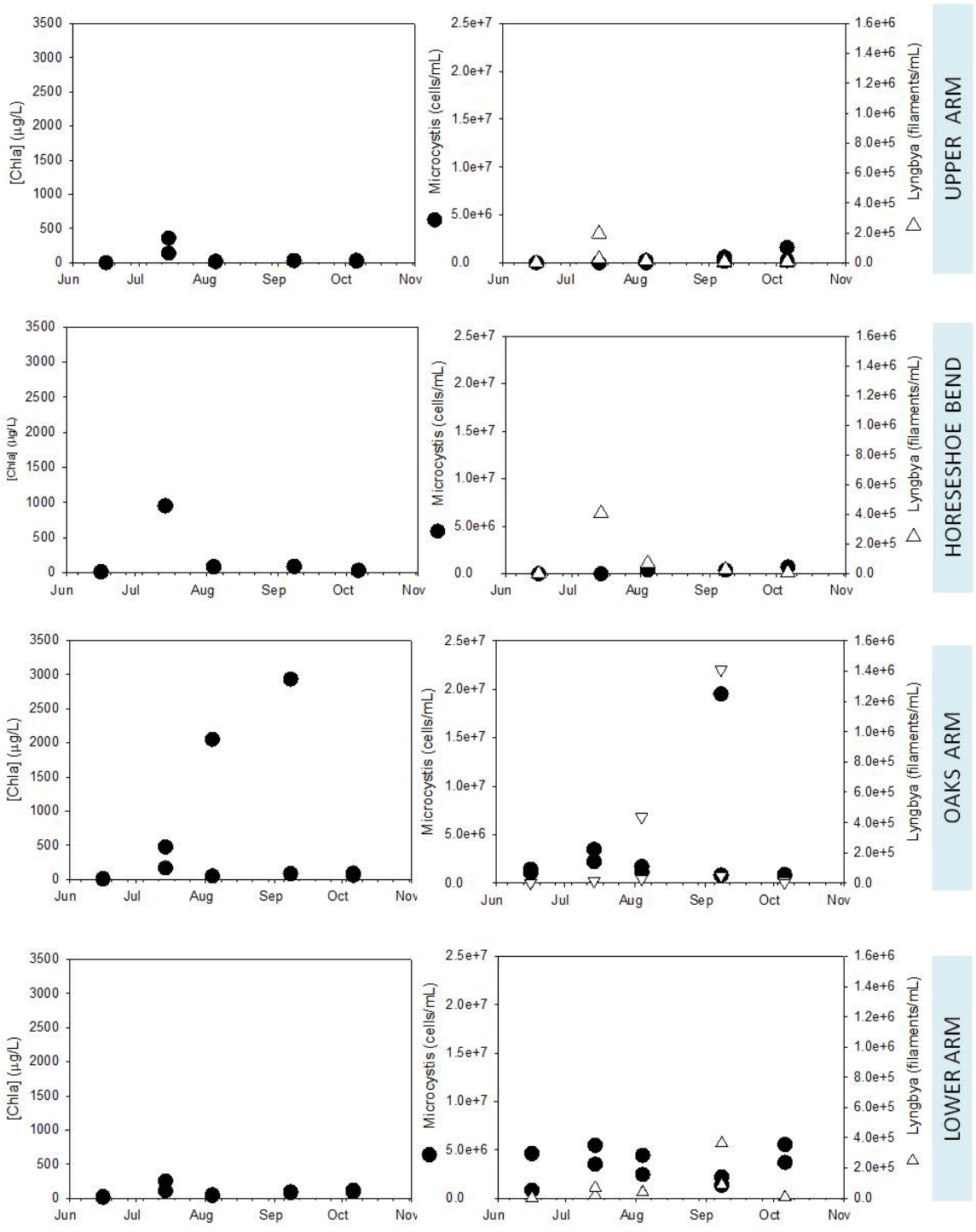

Figure 12: Biological variable time series in Clear Lake during June – October 2011.

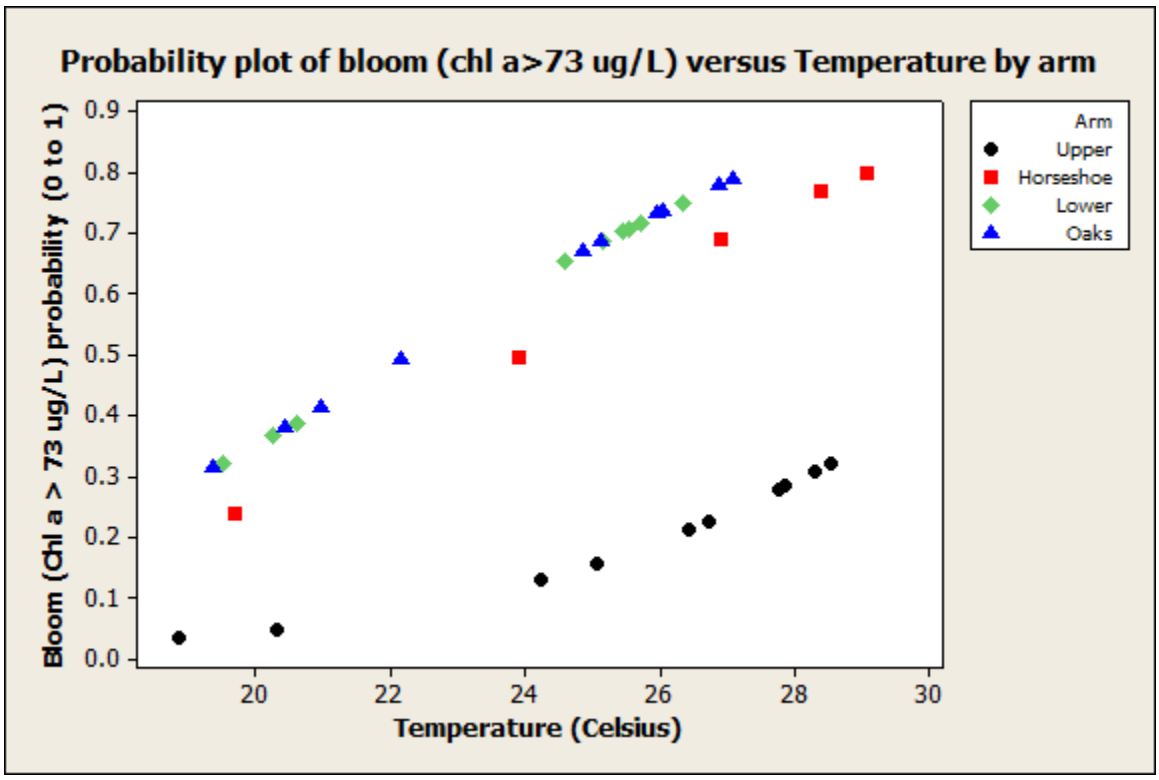

Figure 13: Binary logistic regression model testing the effect of surface water temperature by arm location on bloom (chl *a* > 73 µg/L) incidence in Clear Lake.

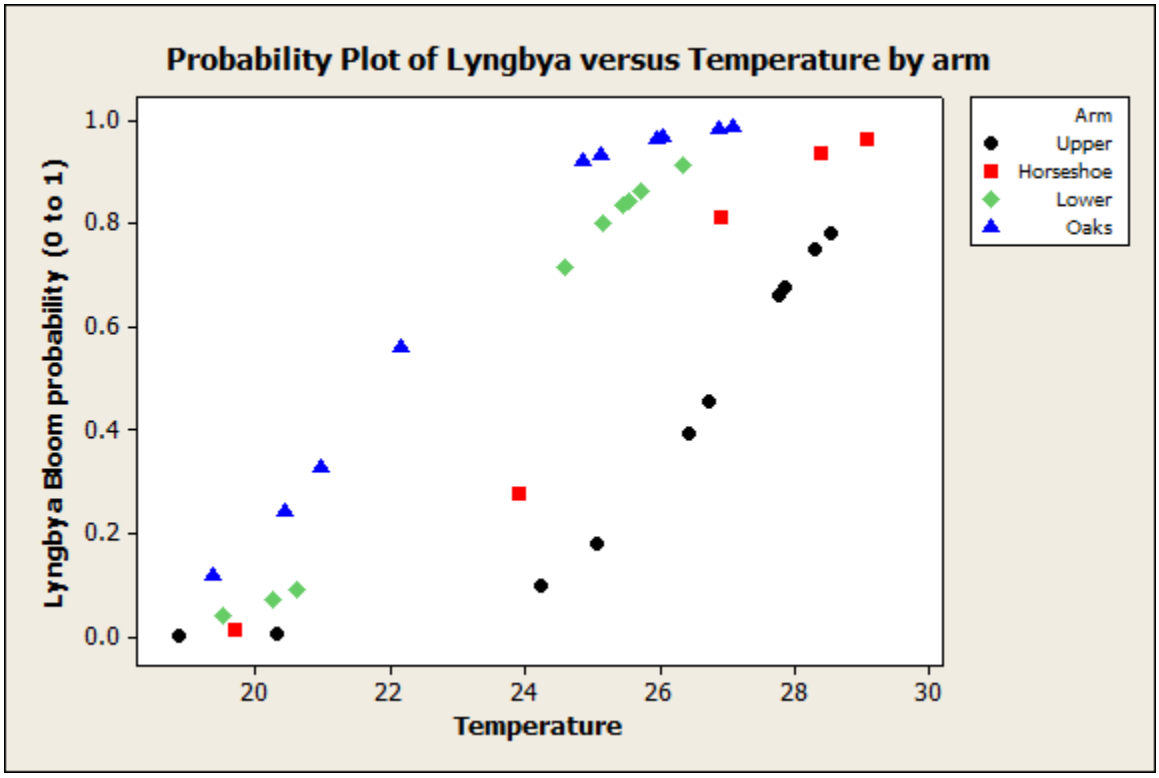

Figure 14: Binary logistic regression model testing the effect of temperature and arm location on *Lyngbya hieronymusii* f. *robusta* bloom (>10,000 filaments/mL) incidence in Clear Lake.

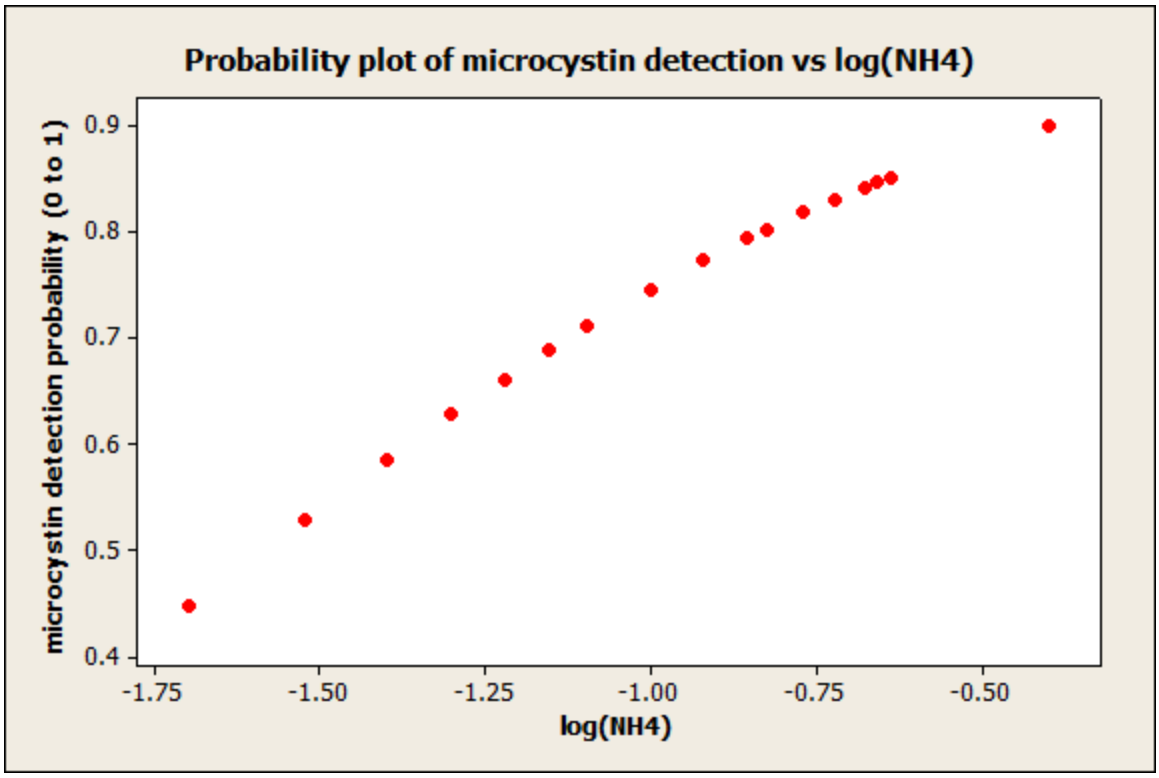

Figure 15: Binary logistic regression model testing the effect of ammonium concentration and arm location on microcystin toxin detection in Clear Lake.

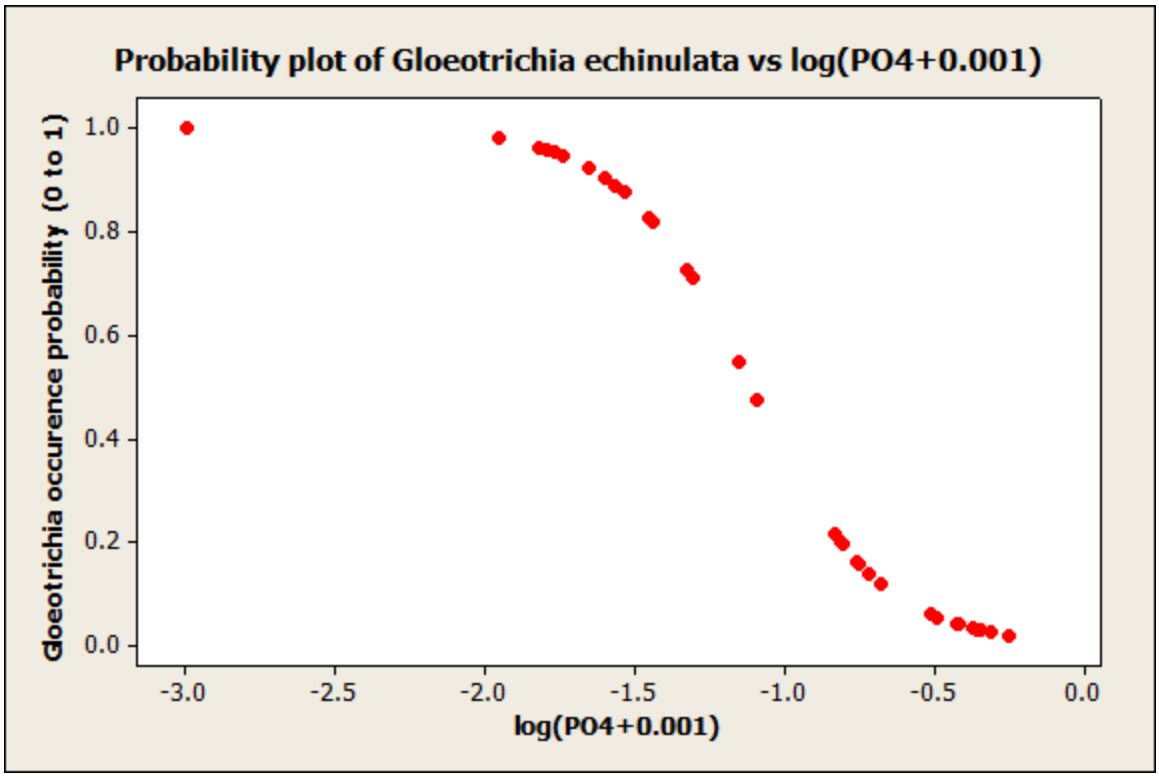

Figure 16: Binary logistic regression model testing the effect of orthophosphate concentration on *Gloeotrichia echinulata* occurrence (presence = 1, absence = 0) in Clear Lake.

Table 1 – Cyanotoxins and known producers (adapted from Carmichael 2001, Castle and Rodgers 2009, WHO 2003). Potentially toxigenic cyanobacteria strain recurrently observed in Clear Lake or the Delta are identified in bold characters.

Cyanotoxins	Known Producers	Mechanism of toxicity
Hepatotoxins		
Microcystins	***Microcystis, Anabaena****, Planktothrix (**Oscillaria**), Nostoc, Haplosiphon, Anabaenopsis, Nodularia, Gloeocapsa, Synechococcus, Eucapsis, Aphanocapsa, Rivularia, Entophysalis, Schizothrix, Phormidium, Microcoleus,* ***Woronichinia, Gloeotrichia.***	protein phosphatase blocker . Bindcovalently to protein phosphatases and cause hemorrhaging of the liver; chronic exposure may result in liver cancer
Nodularins	*Nodularia*	Same as microcystins
Cylindrospermopsins[1]	*Cylindrospermopsis,* ***Aphanizomenon****, Umezakia, Raphidiopsis*	Blocks protein synthesis; substantial cumulative toxicity
Neurotoxins		
Anatoxin-a	*Planktothrix (**Oscillaria**),* ***Anabaena,*** *Plectonema,* ***Aphanizomenon****, Rhaphidiopsis, Hyella*	Blocks post-synaptic depolarization
Anatoxin-a(S)	***Anabaena***	Blocks acetylcholiesterase
Saxitoxins	***Anabaena, Aphanizomenon, Lyngbya****, Cylindrospermopsis, Planktothrix (**Oscillatoria**)*	Blocks sodium channels
Dermatoxins		
Lyngbyatoxin-a	***Lyngbya,*** *Schizothrix, Planktothrix (**Oscillatoria**)*	Activates protein kinase C. Skin irritant and tumor promoter
Aplysiatoxins	***Lyngbya,*** *Schizothrix, Planktothrix (**Oscillatoria**), Microcoleus.*	Same as lyngbyatoxin-a
Lipopolysaccharides	***All cyanobacteria***	

[1] compared with other cyanotoxins, cylindrospermopsin can be found free in water even when cells are healthy (Carmichael 2001)

Table 2 – Hypotheses explaining the success of cyanobacteria in freshwater ecosystems (based on Dokulil and Teubner 2000).

Hypothesis	**Proposed advantageous traits explaining cyanobacteria success**	**References**
Elevated Temperature	Cyanobacteria have higher temperature optima as compared to other algae	Paerl & Huisman (2008)
Low Light	Some cyanobacteria have low light requirements as compared to other algae	Reynolds et al. (1981)
Low CO2/high pH	Cyanobacteria possess carbon concentration mechanisms (favored at high pH)	King (1970), Shapiro (1984)
Low TN:TP ratio	Cyanobacteria thrive at low TN:TP ratios. Some cyanobacteria (e.g. *Anabaena, Aphanizomenon*) can fix atmospheric nitrogen to match the phosphorus uptake when the supplies in dissolved inorganic nitrogen are depleted. Non-nitrogen fixing cyanobacteria (e.g. *Microcystis, Lyngbya*) have low nitrogen requirements or can migrate within the water column to access benthic nitrogen sources.	Schindler (1977) Smith (1983) Ferber et al. (2004)
Nitrogen speciation	Non-nitrogen fixing cyanobacteria are favored by ammonium-nitrogen while eukaryotic phytoplankton develops when nitrate-nitrogen is the main nitrogen source present. Nitrogen-fixing cyanobacteria are favored when nitrogen is depleted. High dissolved inorganic nitrogen (e.g. urea) favors cyanobacteria growth over other algal groups.	Blomquist et al. (1994) Glibert et al. (2011) Glibert et al. (2004)
Phosphorus reserves	Cyanobacteria migrating from the sediment to the water column can store internal phosphorus reserves as polyphosphates which favor them when external phosphorus is low.	Pettersson et al. (1993)
Trace metals	Cyanobacteria, especially nitrogen-fixers, have higher requirements for some trace metals as compared to eukaryotic phytoplankton. Nitrogen-fixation requires iron as a cofactor.	Reuter & Petersen (1987)
Buoyancy	Some cyanobacteria (e.g. *Microcystis*) bear gas-veicles which limit siking loss and enable them to accumulate at intermediate depth where conditions (e.g. nutrients) favor them or to rise to the water surface where light and carbon dioxide are available. Other cyanobacteria (e.g. *Aphanizomenon*) are more dependent on higher turbulence.	Knoechel & Kalff (1975) Reynolds (1987)
Grazing	Cyanobacteria mortality via zooplankton grazing is minimal due to large size of colonial and filamentous cyanobacteria, low lipid content and toxicity. Bivalve mollusk (e.g. *Corbula*) grazing may provide competitive advantage to colonial and filamentous cyanobacteria.	Lampert (1987) Glibert et al. (2011)
Allelopathy	Cyanobacteria secrete organic compounds which suppress the growth of other algae	Murphy et al. (1976) Keating (1978)
Toxin production	Toxigenic cyanobacteria affect natural grazers and other aquatic biota.	
Recruitment from sediments	Cyanobacteria overwinter in the sediments and are resuspended in the water column in Spring, seeding the surface when conditions become favorable.	Rengefors et al. (2004)
Oxygen depletion/anoxia	The increase of the anoxic zone during stratification promotes cyanobacteria recruitment	Trimbee & Prepas (1988)
Fish population composition	Planktivorous fish remove large zooplankton which reduces grazing pressure	Fott et al. (1980)

Table 3 – Relative toxicity between the most common microcystin variants. Molecular formula and intraperitoneal LD_{50}s of several of the commonly occurring microcystin variants (Rinehart et al. 1994).

Microcystin variant	Formula	LD-50 (µg.kg^{-1})
Microcystin-LR	$C_{49}H_{74}N_{10}O_{12}$	50
Microcystin-LA	$C_{46}H_{67}N_7O_{12}$	50
Microcystin-RR	$C_{49}H_{75}N_{13}O_{12}$	600
Microcystin-AR	$C_{49}H_{68}N_{10}O_{12}$	250
Microcystin-LY	$C_{52}H_{71}N_7O_{13}$	90
Microcystin-WR	$C_{54}H_{73}N_{11}O_{12}$	150-200

Table 4 – Station codes, site names, types of station and locations.

Station	SWAMP Codes	Latitude	Longitude	Station Name	Type of Station
Clear Lake					
1	513LAK001	39.02919	-122.85192	Lakeport	Discrete
CL-1	513LAK002	39.06457	-122.86616	Upper Arm	Discrete
2	513LAK003	39.00670	-122.76232	Horseshoe Bend	Discrete & Continuous
3	513LAK004	38.96371	-122.65200	Clearlake (City)	Discrete & Continuous
CL-3	513LAK005	38.96463	-122.68166	Lower Arm	Discrete
4	513LAK006	39.01380	-122.66691	The Keys	Discrete & Continuous
CL-4	513 LAK007	39.01238	-122.70184	Oaks Arm	Discrete
Sacramento/San Joaquin Delta					
D24A	510SOL005	38.15970	-121.68640	Rio Vista (SAC)	Continuous
D12	544CCC001	38.02161	-121.80630	Antioch Ship Channel (SJR)	Discrete
D12A	544CCC002	38.01800	-121.80200	Antioch (SJR)	Continuous
D19	544CCC003	38.04376	-121.61480	Frank's Tract (SJR - flooded island)	Discrete
D28A	544CCC004	37.97048	-121.57300	Old River at Rancho del Rio	Discrete & Continuous
D16	544SAC004	38.09690	-121.66910	Twitchell Island (SJR)	Discrete
D26	544SJC010	38.07664	-121.56690	Potato Point (SJR)	Discrete
D29	544SJC011	38.06600	-121.56200	Prisoners Point (SJR)	Continuous

SJR = San Joaquin River
SAC = Sacramento River

Table 5 – Summary of the Quality Control Samples for the UCSC laboratory analyses as well as completeness of sample analysis.

QA/QC type	Chla	$N\text{-}NH_4$	$N\text{-}NO_3$	$N\text{-}NO_2$	$P\text{-}PO_4$	DOC	Toxins (grab)	Toxins (SPATT)	Microscopy
Field dup	100.0%	100.0%	100.0%	100.0%	100.0%	100%	NA	NA	90%[a]
Lab Dup	100.0%	100.0%	100.0%	100.0%	100.0%	100%	100%	100%	NA
Matrix Spike	NA	100.0%	100.0%	100.0%	100.0%	100%	100%	100%	NA
Matrix Spike dup	NA	100.0%	100.0%	100.0%	100.0%	NA	100%	100%	NA
Trip Blank	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	NA	NA	100%
Lab blank	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100%	100%	100%
Total QA/QC	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100%**	**100%**	**96.7%**
Completeness[b]	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**98.33%**	**100.0%**	**78.5%**	**100%**

[a]Micrcoscopy: One of the field duplicate collected at station 544CCC003 (Delta) on August 19, 2011 had a RPD that exceeded the threshold of 25% for *Anabaena* cell count. The source of variation was due to the colonial nature of this organism as well as its low abundance. All the other field duplicates met the precision requirements.

[b]Completeness:

- one DOC sample was lost during transport (vial was cracked which could have resulted in contamination of the sample so it wasn't analyzed)
- 6 SPATTs were lost in Clear Lake in June and July due to extreme wind conditions. Despite our efforts to re-deploy replacement SPATTs in July using sturdier attachment devices, these SPATTs were also lost. In the future, securing the SPATTs to the chains of the buoys rather than to the buoy floater might reduce loss as the wave traction and turbulence might be reduced at greater depth. However, the goal of this study was to monitor cyanotoxin in surface and this is the reason why we chose to attach the SPATT within a foot depth.

Table 6 – Summary of the Quality Control for the field analyses

Sonde % Pass	**GPS**	**Depth**	**DO (%)**	**DO (mg/L)**	**Temp (°C)**	**EC (mS/cm)**	**pH**	**Turbidity (NTU)**	**Fluorescence**
Clear Lake(Lake County)									
SN 1MW025579	100.0	-	-	-	-	-	-	-	-
02E1047AV	-	100%	71.4	71.4	100.0	100.0	100.0	-	-
Sacramento-San Joaquin Delta (DWR)									
Raymarine RC320	100.0	-	-	-	-	-	-	-	-
06D2439 AE	-	100.0	100.0	100.0	100.0	100.0	100.0	-	-
06G2016 AA	-	-	100.0	100.0	100.0	100.0	100.0	-	-
5495 FRTD	-	-	-	-	-	-	-	-	100.0
6499 RTD	-	-	-	-	-	-	-	92.0	-
Total									
% Pass total	**100.0**	**100.0**	**83.33**	**83.33**	**100.0**	**100.0**	**100.0**	**92.0**	**100.0**

Note:
Despite repetitive attempts to recalibrate the Lake County probe in the field as well as changing the sensor in August and September, the readings remained suspicious (underestimated) and were therefore not included in the metadata base. The issue was solved in October, a new sensor was ordered and recalibrated and gave field measurements within the expected range.

Table 7 – Algal samples used for molecular studies showing the species composition of cyanobacterial species as determined by morphological identification. Data were taken from C. Mioni's Sept 2011 report.

Sampling date	Sample ID	Station ID	MSAE (cell/mL)	APHA (filament/mL)	LYNG (filmt/mL)	ANA (cells/mL)	GLOE (filmt/mL)
Sep-8	CL1(9)	513LAK002	153,468 (52.6%)	ND	5,888 (2.0%)	132,273 (45.4%)	ND
Jun-16	CL3(6)	513LAL005	819,540 (61.8%)	54,950 (4.1%)	2,355 (0.2%)	312,430 (23.6%)	136,983 (10.3%)
Jul-14	CL4(7)	513LAK007	2,219,588 (77.0%)	ND	15,700 (0.5%)	ND	648,606 (22.5%)
Aug-04	S1(8)	513LAK001	ND	245 (0.1%)	10,058 (4.7%)	179,814 (83.5%)	25,267 (11.7%)
Jul-14	S2	513LAK003	ND	ND	406,238 (20.9%)	516,138 (26.6%)	1,018,538 (52.5%)
Jul-07	D16(7)	544SAC004	ND	ND	ND	41,998 (100%)	ND
Sep-19	D16(9)	544SAC004	38,858 (48.4%)	41,409 (51.6%)	ND	ND	ND
Sep-19	D26(9)	544SJC010	ND	21,195 (100%)	ND	ND	ND

Clear Lake samples: CL1, CL3, CL4, S1, S2
San Francisco Delta samples: D16-7, D16-9, D26-9
MSAE: *Microcystis aeruginosa*
APHA: *Aphanizomenon* spp.
LYNG: *Lyngbya* spp.
ANA: *Anabaena* spp.
GLOE: *Gloeotrichia*

Table 8 – Primers used for molecular analyses of algal samples from selected sites in the Sacramento-San Joaquin Delta and Clear Lake.

Primer	Seq (5' - 3')	Target (size)	Reference
pA	AGAGTTTGATCCTGGCTCAG	Cyanobacterial 16S rDNA-ITS (1.5- 2kb)	Edwards et al. 1989
B23S	CTTCGCCTCTGTGTGCCTAGGT		Lepere et al. 2000
CYA108F	ACGGGTGAGTAACRCGTRA	Cyanobacterial 16S rDNA (1.5 kb)	Urbach et al. 2001
CYA16S SCYR	CTTCAYGYAGGCGAGTTGCAGC		
M13Fw	CACGACGTTGTAAAACGAC	Used for sequencing reactions	pGEM T vector®
M13Rv	GGATAACAATTTCACACAGG	Used for sequencing reactions	pGEM T vector®
AlgaeIDSqF1	CGCACGCAAGTGTGAAACTC	Used for sequencing reactions	Designed in this study
AlgaeIDSqF3	GCAAACAGGATTAGATACCC	Used for sequencing reactions	Designed in this study

Table 9 – World Health Organization guideline values for the relative probability of acute health effects during recreational exposure to cyanobacteria and microcystin toxins in freshwater systems (based on WHO 2003, Graham et al. 2009). These guideline thresholds were used to categorize our biological and toxicological data for statistical analyses and logistic modeling as well as to describe relative health risks. Because a TMDL threshold was available for Clear Lake for chlorophyll *a* (Tetra Tech 2004, 73 µg/L), we used this TMDL value as a basis for categorizing chlorophyll *a* data gathered in this system instead of the WHO guideline categories. Similarly, because these guidelines do not apply to large-sized filamentous cyanobacteria such as *Lyngbya*, the threshold of 10,000 filaments/mL was used to categorize bloom versus non bloom conditions in Clear Lake based on our field observations and microscopic analyses.

Relative probability of adverse health effects	Cyanobacteria (cells/mL)	Chl a (µg/L)	Microcystin (µg/L)	Health risks	Typical actions
Low	<20,000	<10	<10	Short-term adverse health outcomes (e.g. skin irritations, gastrointestinal illness)	Post on-site risk advisory signs Inform relevant authorities
Moderate	20,000 – 100,000	10 – 50	10 – 20	Same as above, and Potential for log-term illness with some cyanoHAB species	Same as above, and Watch for scums Discourage swimming and investigate hazard
High	100,000 – 10,000,000	50 – 5,000	20 – 2,000	Same as above, and, Potential for acute poisoing	Immediate action to control contact with possible scum; possible prohibition of swimming and other water contact activities Public health follow up activities
Very High	>10,000,000	> 5,000	> 2,000	Same as above	Same as above

Table 10 – Limnological characteristics in the Sacramento-San Joaquin Delta during the period of June-August 2011. The nutrient concentrations measured during this study were compared to the concentrations measured by the EMP/DWR group at the same stations and timepoints and were comparable. If the concentrations deviated from that determined by the EMP/DWR group, computations were double checked for mistake (e.g. offset correction) and if necessary, samples were reanalyzed. Stations where field duplicates were collected are identified with an asterisk, the values at these stations represent the average of the duplicates.

Date	Station	Temp. (°C)	pH	DO (mg/L)	EC (µS/cm)	Secchi (cm)	[N-NH4] (mg/L)	[N-NO3] (mg/L)	[N-NO2] (mg/L)	[P-PO4] (mg/L)	[DIN]/P	DOC (µM)	[Chla] (µg/L)
6/6/2011	544CCC001	16.10	7.13	8.77	153	88	0.05	0.18	0.01	0.05	4.61	168	2.38
6/7/2011	544SAC004	16.60	7.16	9.04	149	160	0.04	0.20	0.00	0.04	4.97	162	2.21
6/6/2011	544CCC003*	17.10	7.24	8.57	168	160	0.02	0.16	0.00	0.05	3.33	194	2.21
6/7/2011	544SJC010	16.40	7.14	8.79	139	156	0.06	0.21	0.01	0.04	5.80	156	0.85
6/7/2011	544CCC004	17.80	7.37	8.57	202	200	0.02	0.21	0.00	0.06	3.40	229	1.36
7/6/2011	544CCC001	22.09	7.60	7.95	126	92	0.03	0.14	ND	0.05	3.19	493	6.41
7/7/2011	544SAC004	22.50	7.00	7.83	119	120	0.02	0.16	ND	0.05	3.57	Lost	4.88
7/6/2011	544CCC003	22.69	7.87	8.29	119	152	0.02	0.14	ND	0.04	3.83	598	4.43
7/7/2011	544SJC010	22.49	7.26	7.46	115	128	0.05	0.17	ND	0.04	5.02	642	3.82
7/6/2011	544CCC004*	25.04	7.92	7.92	139	180	0.02	0.10	ND	0.06	1.68	699	3.88
8/19/2011	544CCC001	22.00	7.50	8.00	704	60	0.04	0.22	0.00	0.05	5.11	159	11.10
8/18/2011	544SAC004	22.90	7.34	7.80	204	112	0.05	0.21	0.00	0.04	5.64	151	8.83
8/19/2011	544CCC003*	22.70	7.43	7.80	219	124	0.05	0.17	0.00	0.05	3.75	150	7.61
8/18/2011	544SJC010	23.20	7.11	7.60	154	120	0.10	0.24	0.01	0.04	6.86	157	6.68
8/19/2011	544CCC004	24.20	7.83	8.40	176	200	0.05	0.06	ND	0.06	1.78	182	4.98
9/20/2011	544CCC001	21.60	7.94	8.30	318	88	0.04	0.15	0.00	0.05	3.31	120	31.80
9/19/2011	544SAC004	21.90	7.88	8.30	178	196	0.07	0.17	0.01	0.04	5.06	139	22.33
9/20/2011	544CCC003*	22.10	8.06	8.47	186	148	0.05	0.14	0.00	0.04	3.81	120	23.01
9/19/2011	544SJC010	22.00	7.67	7.90	169	192	0.08	0.19	0.01	0.04	5.97	130	9.99
9/20/2011	544CCC004	23.00	8.28	8.70	181	284	0.05	0.08	ND	0.05	2.56	152	12.51
10/17/11	544CCC001	18.30	7.74	8.62	264	96	0.07	0.23	0.01	0.06	4.29	147	6.41
10/18/11	544SAC004	18.60	7.64	8.44	141	208	0.06	0.25	0.01	0.05	5.36	124	4.99
10/17/11	544CCC003	18.60	7.80	8.38	159	212	0.04	0.23	0.01	0.05	4.82	334	5.30
10/18/11	544SJC010*	18.60	7.80	8.52	135	184	0.09	0.27	0.01	0.05	6.91	125	3.59
10/17/11	544CCC004	19.50	7.98	8.34	164	344	0.03	0.16	0.00	0.05	3.46	157	3.28

Table 11 – Cell identification, enumeration and cyanotoxin in discrete surface water samples in the Sacramento-San Joaquin Delta during the period of July-October 2011.

Date	Station	*Microcystis* (cell/mL)	*Aphanizomenon* (cell/mL)	Oscillatoriales (filament/mL)	*Anabaena* (cells/mL)	Anatoxin-a	MCY-LR	MCY-RR	MCY-YR	CYL	PSP
6/6/2011	544CCC001	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6/7/2011	544SAC004	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6/6/2011	544CCC003*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6/7/2011	544SJC010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6/7/2011	544CCC004	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7/6/2011	544CCC001	ND	ND	ND	13738	ND	ND	ND	ND	ND	ND
7/7/2011	544SAC004	ND	ND	ND	41998	ND	ND	ND	ND	ND	ND
7/6/2011	544CCC003	ND	ND	ND	12364	ND	ND	ND	ND	ND	ND
7/7/2011	544SJC010	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7/6/2011	544CCC004*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8/19/2011	544CCC001	ND	2551	ND	ND	ND	ND	ND	ND	ND	ND
8/18/2011	544SAC004	8635	1374	393	ND	ND	ND	ND	ND	ND	ND
8/19/2011	544CCC003*	82131	7458	ND	14621	ND	ND	ND	ND	ND	ND
8/18/2011	544SJC010	ND	393	ND	ND	ND	0.28	ND	ND	ND	ND
8/19/2011	544CCC004	10401	11579	196	ND	ND	ND	ND	ND	ND	ND
9/20/2011	544CCC001	41409	82818	196	ND	ND	ND	ND	ND	ND	ND
9/19/2011	544SAC004	388588	41409	ND	ND	ND	ND	ND	ND	ND	ND
9/20/2011	544CCC003*	ND	72122	ND	3042	ND	ND	ND	ND	ND	ND
9/19/2011	544SJC010	ND	21195	ND	ND	ND	ND	ND	ND	ND	ND
9/20/2011	544CCC004	6673	61426	196	3729	ND	ND	ND	ND	ND	ND
10/17/2011	544CCC001	240014	21980	ND	ND	ND	ND	ND	ND	ND	ND
10/18/2011	544SAC004	ND	1570	ND	ND	ND	ND	ND	ND	ND	ND
10/17/2011	544CCC003	ND	3925	ND	ND	ND	ND	ND	ND	ND	ND
10/18/2011	544SJC010*	ND	393	ND	ND	ND	ND	ND	ND	ND	ND
10/17/2011	544CCC004	ND	13541	393	ND	ND	ND	ND	ND	ND	ND

Table 12 – Molecular identification: Summary of cyanobacterial species and other bacteria found in Clear Lake and Sacramento-San Joaquin Delta. Numbers indicate the number of clones with specific sequences of 20 clones sequenced for each sample.

Date	Station ID	MS -AE	APHA -FL	LYNG -HIE[b]	LYNG -sp.	ANA -LM	ANA -sp.	SYN	BCL -PM	PNB -AL	PNB -sp.	FLV-TF	aPR	RHD-SPH	RHD-sp.	ALG -sp.	ND
Clear Lake																	
Jun-15	513LAK005	3	8			1			8								
Jul-15	513LAK003			1	1			2					12	1	1	1	
Jul-15	513LAK007									18	2						
Aug-15	513LAK001			6				12				1					
Sep-11	513LAK002							20									
Sacramento-San Joaquin Delta																	
Jul-15	544SAC004							13									2[c]
Sep-15	544SAC004		20														
Sep-15	544SJC010		13				7										

Notes:

The obtained 16S rDNA sequences were classified by BLASTN search and phylogenetic trees. Phylogenetic analysis was conducted for classifying closely related taxa such as *Anabaena* vs. *Aphanizomenon*, and *Lyngbya* vs. *Planktothrix*.

DNA sequences which cannot be classified at species level by molecular analysis are indicated as "sp". Further analysis is necessary such as isolation, microscopy, and sequencing for specific identification of these species with potentially unique sequences.

[a] Sequencing reaction did not work for 1 clone from S2(7) and S1(8), and from 5 clones from D16(7)

[b] Either *Lyngbya hieronymusii* or *Planktothrix cryptovaginata*, cannot distinguished by molecular analysis.

[c] These two sequences were designated as unidentified bacteria (maximum identity by BLASTN search <95%)

Table 13 – Solid Phase Toxin Tracking (SPATT) Device– amount of dissolved cyanotoxins adsorbed at the Sacramento-San Joaquin Delta continuous stations

Stations	Date deployed	Date retrieved	---------- ng/g resin ----------					---------- ng/g/day ----------				
			MCY-LR	MCY-RR	MCY-YR	MCY-LA	ANA-A	MCY-LR	MCY-RR	MCY-YR	MCY-LA	ANA-A
544CCC002	6/16/2011	7/13/2011	4.80	1.05	0	0	0	0.18	0.04	0	0	0
544CCC004	6/16/2011	7/13/2011	4.27	0	0	0	0	0.16	0	0	0	0
510SOL005	6/16/2011	7/13/2011	1.19	0	0	0	0	0.04	0	0	0	0
544SJC011	6/16/2011	7/13/2011	2.80	0	0	0	0	0.10	0	0	0	0
544CCC002	7/13/2011	8/15/2011	42.30	0	0	0	0	1.28	0	0	0	0
544CCC004	7/13/2011	8/15/2011	7.00	0	0	0	0	0.21	0	0	0	0
510SOL005	7/13/2011	8/15/2011	17.60	0	0	0	0	0.53	0	0	0	0
544SJC011	7/13/2011	8/15/2011	10.20	0	0	0	0	0.31	0	0	0	0
544CCC002	8/15/2011	9/9/2011	1596.79	10.15	0	683.59	0	63.87	0.41	0	27.34	0
544CCC004	8/15/2011	9/9/2011	768.75	4.34	0	245.02	0	30.75	0.17	0	9.80	0
510SOL005	8/15/2011	9/9/2011	-	-	-	-	-	-	-	-	-	-
544SJC011	8/15/2011	9/9/2011	252.35	0	0	91.30	0	10.09	0	0	3.65	0
544CCC002	9/9/2011	10/10/2011	227.13	16.67	0	119.81	0	7.33	0.54	0	3.86	0
544CCC004	9/9/2011	10/10/2011	279.16	12.15	0	170.73	0	9.01	0.39	0	5.51	0
510SOL005	9/9/2011	10/10/2011	35.32	10.18	0	0	0	1.14	0.33	0	0	0
544SJC011	9/9/2011	10/10/2011	113.58	12.89	0	55.00	0	3.66	0.42	0	1.77	0

Table 14 – ANOVA testing the effects of physico-chemical variables on chlorophyll *a* in the Sacramento-San Joaquin Delta during June-October 2011. Predicted plot had a R^2 of 0.65 (P=0.0272, RMSE = 0.2871). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). Only temperature was a statistically significant covariate of chlorophyll *a*.

Variable	Nparm	DF	Sum of squares	F ratio	Prob>F
Log(Temperature)	1	1	0.464	5.6270	**0.0326***
Log(EC)	1	1	0.302	3.6669	0.0762
Log(NTU)	1	1	0.001	0.0150	0.9041
Log(Secchi)	1	1	0.002	0.0276	0.8703
Log(NH4)	1	1	0.153	1.8553	0.1947
Log(NO3)	1	1	0.025	0.2993	0.5929
Log(PO4)	1	1	0.118	1.4323	0.2513
Log(DIN/DIP ratio)	1	1	0.052	0.6278	0.4414

Table 15 –ANOVA testing the effects of physico-chemical variables on *Aphanizomenon flos-aquae* abundance (transformed as Log_{10}(Aphanizomenon+1) in the Sacramento-San Joaquin Delta during June-October 2011. Predicted plot had a R^2 of 0.86 (P=0.0003, RMSE = 1.01). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). Only EC and Ammonium concentrations were statistically significant covariates of *Aphanizomenon flos-aquae* abundance.

Variable	Nparm	DF	Sum of squares	F ratio	Prob>F
Log(Temperature)	1	1	2.378	2.3528	0.1490
Log(EC)	1	1	13.578	13.4342	**0.0029****
Log(NTU)	1	1	0.598	0.5923	0.4553
Log(Secchi)	1	1	0.987	0.9763	0.3411
Log(NH4)	1	1	7.278	7.2003	**0.0188***
Log(NO3)	1	1	0.219	0.2171	0.6490
Log(PO4)	1	1	0.467	0.4615	0.5088
Log(DIN/DIP ratio)	1	1	0.384	0.3804	0.5481
Log(Microcystis+1)	1	1	3.328	3.2927	0.0927

Table 16 – ANOVA testing the effects of physico-chemical variables on *Microcystis* abundance (transformed as Log_{10}(Microcystis+1)) in the Sacramento-San Joaquin Delta during June-October 2011. Predicted plot had a R^2 of 0.44 (P=0.2894, RMSE = 1.99). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). No significant correlation was found.

Variable	Nparm	DF	Sum of squares	F ratio	Prob>F
Log(Temperature)	1	1	0.000	0.0000	0.9977
Log(EC)	1	1	8.563	2.1548	0.1642
Log(NTU)	1	1	1.529	0.3848	0.5450
Log(Secchi)	1	1	0.724	0.1822	0.6759
Log(NH4)	1	1	14.905	3.7509	0.0732
Log(NO3)	1	1	0.920	0.2316	0.6378
Log(PO4)	1	1	1.051	0.2646	0.6150
Log(DIN/DIP ratio)	1	1	4.151	1.0447	0.3241

Table 17 – Model specifications and diagnostics for the logistic regression models developed for the Sacramento-San Joaquin Delta. The likehood ratio test is a test of the null hypothesis that the predictor variable coefficients are zero (i.e. have no predictive value) and can be evaluated for significance as a deviate chi-squared. An odds ratio compares the odds of two events, where the odds of an event equals the probability the event occurs divided by the probability that it does not occur.

Model	**Bloom (chl *a* > 10µg/L)**	***Aphanizomenon* occurrence**
Response variable	Log(Chlorophyll a)	Log(Aphanizomenon+1)
Threshold	chla = 10µg/L	Log(Aphanizomenon+1)>0
Predictor variable(s)	Log(temperature)	Log(EC), Log(NH4)
N (total cases)	25	25
N (event cases)	6	15
Likehood-ratio statistics	-12.375	-5.747
p-value	0.094	0.000
Odds ratio	Log(temperature) = 16466289.87 (p = 0.148)	Log(EC): 6.385 E+15 (*p* = 0.104) Log(NH4): 791658.08 (*p* = 0.051)

Table 18 – Limnological characteristics in Clear Lake during the period of June-October 2011.

Date	Station	Temp. (°C)	pH	DO (mg/L)	EC (µS/cm)	Secchi (cm)	[N-NH4] (mg/L)	[N-NO3] (mg/L)	[N-NO2] (mg/L)	[P-PO4] (mg/L)	[DIN]/P	DOC (µM)	[Chla] (µg/L)
6/16/2011	513LAK001	24.22	8.25	6.05	278	>390	0.03	ND	ND	0.01	2.31	280	1.63
6/16/2011	513LAK002*	25.07	8.33	7.41	278	570	0.05	ND	ND	0.01	3.45	275	2.91
6/16/2011	513LAK003	23.89	8.24	7.38	279	450	0.05	ND	ND	0.01	4.65	276	10.22
6/16/2011	513LAK004	26.33	8.08	NA	296	290	0.06	ND	ND	0.02	2.56	304	15.84
6/16/2011	513LAK005	20.60	8.21	10.10	293	360	0.07	ND	ND	0.03	2.53	283	27.89
6/16/2011	513LAK006	22.14	8.13	7.58	295	320	0.04	ND	ND	0.02	2.61	277	14.51
6/16/2011	513LAK007	20.95	8.27	10.25	280	380	0.05	ND	ND	0.02	2.40	281	7.77
7/14/2011	513LAK001	28.54	8.99	8.60	281	N/A	0.02	ND	ND	0.04	0.60	1775	358.40
7/14/2011	513LAK002	26.74	8.79	8.24	284	150	0.03	0.01	ND	0.08	0.47	2143	140.61
7/14/2011	513LAK003	26.90	9.10	10.50	276	20	0.02	0.01	ND	ND	0.00	2005	952.63
7/14/2011	513LAK004	24.60	8.73	6.14	287	170	0.23	ND	ND	0.05	4.44	2312	112.22
7/14/2011	513LAK005	25.16	9.03	9.43	279	80	0.03	ND	ND	ND	0	2150	254.65
7/14/2011	513LAK006*	25.12	8.86	7.97	283	110	0.04	ND	ND	0.02	2.31	2281	476.30
7/14/2011	513LAK007	24.85	8.86	10.06	279	N/A	0.03	ND	ND	ND	0	2084	169.76
8/04/2011	513LAK001*	28.30	8.80	9.05	294	180	0.02	ND	ND	0.15	0.14	306	21.15
8/04/2011	513LAK002	26.42	8.66	8.38	293	310	0.02	0.01	ND	0.17	0.20	309	15.47
8/04/2011	513LAK003	29.06	8.83	8.98	295	50	0.04	ND	ND	0.07	0.49	349	82.45
8/04/2011	513LAK004	25.72	8.76	N/A	296	140	0.15	ND	ND	0.02	5.78	335	52.76
8/04/2011	513LAK005	25.54	8.89	N/A	289	210	0.03	ND	ND	0.03	1.07	317	43.46
8/04/2011	513LAK006	27.08	8.81	N/A	296	25	0.04	0.01	ND	0.05	0.95	366	2050.61
8/04/2011	513LAK007	29.95	8.98	9.84	285	140	0.03	ND	ND	0.03	0.75	319	51.98
9/08/2011	513LAK001	27.86	8.55	N/A	305	225	0.04	ND	ND	0.43	0.08	312	33.80
9/08/2011	513LAK002	27.78	7.43	N/A	305	250	0.02	ND	ND	0.45	0.05	285	30.65
9/08/2011	513LAK003	28.40	8.80	N/A	302	125	0.02	ND	ND	0.32	0.06	297	88.86
9/08/2011	513LAK004	25.45	8.12	N/A	316	125	0.40	ND	0.00	0.15	2.33	349	94.58
9/08/2011	513LAK005	25.45	8.42	N/A	309	200	0.05	ND	ND	0.15	0.30	359	80.93
9/08/2011	513LAK006*	26.88	8.71	N/A	306	0	0.12	ND	ND	0.18	0.60	480	2933.51
9/08/2011	513LAK007	26.04	8.60	N/A	301	120	0.02	ND	ND	0.31	0.06	279	86.63
10/6/2011	513LAK001*	18.84	8.37	7.19	355	240	0.08	0.02	ND	0.56	0.04	440	24.66
10/6/2011	513LAK002	20.32	8.20	6.57	359	170	0.14	0.01	ND	0.42	0.03	377	35.11
10/6/2011	513LAK003	19.68	8.41	6.46	357	190	0.10	0.03	ND	0.49	0.07	379	32.26
10/6/2011	513LAK004	19.50	7.72	7.03	368	170	0.17	0.04	ND	0.19	0.22	353	94.92
10/6/2011	513LAK005	20.26	7.90	6.84	367	180	0.19	0.02	ND	0.21	0.11	353	116.60
10/6/2011	513LAK006	19.36	8.10	7.5	359	170	0.22	0.07	0.00	0.37	1.09	338	93.97
10/6/2011	513LAK007	20.43	7.97	6.32	359	190	0.21	0.04	0.00	0.38	1.00	400	59.79

Table 19 – Cell identification, enumeration (as measured by epifluorescence microscopy, Cécile Mioni/UCSC) and toxin (as measured by LC/MS, Raphel Kudela/UCSC) in Clear Lake surface water samples collected in June-October 2011.

Date	Station	*MSAE* (cell/mL)	*APHA-FL* (cell/mL)	*Lyngbya* (filament/mL)	*ANA-SP* (cells/mL)	*Gloeotrichia* (filament/mL)	Ana-a	MCY-LR	MCY-RR	MCY-YR	MCY-LA
6/16/2011	513LAK001	ND	6280	393	44745	141300	ND	ND	ND	ND	ND
6/16/2011	513LAK002*	ND	5888	393	49455	111470	ND	ND	ND	ND	ND
6/16/2011	513LAK003	ND	27475	393	83603	281619	ND	ND	ND	ND	ND
6/16/2011	513LAK004	4612268	24335	1178	14523	208418	ND	*0.08*	ND	ND	ND
6/16/2011	513LAK005	819540	54950	2355	312430	136983	ND	0.20	ND	ND	ND
6/16/2011	513LAK006	1434195	4710	1178	ND	142870	ND	*0.17*	ND	ND	ND
6/16/2011	513LAK007	1059750	10990	1963	7850	51418	ND	ND	ND	ND	ND
7/14/2011	513LAK001	ND	ND	194288	735938	506325	ND	ND	ND	ND	ND
7/14/2011	513LAK002	ND	ND	24531	254144	535763	ND	ND	ND	ND	ND
7/14/2011	513LAK003	ND	ND	406238	516138	1018538	ND	*0.07*	ND	ND	ND
7/14/2011	513LAK004	3512875	2944	18644	370913	209006	ND	*0.10*	ND	ND	ND
7/14/2011	513LAK005	5463600	981	69669	290450	653513	ND	*0.11*	ND	0.16	ND
7/14/2011	513LAK006*	3477059	1472	13247	266823	1364428	ND	*0.10*	0.03	ND	ND
7/14/2011	513LAK007	2219588	ND	15700	ND	648606	ND	ND	ND	ND	ND
8/04/2011	513LAK001*	ND	245	10058	179814	25267	ND	ND	ND	ND	ND
8/04/2011	513LAK002	214403	ND	12266	426353	ND	ND	ND	ND	ND	ND
8/04/2011	513LAK003	383669	ND	70650	212931	ND	ND	0.11	ND	ND	ND
8/04/2011	513LAK004	4425438	981	39250	101069	981	0.43	0.54	ND	ND	ND
8/04/2011	513LAK005	2440369	981	38269	119713	ND	ND	0.36	ND	ND	ND
8/04/2011	513LAK006	1701488	1963	435675	308113	ND	ND	0.25	ND	ND	ND
8/04/2011	513LAK007	1136288	ND	28456	419975	1963	2.25	*0.14*	ND	ND	ND
9/08/2011	513LAK001	580508	2355	1963	178588	102050	ND	*0.17*	ND	ND	ND
9/08/2011	513LAK002	153468	ND	5888	132273	ND	ND	ND	ND	ND	ND
9/08/2011	513LAK003	365025	ND	27083	107153	ND	ND	*0.19*	ND	ND	ND
9/08/2011	513LAK004	2190150	ND	363063	109900	ND	ND	1.09	*0.14*	ND	ND
9/08/2011	513LAK005	1324688	3925	87331	273769	ND	ND	0.87	ND	ND	ND
9/08/2011	513LAK006*	19507250	44156	1408094	706500	ND	ND	29.61	3.30	11.08	ND
9/08/2011	513LAK007	835044	3925	49063	228631	ND	ND	0.61	*0.08*	ND	ND
10/6/2011	513LAK001*	232949	393	3140	176233	ND	ND	ND	ND	ND	ND
10/6/2011	513LAK002	1580205	ND	6280	23943	ND	ND	ND	ND	ND	ND
10/6/2011	513LAK003	715528	1178	7065	183690	ND	ND	ND	ND	ND	ND
10/6/2011	513LAK004	5538175	23550	6869	590713	ND	ND	2.18	2.57	1.86	1.24
10/6/2011	513LAK005	3692444	2944	5888	384650	ND	ND	1.30	1.72	ND	ND
10/6/2011	513LAK006	883910	785	10205	56520	ND	ND	0.75	0.28	2.30	2.09
10/6/2011	513LAK007	804625	785	2748	88705	ND	ND	0.80	ND	ND	1.51

Table 20 – Solid Phase Toxin Tracking (SPATT) Device– amount of dissolved cyanotoxin adsorbed at Clear Lake continuous stations. (SPATT deployed in June and July were lost due to extreme wind conditions)

Station	Date deployed	Date retrieved	---------- ng/g resin ----------					---------- ng/g/day ----------				
			MCY-LR	MCY-RR	MCY-YR	MCY-LA	ANA-A	MCY-LR	MCY-RR	MCY-YR	MCY-LA	ANA-A
513LAK003	8/4/2011	9/8/2011	39.53	11.71	0	0	0	1.13	0.33	0	0	0
513LAK004	8/4/2011	9/8/2011	216.45	9.37	0	0	0	6.18	0.27	0	0	0
513LAK006	8/4/2011	9/8/2011	255.33	1.70	0	0	0	7.30	0.05	0	0	0
513LAK003	9/8/2011	10/6/2011	757.80	21.44	9.84	9.75	0	27.06	0.77	0.35	0.35	0
513LAK004	9/8/2011	10/6/2011	1782.34	42.32	16.87	17.02	0	63.65	1.51	0.60	0.61	0
513LAK006	9/8/2011	10/6/2011	1467.74	32.70	24.82	19.79	0	52.42	1.17	0.89	0.71	0

Table 21 – ANCOVA testing the effects of physico-chemical variables on chlorophyll *a* in Clear Lake during June-October 2011. Predicted plot had a R^2 of 0.55 (P=0.0014*, RMSE = 0.5041). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). Temperature, Nitrate and Arm location were statistically significant covariates of chlorophyll *a*.

Variable	Nparm	DF	Sum of squares	F ratio	Prob>F
Temperature	1	1	5.5473858	20.2158	**0.0001****
Log(NH4)	1	1	0.2686018	0.9788	0.3313
Log(NO3+0.001)	1	1	3.6638465	13.3518	**0.0011****
Log(PO4+0.001)	1	1	0.1209110	0.4406	0.5124
Arm	3	3	3.5651670	4.3307	**0.0129***

Table 22 – ANCOVA testing the effects of physico-chemical variables on *Lyngbya hieronymusii* f. *robusta* in Clear Lake during June-October 2011. Predicted plot had a R^2 of 0.54 (P = 0.002*, RMSE = 0.68449). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). Model shows that temperature and Nitrate+Nitrite were statistically significant covariates of L. *hieronymusii* f. *robusta*abundance. Arm location was a marginally significant covariate.

Variable	Nparm	DF	Sum of squares	F ratio	Prob>F
Temperature	1	1	11.395776	24.3226	**<0.0001****
Log(NH4)	1	1	0.342931	0.7319	0.3998
Log(NO3+0.001)	1	1	3.563441	7.6056	**0.0103***
Log(PO4+0.001)	1	1	0.004753	0.0101	0.9205
Arm	3	3	3.939263	2.8026	0.0589

Table 23 – ANCOVA testing the effects of physico-chemical variables on *Microcystis aeruginosa* in Clear Lake during June-October 2011. Predicted plot had a R^2 of 0.62 (P=0.0002*, RMSE = 1.7466). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). Orthophosphate and Arm location were statistically significant covariates of *M. aeruginosa*.

Variable	Nparm	DF	Sum of squares	F ratio	Prob>F
Temperature	1	1	0.942669	0.3090	0.5829
Log(NH4)	1	1	0.609498	0.1998	0.6585
Log(NO3+0.001)	1	1	0.852703	0.2795	0.6013
Log(PO4+0.001)	1	1	30.872511	10.1195	**0.0037***
Arm	3	3	83.854456	9.1620	**0.0002****

Table 24 – ANCOVA testing the effects of physico-chemical variables on *Gloeotrichia echinulata* in Clear Lake during June-October 2011. Predicted plot had a R^2 of 0.76 (P=0.0367*, RMSE = 1.9573). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). Orthophosphate was the only statistically significant covariates of *G. echinulata*

Variable	Nparm	DF	Sum of squares	F ratio	Prob>F
Log(Temperature)	1	1	0.22597	0.0799	0.7796
Log(NH4)	1	1	0.08619	0.0305	0.8627
Log(NO3+0.001)	1	1	2.13904	0.7565	0.3921
Log(PO4+0.001)	1	1	105.75230	37.4031	**<0.0001****
Arm	3	3	11.86670	1.3990	0.2646

Table 25 – ANCOVA testing the effects of physico-chemical variables on *Aphanisomenon flos-aquae* in Clear Lake during June-October 2011. Predicted plot had a R^2 of 0.353 (P = 0.0778, RMSE = 1.574974). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). The model was marginally significant. Temperature was the best explaining variable among the physico-chemical variables considered.

Variable	DF	Sum of squares	F ratio	Prob>F
Log(Temperature)	1	14.088424	5.6796	**0.0245***
Log(NH4)	1	0.258896	0.1044	0.7491
Log(NO3+0.001)	1	6.530316	2.6326	0.1163
Log(PO4+0.001)	1	0.541811	0.2184	0.6440
Arm	3	6.584032	0.8848	0.4614

Table 26 – ANCOVA testing the effects of physico-chemical variables on *Anabaaena lemmermanii* in Clear Lake during June-October 2011. Predicted plot had a R^2 of 0.3807 (P = 0.0502, RMSE = 1.154081). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). Temperature and orthophosphate were the best explaining variable among the physic-chemical variables considered but were only marginally significant covariates of *A. lemmermanii* abundance.

Variable	DF	Sum of squares	F ratio	Prob>F
Log(Temperature)	1	5.0125119	3.7634	0.0629
Log(NH4)	1	7.6205E-10	0.0000	1.0000
Log(NO3+0.001)	1	3.7836467	2.8408	0.1034
Log(PO4+0.001)	1	4.2432725	3.1859	0.0855
Arm	3	7.1882126	1.7990	0.1712

Table 27 – ANCOVA testing the effects of environmental variables on microcystin toxins in Clear Lake during June-October 2011. Predicted plot had a R^2 of 0.773016 (P = 0.0001, RMSE = 0.383556). Statistically significant values are indicated in bold (*=p < 0.05, ** = p < 0.01). The *Gloeotrichia echinulata* abundance was the only significant covariate for microcystin toxins (indirect correlation). Ammonia, Arm location and *Aphanizomenon flos-aquae* abundance were marginally significant covariates.**

Variable	DF	Sum of squares	F ratio	Prob>F
Log(Temperature)	1	0.1602807	1.0895	0.3079
Log(NH4)	1	0.5357546	3.6417	0.0695
Log(NO3+0.001)	1	0.1122573	0.7631	0.3918
Log(PO4+0.001)	1	0.0113770	0.0773	0.7835
Arm	3	1.1976146	2.7136	0.0695
Log(Microcystis+1)	1	0.3250601	2.2096	0.1513
Log(Lyngbya+1)	1	0.2066095	1.4044	0.2486
Log(Gloeotrichia+1)	1	0.9360302	6.3626	**0.0194***
Log(Aphanizomenon+1)	1	0.4804948	3.2661	0.0844
Log(Anabaena+1)	1	0.0125257	0.0851	0.7732

Table 28 – Model specifications and diagnostics for the logistic regression models developed for Clear Lake. The likehood ratio test is a test of the null hypothesis that the predictor variable coefficients are zero (i.e. have no predictive value) and can be evaluated for significance as a deviate chi-squared. An odds ratio compares the odds of two events, where the odds of an event equals the probability the event occurs divided by the probability that it does not occur.

Model	**Bloom (chl *a* > 73 µg/L)**	***Lyngbya hieronymusii f. robusta***
Response variable	Log(Chlorophyll a)	Log(Lyngbya hieronymusii f. robusta)
Threshold	chla = 73µg/L	Log((Lyngbya hieronymusii f. robusta))>10,000
Predictor variable(s)	temperature, arm	temperature, arm
Categorical variable	arm	arm
N (total cases)	35	35
N (event cases)	17	20
Likehood-ratio statistics	-19.855	-13.158
p-value model	0.067	**0.000****
Odds ratio	Temperature =1.31 (p = 0.064) Arm (Horseshoe) = 7.33 (p = 0.121) Arm(Lower) = 11.50 (p = **0.034***) Arm(Oaks) = 11.63 (p = **0.035***)	Temperature: 2.24 (*p* = **0.003**) Arm(Horseshoe): 4.56 (p = 0.377) Arm(Lower): 16.97 (p = **0.045**) Arm(Oaks) = 62.34 (p = **0.028**)

Model	**Toxicity (mcy > 0 µg/L)**	***Gloeotrichia echinulata***
Response variable	Log(microcystin)	Log(Gloeotrichia + 1)
Threshold	Microcystins > 0µg/L	Log((Gloeotrichia))>0
Predictor variable(s)	Log(NH4)	Log(PO4+0.001)
Categorical variable	none	none
N (total cases)	35	35
N (event cases)	22	18
Likehood-ratio statistics	-21.510	-10.276
p-value model	0.075	**0.000****
Odds ratio	Log(NH4) =1.65 (p =0.099)	Log(PO4+0.001): 0.01 (*p* = **0.001****)

Appendix –

1. **Intercomparison of toxicology analyses**
2. **Greenwater lab taxonomy and toxicology analyses**
3. **EMP/DWR Monitoring Instrumentation**

CLEAR LAKE

Date	Station	Station ID	sample	method	MC-LR	MC-dLR	MC-RR	MC-dRR	MC-YR	MC-LA	MC-LW	MC-LF	MC-LY	Ana	Lab
8/4/2011	S2	513LAK003	sonicated	LCMS	ND	-	ND		ND	ND	-	-	-	ND	GW
8/4/2011	S2	513LAK003	sonicated	LCMS	*0.07*	-	ND	-	ND	ND	-	-	-	2.25	UCSC
8/4/2011	CL4	513LAK007	sonicated	LC/MSMS	26.0	3.09	54.8	12.0	7.33	10.5	0.252	0.262	0.111	N/D	FGRC
8/4/2011	CL4	513LAK007	sonicated	LCMS	0.1	-	ND	-	ND	ND	-	-	-	2.25	UCSC
9/8/2011	S1	513LAK001	sonicated	LCMS	*0.17*	-	ND	-	ND	ND	-	-	-	-	UCSC
9/8/2011	S1	513LAK001	not sonicated	ELISA	<0.4	-	-	-	-	-	-	-	-	-	LCWRD
9/8/2011	CL1	513LAK002	sonicated	LCMS	ND	-	ND	-	ND	ND	-	-	-	-	UCSC
9/8/2011	CL1	513LAK002	not sonicated	ELISA	<0.4	-	-	-	-	-	-	-	-	-	LCWRD
9/8/2011	S2	513LAK003	sonicated	LCMS	*0.19*	-	ND	-	ND	ND	-	-	-	-	UCSC
9/8/2011	S2	513LAK003	not sonicated	ELISA	<0.4	-	-	-	-	-	-	-	-	-	LCWRD
9/8/2011	S3	513LAK004	sonicated	LCMS	1.09	-	*0.14*	-	ND	ND	-	-	-	-	UCSC
9/8/2011	S3	513LAK004	not sonicated	ELISA	1.58	-	-	-	-	-	-	-	-	-	LCWRD
9/8/2011	CL3	513LAK005	sonicated	LCMS	1.09	-	*0.14*	-	ND	ND	-	-	-	-	UCSC
9/8/2011	CL3	513LAK005	not sonicated	ELISA	1.58	-	-	-	-	-	-	-	-	-	LCWRD
9/8/2011	S4	513LAK006	not sonicated	LC/MSMS	9.71	1.52	0.35	ND	0.31	1.69	ND	ND	ND	ND	FGRC
9/8/2011	S4	513LAK006	sonicated	LC/MSMS	2.56	0.39	0.08	ND	N/D	0.26	ND	ND	ND	ND	FGRC
9/8/2011	S4	513LAK006	sonicated	LC/MS	29.61	-	3.30	-	11.08	N/D	-	-	-	-	UCSC
9/8/2011	S4	513LAK006	not sonicated	ELISA	>2.5	-	-	-	-	-	-	-	-	-	LCWRD
9/8/2011	CL4	513LAK007	sonicated	LC/MS	0.61	-	*0.08*	-	ND	N/D	-	-	-	-	UCSC
9/8/2011	CL4	513LAK007	not sonicated	ELISA	1.90	-	-	-	-	-	-	-	-	-	LCWRD
10/6/2011	S2	513LAK003	sonicated	LCMS	ND	-	ND	-	ND	ND	-	-	-	-	UCSC
10/6/2011	S2	513LAK003	not sonicated	ELISA	<0.4	-	-	-	-	-	-	-	-	-	LCWRD
10/6/2011	S3	513LAK004	sonicated	LC/MSMS	0.79	ND	0.27	ND	0.17	0.64	ND	ND	ND	ND	FGRC
10/6/2011	S3	513LAK004	sonicated	LC/MS	2.18	-	2.57	-	1.86	1.24	-	-	-	-	UCSC
10/6/2011	S3	513LAK004	not sonicated	ELISA	2.40	-	-	-	-	-	-	-	-	-	LCWRD
10/6/2011	CL3	513LAK005	sonicated	LC/MSMS	0.66	ND	0.27	ND	0.21	0.44	ND	ND	ND	ND	FGRC
10/6/2011	CL3	513LAK005	sonicated	LC/MS	1.30	-	1.72	-	ND	ND	-	-	-	-	UCSC
10/6/2011	CL3	513LAK005	not sonicated	ELISA	1.75										LCWRD
10/6/2011	S4	513LAK006	sonicated	LC/MSMS	0.50	ND	0.04	ND	ND	1.72	ND	ND	ND	ND	FGRC
10/6/2011	S4	513LAK006	sonicated	LC/MS	0.75	-	0.28	-	2.30	2.09	-	-	-	-	UCSC
10/6/2011	S4	513LAK006	not sonicated	ELISA	0.65	-	-	-	-	-	-	-	-	-	LCWRD
10/6/2011	CL4	513LAK006	sonicated	LC/MS	0.80	-	ND	-	ND	1.51	-	-	-	-	UCSC
10/6/2011	CL4	513LAK006	not sonicated	ELISA	0.43	-	-	-	-	-	-	-	-	-	LCWRD
10/6/2011	Redbud	NA	sonicated	LC/MSMS	10.75	1.48	6.25	0.95	3.20	9.25	ND	ND	0.09	ND	FGRC
10/6/2011	Redbud	NA	sonicated	LC/MS	74.07	-	131.89	-	52.79	24.84	-	-	-	-	UCSC
10/6/2011	Redbud	NA	not sonicated	ELISA >2.5		-	-	-	-	-	-	-	-	-	LCWRD

DELTA

Date	Station	Station ID			MC-LR	MC-dLR	MC-RR	MC-dRR	MC-YR	MC-LA	MC-LW	MC-LF	MC-LY	Ana	Lab
9/20/2011	D12	544CCC001	sonicated	LC/MSMS	0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND	FGRC
9/20/2011	D12	544CCC001	sonicated	LC/MS	ND	-	ND	-	ND	ND	-	-	-	-	UCSC
9/19/2011	D16	544SAC004	sonicated	LC/MSMS	0.07	ND	ND	ND	ND	0.04	ND	ND	ND	ND	FGRC
9/19/2011	D16	544SAC004	sonicated	LC/MS	ND	-	ND	-	ND	ND	-	-	-	-	UCSC

Clear Lake Algal Analysis Report

Prepared: August 19, 2011
Prepared By: GreenWater Laboratories

Samples: 7 (Collected on 8/4/11)

1. CL1
2. CL3
3. CL4
4. STN1
5. STN2
6. STN3
7. STN4

Sample 1: CL1

Total cell numbers in the CL1 sample collected on 8/4/11 were 76,832 cells/mL. Blue-green algae (Cyanobacteria; 76,488 cells/mL) were the dominant algal group in the sample accounting for 99.6% of total cell numbers. Other algal groups in the sample were diatoms (Bacillariophyta; 89 cells/mL), green algae (Chlorophyta; 195 cells/mL), cryptophyes (Cryptophyta; 6 cells/mL) and microflagellates (Miscellaneous; 54 cells/mL). The most abundant species were picoplanktonic (<2.5µm in size) cyanophytes (44,233 cells/mL) and an unknown cyanophyte colony (26,765 cells/mL; Figs. 1-2). This cyanophyte colony most closely resembled *Aphanothece* or *Cyanodictyon*.

The density of *Lyngbya* sp. (Figs. 3-4) in the CL1 sample was 3,546 cells/mL. The *Lyngbya* species is from the complex of planktonic *Lyngbya* species containing aerotopes (gas vacuoles) that includes *L. hieronymusii*, *L. birgei* and L. *robusta*. Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 147 cells/mL (0.2% of total cell numbers). PTOX Cyano species present included *Woronichinia naegeliana* (105 cells/mL; Fig. 5) and *Anabaena* sp. (42 cells/mL; Fig. 6).

Sample 2: CL3

Total cell numbers in the CL3 sample collected on 8/4/11 were 16,797 cells/mL. Blue-green algae (Cyanobacteria; 16,041 cells/mL) were the dominant algal group in the sample accounting for 95.5% of total cell numbers. Other algal groups in the sample were diatoms (Bacillariophyta; 431 cells/mL), green algae (Chlorophyta; 128 cells/mL), cryptophyes (Cryptophyta; 46 cells/mL) and microflagellates (Miscellaneous; 151 cells/mL). The most abundant species was *Lyngbya* sp. (8,528 cells/mL).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) in the CL3 sample were 3,402 cells/mL (20.3% of total cell numbers). PTOX Cyano species present included *Microcystis botrys* (1,820 cells/mL; Fig. 7), *Aphanizomenon* cf. *flos-aquae* (1,573 cells/mL; Fig. 8) and *Anabaena* sp. (9 cells/mL).

Sample 3: CL4
Total cell numbers in the CL4 sample collected on 8/4/11 were 28,931 cells/mL. Blue-green algae (Cyanobacteria; 27,636 cells/mL) were the dominant algal group in the sample accounting for 95.5% of total cell numbers. Other algal groups in the sample were diatoms (Bacillariophyta; 1,040 cells/mL), green algae (Chlorophyta; 122 cells/mL), cryptophyes (Cryptophyta; 11 cells/mL), microflagellates (Miscellaneous; 121 cells/mL) and dinoflagellates (Pyrrhophyta; 2 cells/mL). The most abundant species was *Lyngbya* sp. (18,007 cells/mL).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) in the CL4 sample were 2,108 cells/mL (7.3% of total cell numbers). PTOX Cyano species present included *Anabaena* sp. (1,497 cells/mL), *Microcystis botrys* (379 cells/mL) and *Woronichinia naegeliana* (232 cells/mL).

Sample 4: STN1
Total cell numbers in the STN1 sample collected on 8/4/11 were 34,074 cells/mL. Blue-green algae (Cyanobacteria; 32,131 cells/mL) were the dominant algal group in the sample accounting for 94.3% of total cell numbers. Other algal groups in the sample were diatoms (Bacillariophyta; 47 cells/mL), green algae (Chlorophyta; 1,489 cells/mL), cryptophyes (Cryptophyta; 113 cells/mL), euglenophytes (Euglenophyta; 2 cells/mL), microflagellates and unknown unicells (Miscellaneous; 290 cells/mL) and dinoflagellates (Pyrrhophyta; 1 cell/mL). The most abundant species were picoplanktonic (<2.5µm in size) cyanophytes (18,899 cells/mL) and an unknown cyanophyte colony (8,383 cells/mL). This cyanophyte colony most closely resembled *Aphanothece* or *Cyanodictyon*.

The density of *Lyngbya* sp. in the STN1 sample was 2,809 cells/mL. Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) were 620 cells/mL (1.8% of total cell numbers). PTOX Cyano species present included *Anabaena* sp. (612 cells/mL) and *Aphanizomenon* cf. *flos-aquae* (8 cells/mL).

Sample 5: STN2
Total cell numbers in the STN2 sample collected on 8/4/11 were 338,483 cells/mL. Blue-green algae (Cyanobacteria; 193,372 cells/mL) and unknown unicells and flagellates (Miscellaneous; 141,773 cells/mL) were the dominant algal groups in the sample accounting for 57.1% and 41.9% of total cell numbers respectively. Other algal groups in the sample were diatoms (Bacillariophyta; 553 cells/mL), green algae (Chlorophyta; 1,947 cells/mL), cryptophyes (Cryptophyta; 749 cells/mL) and euglenophytes (Euglenophyta; 91 cells/mL). The most abundant species were an unknown unicell (140,026 cells/mL; Figs. 9-10), picoplanktonic (<2.5µm in size) cyanophytes (85,116 cells/mL) and *Lyngbya* sp. (81,656 cells/mL). The algal nature of the unknown unicell at this point is still not certain.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) in the STN2 sample were 2,427 cells/mL (0.7% of total cell numbers). PTOX Cyano species present included *Anabaena* sp. (1,293 cells/mL), *Gloeotrichia echinulata* (612 cells/mL; Fig.11) and *Microcystis botrys* (522 cells/mL).

Sample 6: STN3

Total cell numbers in the STN3 sample collected on 8/4/11 were 162,811 cells/mL. Unknown unicells and flagellates (Miscellaneous; 97,565 cells/mL) and blue-green algae (Cyanobacteria; 63,326 cells/mL) were the dominant algal groups in the sample accounting for 59.9% and 38.9% of total cell numbers respectively. Other algal groups in the sample were diatoms (Bacillariophyta; 1,101 cells/mL), green algae (Chlorophyta; 700 cells/mL), cryptophyes (Cryptophyta; 119 cells/mL) and dinoflagellates (Pyrrhophyta; 1 cell/mL). The most abundant species were an unknown unicell (same species as at STN2; 96,476 cells/mL), *Lyngbya* sp. (20,240 cells/mL) and an unknown cyanophyte colony (14,970 cells/mL; Fig. 12) most closely resembling *Aphanothece* or *Rhabdoderma*.

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) in the STN3 sample were 4,306 cells/mL (2.6% of total cell numbers). PTOX Cyano species present included *Microcystis botrys* (3,648 cells/mL), *Aphanizomenon* cf. *flos-aquae* (544 cells/mL) and *Anabaena* sp. (114 cells/mL).

Sample 7: STN4

Total cell numbers in the STN4 sample collected on 8/4/11 were 580,138 cells/mL. Blue-green algae (Cyanobacteria; 537,994 cells/mL) were the dominant algal group in the sample accounting for 92.7% of total cell numbers. Other algal groups in the sample were diatoms (Bacillariophyta; 19,247 cells/mL), green algae (Chlorophyta; 7,335 cells/mL), golden-brown algae (Chrysophyta; 454 cells/mL), cryptophyes (Cryptophyta; 572 cells/mL), euglenophytes (Euglenophyta; 5 cells/mL), unknown unicells and flagellates (Miscellaneous; 14,517 cells/mL) dinoflagellates (Pyrrhophyta; 10 cells/mL) and yellow-green algae (Xanthophyta; 5 cells/mL). The most abundant species were *Aphanocapsa* sp. (218,205 cells/mL; Fig. 13) and *Lyngbya* sp. (180,325 cells/mL).

Total numbers of potentially toxigenic cyanobacteria (PTOX Cyano) in the STN4 sample were 16,587 cells/mL (2.9% of total cell numbers). PTOX Cyano species present included *Gloeotrichia echinulata* (6,124 cells/mL), *Aphanizomenon* cf. *flos-aquae* (4,990 cells/mL), *Anabaena* sp. (2,268 cells/mL) and *Microcystis botrys* (3,205 cells/mL).

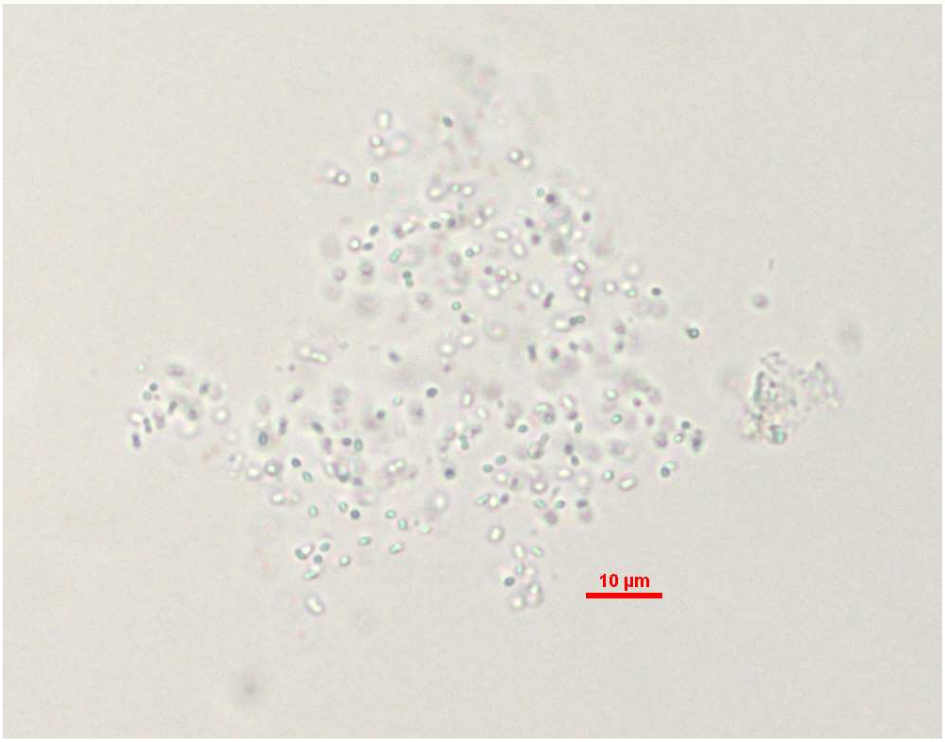

Fig. 1 *Aphanothece*/*Cyanodictyon* sp.400X (scale bar = 10µm)

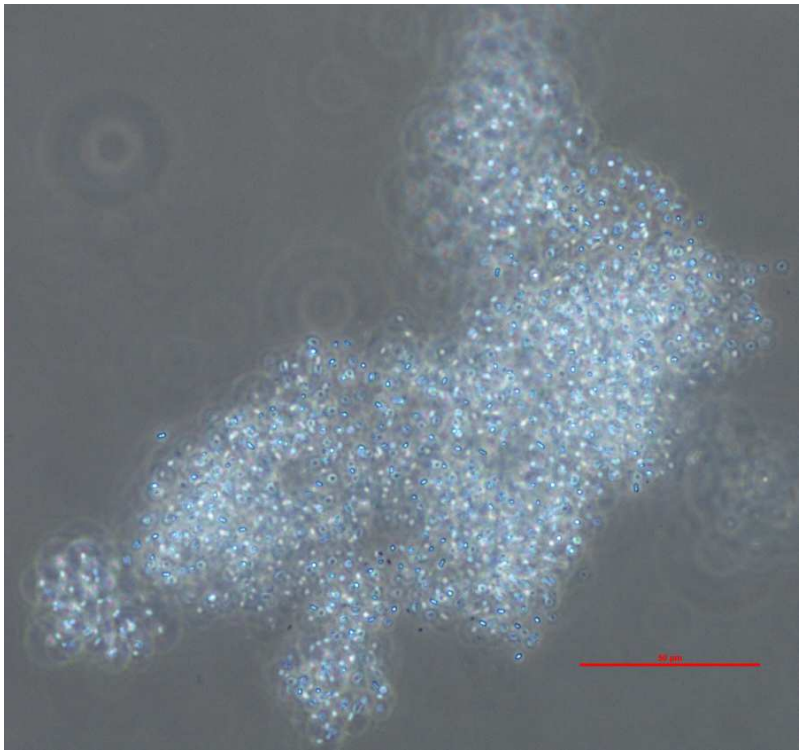

Fig. 2 *Aphanothece*/*Cyanodictyon* sp.400X (scale bar = 50µm)

Fig. 3 *Lyngbya* sp. 400X (scale bar = 10µm)

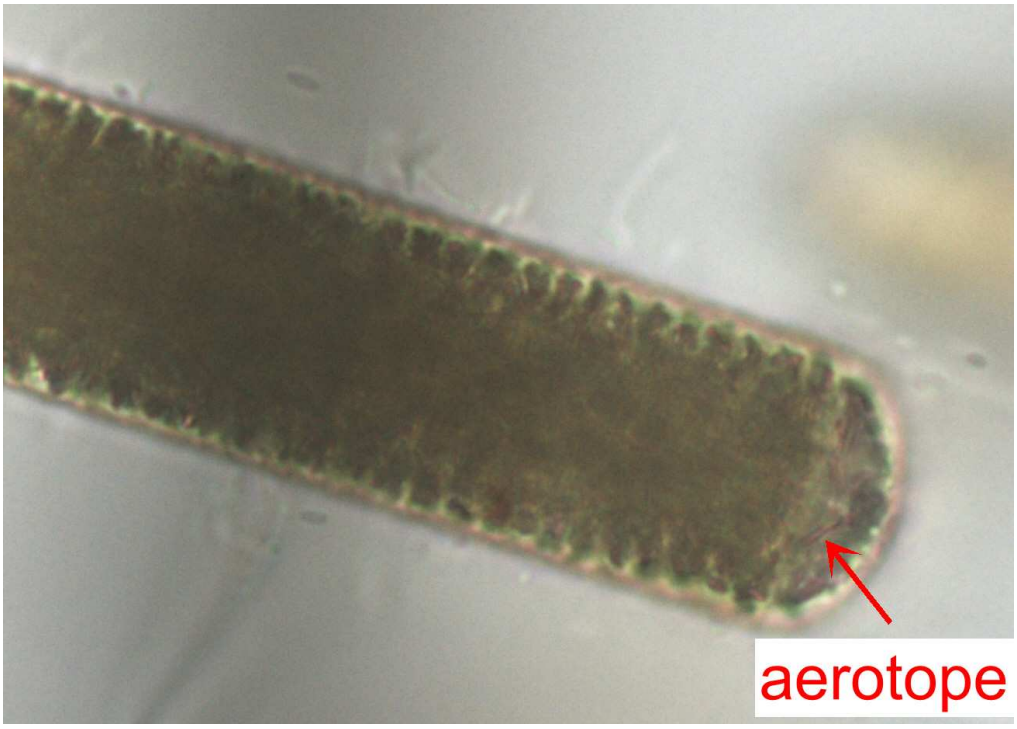

Fig. 4 *Lyngbya* sp. showing aerotope 1000X

Fig. 5 *Woronichinia naegeliana* 400X (scale bar = 20µm)

Fig. 6 *Anabaena* sp. 400X (scale bar = 10µm)

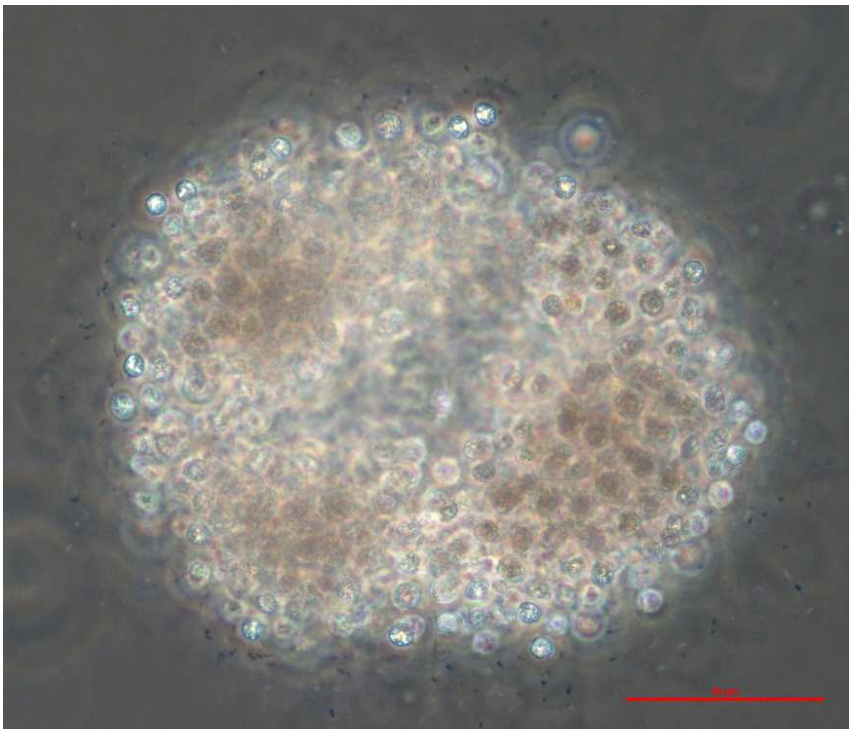

Fig. 7 *Microcystis botrys* 400X (scale bar = 50µm)

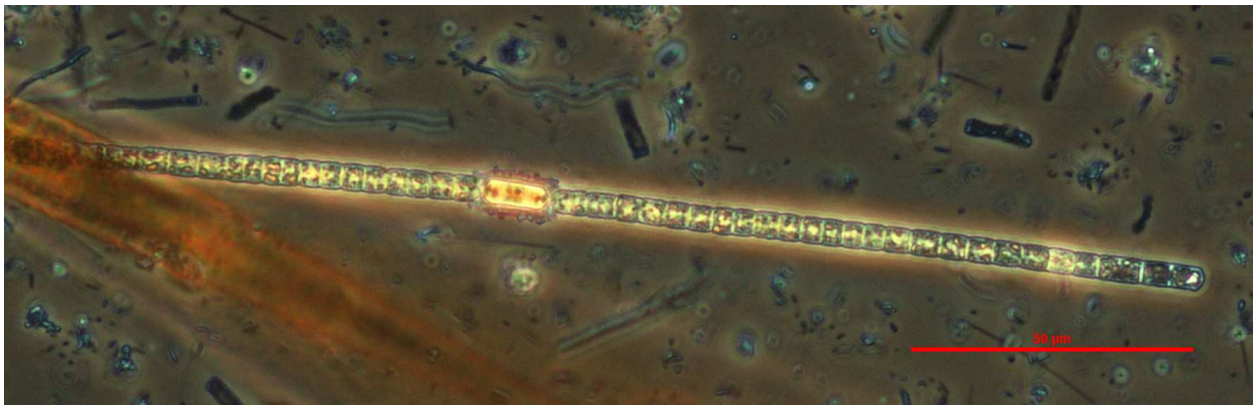

Fig. 8 *Aphanizomenon* cf. *flos-aquaae* 400X (scale bar = 50µm)

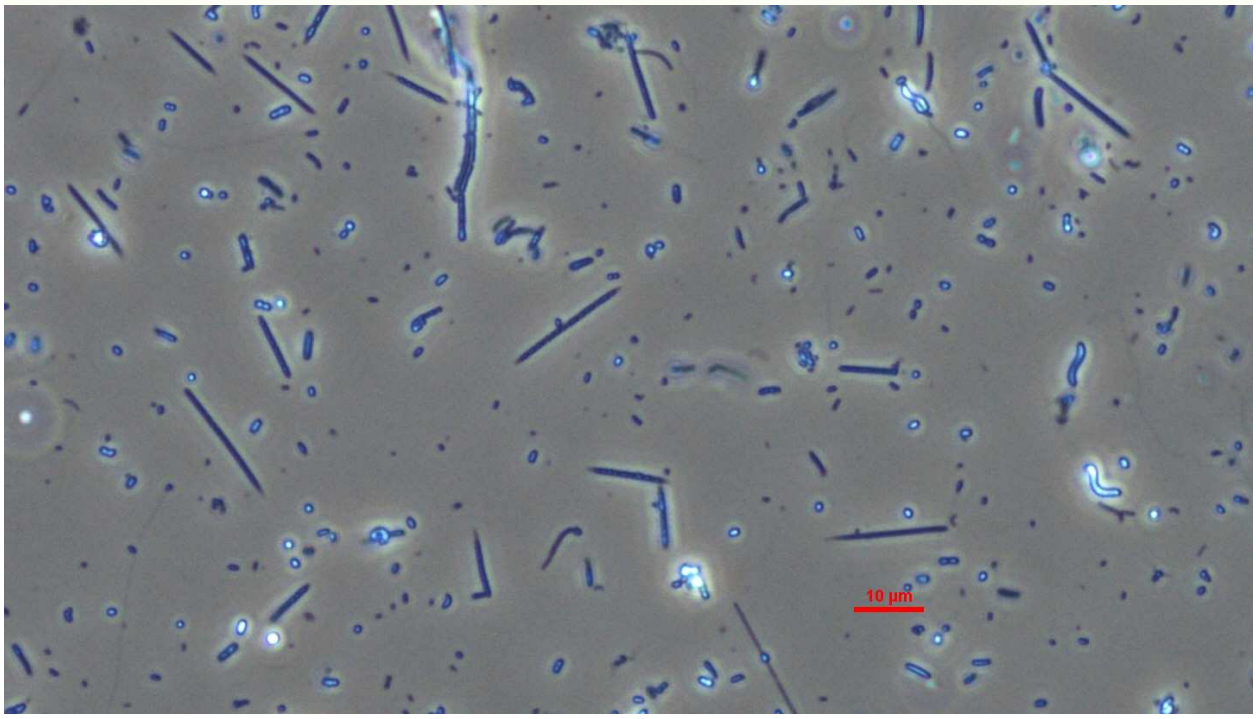

Fig. 9 unknown unicells 400X (scale bar = 10µm)

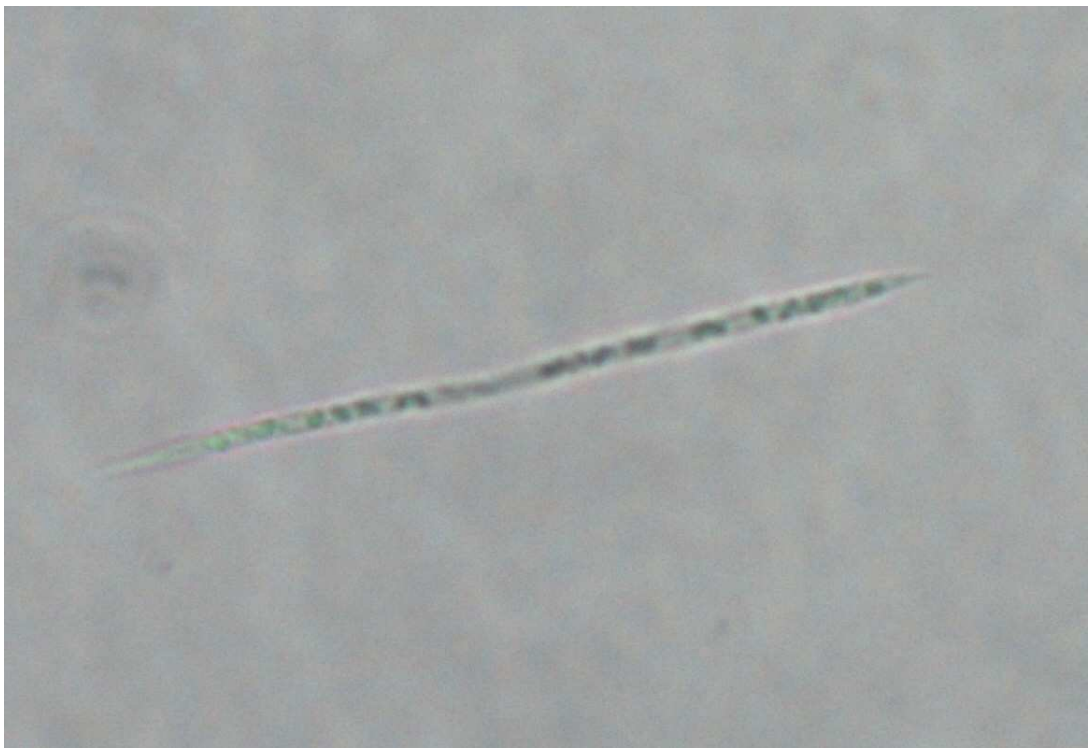

Fig. 10 unknown unicell 1000X

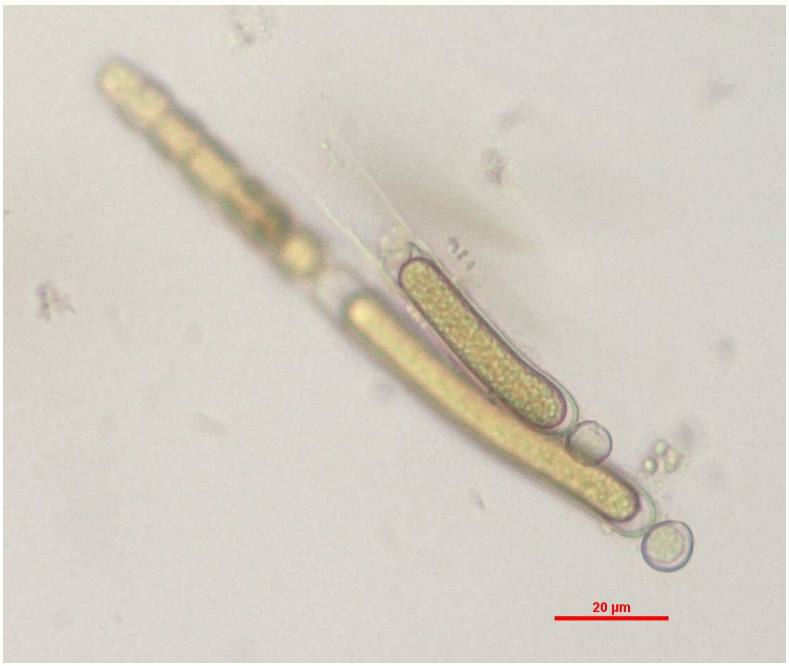

Fig. 11 *Gloeotrichia echinulata* 400X (scale bar = 20µm)

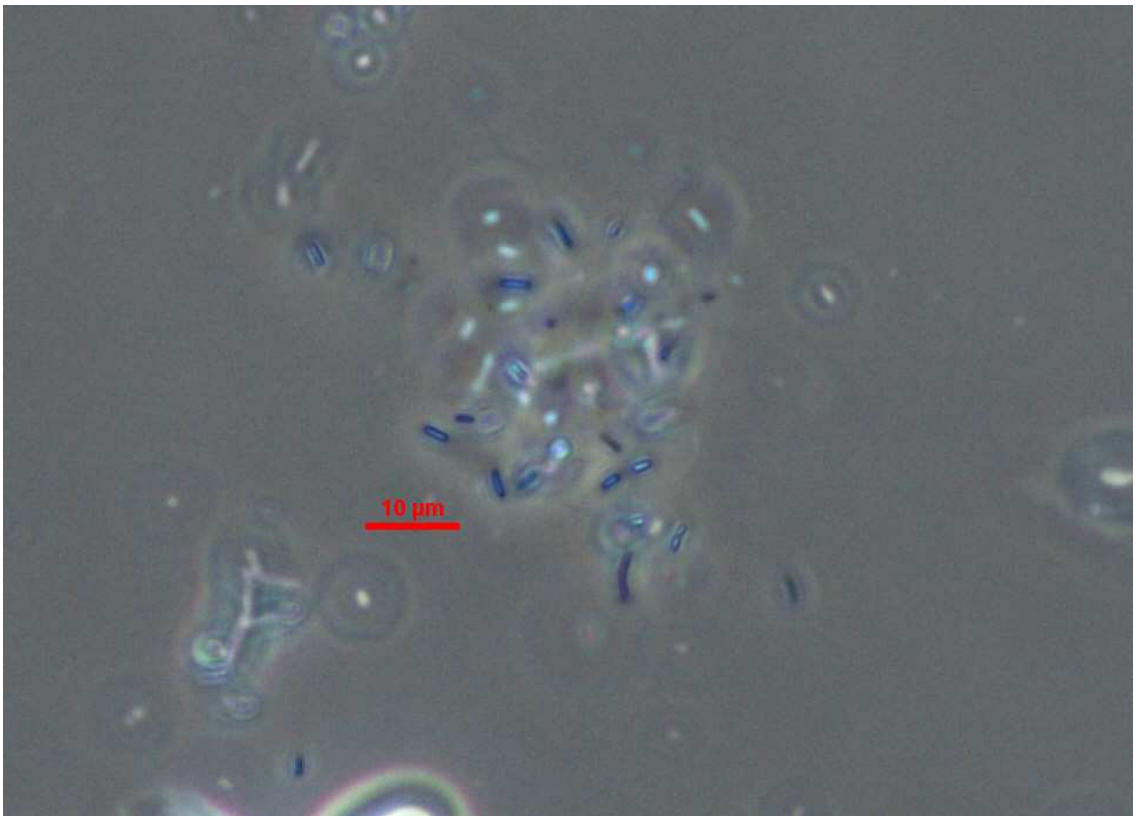

Fig. 12 *Aphanothece/Rhabdoderma* sp. 400X (scale bar = 10µm)

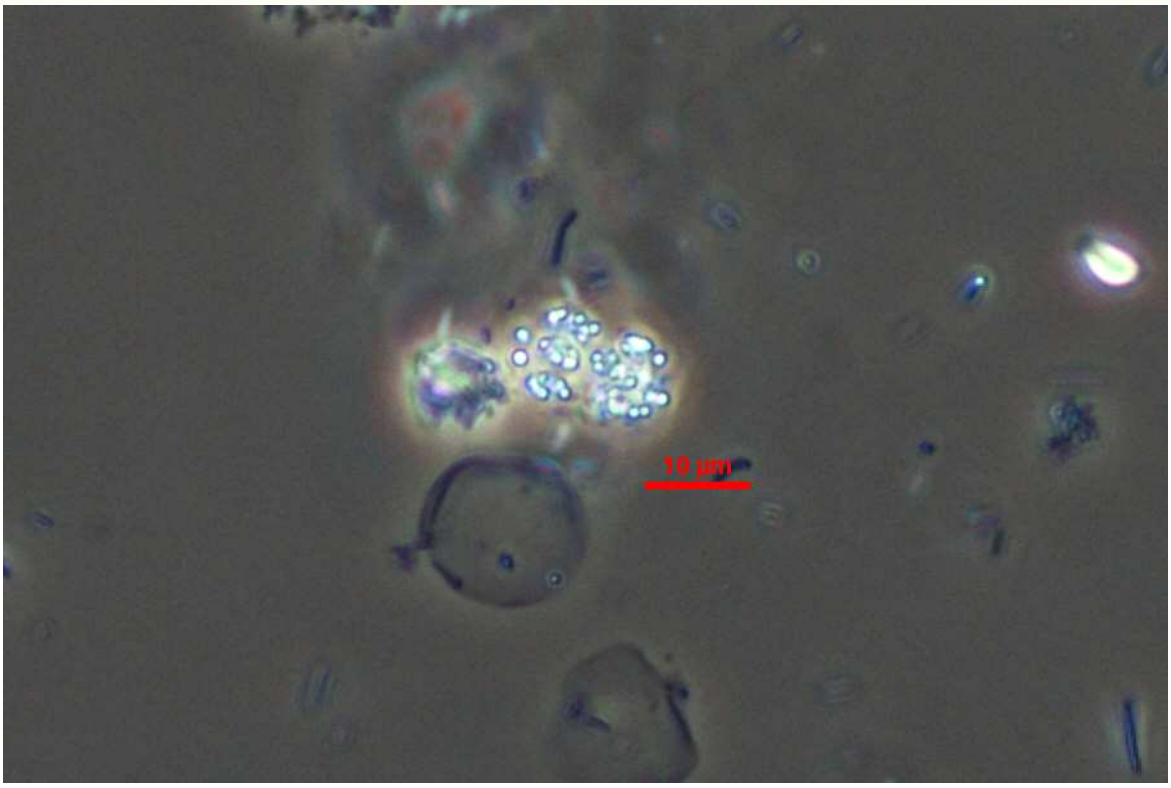

Fig. 13 *Aphanocapsa* sp. 400X (scale bar = 10µm)

Anatoxin-a, Cylindrospermopsin, Lyngbyatoxin-a, Microcystins, Paralytic Shellfish Toxin Data Report

Project: County of Lake Water Resources, California

Sample Identification	Sample Collection Date
Clear Lake	8/4/11

Toxins – Anatoxin-a (ANTX), cylindrospermopsin (CYN), Lyngbyatoxin-a (LTA), microcystins (MC), paralytic shellfish toxins (PSPs, saxitoxins)

Sample Preparation

ANTX-A, CYN, LTA, MC, PSPs

The sample was ultra-sonicated to lyse cells and release toxins. Solid phase extraction (SPE) was utilized for ANTX-A, LTA, MC (Strata) and CYN (Carbograph) clean-up and preconcentration (100x). Duplicate samples (lab fortified matrices, LFM) were spiked at 0.1 µg/L ANTX-A, 0.1 µg/L CYN and 1.0 µg/L each of MCLA, MCLR, MCRR, MCYR and LTA, which provided quantitative capability and additional qualitative confirmation.

For PSPs, 100 mLs of sample was frozen and lyophilized at -50 ˚C. A duplicate LFM sample was prepared, spiked at 1 ppb STX. The lyophilized material was reconstituted in 75% acidified MeOH and blown to dryness. Samples were reconstituted in 1 mL of 0.003 M HCl for a 100x pre-concentration. The peroxide oxidation procedure used was the same as the AOAC first action 2005.06 method for determination of PSPs in shellfish. Standards used to calibrate this method included saxitoxin (STX), decarbamoylsaxitoxin (dcSTX), gonyautoxin 2&3 (GTX2&3), gonyautoxin 5 (GTX5), decarbamoylgonyautoxin 2&3 (dcGTX2&3) and N-sulfocarbamoyl-gonyautoxin-2 and -3 (C1&2).

Analytical Methodology

ANTX-A, CYN, LTA

Liquid chromatography/ mass spectrometry/ mass spectrometry (LC/MS/MS) was utilized for the determination of both CYN, ANTX-A and LT. The $[M+H]^+$ ions for ANTX-A (*m/z* 166), CYN (*m/z* 416) and LTA (*m/z* 438.5) were fragmented and the major product ions for ANTX-A (*m/z* 149, 131, 107, 91), CYN (*m/z* 336, 318, 274, 194) and LTA (*m/z* 410, 393) provided both specificity and sensitivity. The current methodologies established a limit of detection (LOD) of 0.05 µg/L and a limit of quantification (LOQ) of 0.1 µg/L for both CYN and ANTX-A and a LOD of 0.5 µg/L and a LOQ of 1.0 µg/L for LTA.

MC

Liquid chromatography/mass spectrometry (LC/MS) was utilized for the determination of four of the most common microcystin variants and their relative abundance. The $[M+H]^+$ ions for LA (*m/z* 910.5), LR (*m/z* 995.5), RR (*m/z* 519.5) and YR (*m/z* 1045.6) provided both specificity and sensitivity. The LOD and LOQ for each variant are 0.5 µg/L and 1.0 µg/L, respectively.

PSPs

The AOAC Official Method 2005.06 Paralytic Shellfish Poisoning Toxins method was utilized for separation of the toxins. Fluorescence was monitored with an excitation of 340 nm and an emission of 396 nm. Samples were evaluated by comparing peak retention times to standards, standard addition techniques and non-oxidized samples. The LODs for PSP toxins are 0.2 µg/L STX, 0.1 µg/L C1C2, 0.1 µg/L dcGTX2&3, 0.1 µg/L dcSTX, 0.5 GTX 2&3, 1 µg/L GTX5, 1 µg/L dcNEO. The LOQs for PSP toxins are 0.4 µg/L STX, 0.2 µg/L C1C2, 0.2 µg/L dcGTX2&3, 0.2 µg/L dcSTX, 1 GTX 2&3, 2 µg/L GTX5, 2 µg/L dcNEO.

Summary of Results

Sample	Anatoxin (µg/L)	CYN (µg/L)	LTA (µg/L)	MC (LA-LR-RR-YR) (µg/L)	PSPs (µg/L)
Clear Lake	ND	ND	ND	ND	ND

ND = Not detected above the detection limit

Discussion

All spike recoveries were acceptable for all analyses. Anatoxin-a, cylindrospermopsin, lyngbyatoxin-a, microcystins (LA, LR, RR & RR) and PSPs (dcSTX, dcNEO, GTX2&3, GTX5, dcGTX2&3, STX, C1&C2) were not detected above our method detection limits. A trace amount of microcystin(s) was detected with the use of an enzyme linked immunosorbent assay (ELISA) but the value (< 0.3 µg/L) was below the LOD/LOQ of the LC/MS methodology.

References:
AOAC, 2005. Paralytic shellfish poison. Biological method. First action 2005.06. Official Methods of Analysis of the AOAC. Method 49.10.03.

Lawrence, J.F., Niedzwiadek, B., Menard, C., 2005. Quantitative determination of paralytic shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection: collaborative study. J. AOAC Int. 88, 1714-1732.

Submitted by: 
Mark T. Aubel, Ph.D.

Date: 8/24/11

Submitted to: Thomas Smythe
County of Lake Water Resources
Tom_s@co.lake.ca.us

STANDARD OPERATING PROCEDURE (SOP)

Title: Analysis of Water Temperature (WT), Specific Conductance (SpC), Dissolved Oxygen (DO), Sample depth, pH, Turbidity (Turb), *in vivo* Chlorophyll Fluorescence (Chl), of Ambient Surface Water using Yellow Springs Instruments (YSI) 6-series Sonde;

Technician:_____
Date:_____

Section Chief:_____
Date:_____

Branch Chief :_____
Date:_____

Quality Assurance Review:_____
Date:_____

Effective Date: _____

1.0 PURPOSE

This describes the sampling and analytical procedures for *in situ* continuous automated analysis for WT, SpC, DO, Sample Depth, pH, Turb and Chl of ambient water during discrete monthly water quality monitoring in the Sacramento-San Joaquin Estuary.

2.0 SCOPE AND APPLICABILITY

2.1 These procedures are intended for use in the Bay-Delta Monitoring and Analysis Section monthly Discrete Water Quality Monitoring Network.

2.2 This data will be used by DWR operations to meet the standards set in State Water Resources Control Board Decision 1641 and to establish baseline conditions, identify trends, and characterize seasonal variations in water quality.

2.3 WT, SpC, DO, pH, Turb, Chl data meeting QA/QC objectives may be used for the Federal Clean Water Act Sections 305(b) Report and 303(d) Lists.

2.4 Provide timely water quality for water management decisions.

2.5 Provide continuous water quality to the public (via internet) for water bodies of interest.

2.6 The working ranges of the sensors are listed in Table 1.

Table 1

Constituent	**Working Range |accuracy**
WT	-5° to 45° degrees Celsius | ± 0.15 °C
SpC	0 – 100,000 µS/cm | ± 0.5% of reading + 1µS/cm
DO*	0 – 50 mg/L | 0-20 mg/L ±1% reading or ±0.1 mg/L whichever is greater; 20-50 mg/L ± 15% of reading
Sample Depth	0 – 61 meters | ± 0.4 ft
pH	0 – 14 pH units | ± 0.2 units
Turbidity	0 – 1000 NTU | ± 2% of reading or 0.3 NTU whichever is greater
Chlorophyll Fluorescence	0 – 400 µg/L or 0 – 100% | none provided

3.0 METHOD SUMMARY

3.1 The research vessel lab is equipped with an YSI 6600 data sonde and Turner 10-AU instruments for collecting longitudinal water quality profiles in the upper San Francisco Estuary as part of the Department of Water Resources discrete water quality monitoring program. These instruments includes a flow-through system and a computer to collect geographically referenced in-situ water quality data including WT, SpC, DO, pH, Turbidity, and chlorophyll fluorescence. Relative fluorescence values (RFU) are determined using the Turner 10-AU fluorometer.

3.2 DO concentrations is measured by an optical luminescence sensor (EPA method 360.3).

3.3 Water temperature is measured by a resistance thermistor.

3.4 The SpC sensor is a flow cell with four electrodes. Two of the electrodes are current driven, and two are used to measure voltage drop. The voltage drop is converted to conductance in milimhos. This value then multiplied by the cell constant. Conductivity/specific conductance is measured through a 5.0/cm cell using alternating current. Conductivity measurements are

temperature corrected to 25.0° C and are reported as SpC.

3.5 The optical (luminescence) DO sensor is based upon dynamic fluorescence quenching of a luminophore (luminescence dye molecule) by oxygen (EPA 360.3). A blue light is irradiated on the luminophore which causes the luminophore to luminesce. The duration of the luminophore luminescence is inversely proportional to the amount of oxygen present. The luminescence is measured by a photodiode. During the measurement the luminophore is irradiated with red light and this measurement is used as a reference. The DO concentration is calculated using a polynomial regression equation. The optical DO sensor does not consume oxygen at the sensor and has no flow dependence.

3.6 The pH is measured using a combination electrode consisting of a proton selective glass reservoir filled with a buffer at approximately a pH 7 and a Ag/AgCl reference electrode utilizing a gel electrolyte.

3.7 The turbidity sensor uses a light emitting diode (LED) which produces radiation in the near infrared region of the spectrum with a wave length between 830 and 890 nm and a high sensitivity photodiode detector positioned 90 degrees to the emitted light source.

3.8 The fluorometric sensor induces chlorophyll molecules to fluoresce *in vivo* (without disrupting cells) using a blue LED with a peak wavelength of approximately 470 nanometers (nm). Once the chlorophyll molecules are fluoresced, the chlorophyll emits light in the 650 -700 nm region of the spectrum. The amount of fluorescence is measured by a photo diode that is screened by an optical filter that prevents 470 nm excitation light from being detected when it is back-scattered off of particles in the water. The sensor operates under whole-cell, heterogeneous conditions. The sensor measures overall fluorescence which includes chlorophyll *–a, -b, -c,* pheophytin *–a,* and non chlorophyll interfering species that fluoresces above 630 nm.

4.0 LIMITATIONS

4.1 Sonde deployment structures and platforms can be susceptible to damage due to vandalism and extreme adverse environmental conditions. Data loss can occur as a result. All steps are made to minimize these effects.

4.2 In some bodies of water (or due to seasonal variations in water quality) sensor fouling can occur rapidly decreasing sonde deployment times. YSI 6600 sondes are equipped with a brush and wiper pads to mechanically clean the DO, Turbidity, Chlorophyll, pH and water temperature sensor tips. This capability can increase data gaps since data cannot be collected during this process.

4.3 Errors in DO, SpC, fluorescence, and pH measurements can result due to a faulty temperature

sensor.

4.4 The depth sensor is non-vented. The sonde firmware uses atmospheric pressure at the time of calibration, changes in atmospheric pressure between calibrations appears as changes in depth. The error is equal to 0.045 feet for every 1 millimeter mercury (mm Hg) change in atmospheric pressure.

4.5 Expired standards shall not be used

5.0 SAFETY

5.1 Operators will be responsible to read and be familiar with the Bay-Delta Monitoring and Analysis Section Safety plan as well as the Material Safety Data Sheets for all chemicals used in Section operations.

6.0 EQUIPMENT

6.1 Equipment

6.1.1 YSI 6600-Series Sondes with SpC, temperature, DO, pH, turbidity, chlorophyll fluorescence, and depth

6.1.2 YSI field cable

6.1.3 Calibration worksheets

6.1.4 Calibration cup

6.1.5 Ring stand and clamp

6.1.6 Thermometer certified or traceable to National Institute of Standards and Technology (NIST) with 0.1°C resolution

6.2 Standards and Reagents

6.2.1 EC, pH, and turbidity calibration standards

6.2.2 When calibrating for natural waters, use pH standard solutions with ph values of 7.0 and 10.0 pH units.

7.0 PROCEDURE

Before the sonde is deployed, the sensors are cleaned and calibrated in the RTM Lab. Dissolved oxygen is calibrated at the beginning of each sampling day using a Winkler titration.

Once the sonde is deployed, Bay-Delta Monitoring and Analysis staff monitors these instruments to evaluate operational status and verifies values using other calibrated field instruments. If errors are found the sonde is either replaced, recalibrated or removed from service.

7.1 Calibration (reference established YSI6600 calibration procedure)

7.1.1 Sondes are calibrated at DES RTM Lab a minimum of once every month. More frequent sonde calibrations may be necessary depending on sensor fouling rates and instrument drift.