Unprecedented Bloom of Toxin-Producing Cyanobacteria in the Southern Bay-Delta Estuary and its Potential Negative Impact on the Aquatic Food-Web

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Table of Acronyms

BDT	Brandt Bridge station
BOD	Biochemical oxygen demand
CBOD	Carbonaceous biochemical oxygen demand
CDEC	California Data Exchange Center
CFS	Cubic feet per second
Chl-a	Chlorophyll-a
CIMIS	California Irrigation Management Information System
Cl	Chloride
DOC	Dissolved organic carbon
DWSC	Deep water ship channel
DWR	Department of Water Resources
EERP	Ecological Engineering Research Program
ELISA	Enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
IC	Inorganic carbon
MC	Microcystin
MSS	Mineral suspended solids
NBOD	Nitrogenous biochemical oxygen demand
NO_3-N	Dissolved nitrate + nitrite as nitrogen
NOAA	National Oceanic and Atmospheric Administration
NOAEL	no observed adverse effect level
PO_4-P	Dissolved orthophosphate as phosphorus
POD	Pelagic organism decline
SD	Standard Deviation
SiO2 Si	Dissolved silicate as silicon
SM	Standard Methods
south Delta	Southern San Francisco Bay-Delta Estuary
SUVA	Specific ultraviolet absorbance
TAN	Total ammonia + ammonium nitrogen
TN	Total nitrogen
TP	Total phosphorus
TSS	Total suspended solids
VAMP	Vernalis Adaptive Management Program
VSS	Volatile suspended solids
WHO	World Health Organization
YSI	Yellow Springs International

Introduction

Cyanobacterial blooms impair beneficial uses of surface waters around the world and are becoming more frequent due to eutrophication of surface waters (Chorus and Bartram, 1999). Blooms can form thick mats on the water's surface that are unsightly, malodorous, and can cause odor and taste problems that are costly to get rid of in drinking water supplies (Carmichael, 1994; Chorus and Bartram, 1999; Jacoby et al., 2000). In addition to aesthetic problems, many species produce toxins known as cyanotoxins. Cyanotoxins can be harmful for humans, cattle, pets, and aquatic wildlife (Carmichael, 1994; Watanabe et al., 1995; Codd, 2000; Park et al., 2001; Aguete et al., 2003; Chen et al., 2004; Sivonen, 2007; Lehman et al., 2010). In 1996, 60 people died and another 66 patients were found to have non-lethal symptoms of neurotoxicity and hepatotoxicity after receiving dialysis using water from a lake with a large cyanobacterial bloom in Brazil (Pouria et al., 1998). Between 1999 - 2008, 21 threatened southern sea otters were found dead off the coast of California in Monterey Bay and their deaths have been linked through trophic transfer to microcystin originally released from a freshwater lake and its tributaries near the coast (Miller et al., 2010). Although cyanotoxins poisoning is not widely recognized among veterinarians, there have been documented cases of cattle and other livestock deaths in North and South America, Europe, Australia, and Africa linked to consumption of water contaminated with cyanobacteria (Briand et al., 2003).

The most common and widely studied group of cyanotoxins are microcystins, cyclic heptapeptides with approximately 90 congeners (Pearson et al., 2010). The most common variety found in the San Francisco Bay Delta from past studies is microcystin LR (Lehman et al., 2005; Lehman, 2008). Microcystins are produced by *Microcystis*, found in fresh and brackish waters (<10 g L^{-1} salinity), and other cyanobacteria including *Anabaena*, *Oscillatoria*, *Nodularia*, *Nostoc*, *Cyilndrospermopisi*, and *Umezakia* (An and Carmichael, 1994; AWWA, 1995; Park et al., 2001; Pearson et al., 2010; Tonk, 2007).

Cyanotoxins have been found in the tissues of fish, shellfish and other aquatic animals in areas with cyanobacterial blooms (Magalhaes et al., 2001; Ibelings and Chorus, 2007; Lehman et al., 2010; Miller et al., 2010). Bioaccumulation of these toxins has led to concerns of food safety, especially among subsistence fishing communities such as the Yurok Indians living in the Klamath River Basin where mussel tissues were measured to have microcystin concentrations above 100 ng g^{-1} wet weight in 2007 (Kann, 2006; Ibelings and Chorus, 2007; Kann and Corum, 2009; Kann et al., 2010).

The World Health Organization (WHO) has set a limit of 1,000 ng L^{-1} of microcystin for drinking water. This limit is based on an average body weight of 60 kg, average intake of 2 L of water per day, and the provisional limit of 40 ng (kg body weight day)$^{-1}$ as a no observed adverse effect level (NOAEL) on humans (Chorus and Bartram, 1999). The California Environmental Protection Agency (EPA) Office of Environmental Health Hazard Assessment lists the microcystin action level for human swimming as 800 ng L^{-1} microcystin and the action level for human fish consumption at 10 ng g^{-1} wet weight (Butler et al., 2012). Recreational limits were based on 7 to 10 year old swimmers who would be most highly exposed and food limits were based on average adult consumption of fish in California, fraction of microcystin expected to be absorbed, and an average body weight of 70 kg (Butler et al., 2012).

Cyanobacterial blooms can cause fish kills and have been suspected of negatively affecting the aquatic food chain even at relatively low concentrations (Christoffersen, 1996; Fischer and Dietrich, 1999; Kaebernick and Neilan, 2001; Vanderploeg et al., 2001; Lehman et al., 2005; Lehman, 2008; Ger et al., 2010; Lehman et al., 2010). In the Sacramento-San Joaquin Delta of California, blooms of *Microcystis* were first documented in 1999 (Lehman et al., 2005). The Sacramento-San Joaquin Delta is an expansive fresh-water tidal estuary that is part of the San Francisco Estuary, the largest estuary on the US Pacific coast (Sommer et al., 2007). Microcystin has been suspected as one plausible cause of the pelagic organism decline (POD) seen in the San Francisco Bay Delta over the last decade (Armor et al., 2006; Baxter et al., 2008; Brooks et al., 2012). It has been proposed that microcystin could be contributing to POD directly though poisoning of fish or indirectly by negatively affecting zooplankton and other food sources (Armor et al., 2006; Baxter et al., 2008; Deng et al., 2010; Lehman et al., 2010; Brooks et al., 2012).

Zooplankton are an important part of the food web in the Delta and the copepods *E. affinis* and *P. forbesi* are especially important food sources for larval POD species (Baxter et al., 2008; Ger et al., 2010). Delta and longfin smelt, threatened POD species, feed on rotifers and other small organisms the first weeks after hatching, but only when these organisms are present in high densities (Armor et al., 2006; Deng et al., 2010). Microcystin and other toxins produced by cyanobacterial blooms have been shown to negatively affect zooplankton (copepods, cladocerans, and rotifers) survival and fecundity in laboratory studies through feeding deterrence, low nutritional quality, and direct toxicity (Christoffersen, 1996; Kaebernick and Neilan, 2001; Ferrao-Filho et al., 2002; Wilson et al., 2006; Ka et al., 2012). Microcystin has also been found to bioaccumulate in zooplankton collected in areas with cyanobacterial blooms (Ferrao-Filho et al., 2002; Lehman et al., 2005). In studies by others, observed maximum microcystin concentrations in the Sacramento-San Joaquin Delta were below 100 ng L^{-1} in 2004 and 2005, and near or below 1,000 ng L^{-1} in the summers of 2007 and 2008 (Lehman et al., 2005; Lehman et al., 2010; Lehman et al., 2013). Microcystin has been detected in zooplankton and fish tissue in the Sacramento-San Joaquin Delta where *Microcystis* blooms have occurred (Lehman, 2008; Lehman et al., 2010; Lehman et al., 2013).

The factors affecting and supporting cyanobacterial blooms have been widely studied (Robarts and Zohary, 1987; Kotak et al., 1993; Carmichael, 1994; Chorus and Bartram, 1999; Jacoby et al., 2000; Downing et al., 2001; Kaebernick and Neilan, 2001; Gupta et al., 2003; Husisman et al., 2004; Lehman, 2008; Butler et al., 2012). Blooms may occur in one year and be absent in subsequent years (Jacoby et al., 2000; Chorus and Schauser, 2011). High temperature (>18°C, optimally ≥25°C) has been identified as a factor favoring *Microcystis* and other cyanobacterial growth, with the largest blooms occurring in summer months (Robarts and Zohary, 1987; Carmichael, 1994; Chorus and Bartram, 1999; Kaebernick and Neilan, 2001; Gupta et al., 2003; Lehman, 2008; O'Neila et al., 2012; Paerl and Otten, 2013). Some studies found that a low N:P molar ratio is important to bloom growth and other studies have reported that high levels of nitrogen and phosphorous support growth of cyanobacteria (Carmichael, 1994; Chorus and Bartram, 1999; Jacoby et al., 2000; Downing et al., 2001; Briand et al., 2003; Gupta et al., 2003; Husisman et al., 2004; Lehman, 2008; Moisander et al., 2009b; Zillen and Conley, 2010; Paerl et al., 2011; Butler et al., 2012; Paerl and Otten, 2013).

Low flows, low turbulence, low mixing and stratification enhanced by warm temperatures have also been found to promote cyanobacterial blooms (Paerl, 1988; Carmichael, 1994; Chorus and Bartram, 1999; Jacoby et al., 2000; Gupta et al., 2003; Husisman et al., 2004; O'Neila et al., 2012; Paerl and Otten, 2013). Vertical water stability may give cyanobacteria an advantage because unlike other algae species, cyanobacteria have gas vacuoles within their cells that allow them to control their buoyancy and to adjust their depth for optimal conditions of light exposure and nutrient levels (Paerl, 1988; Chorus and Bartram, 1999). A combination of periods of high flow bringing in nutrients, followed by low flows and high residence times have been found to promote cyanobacterial blooms (Paerl and Otten, 2013). Other factors that may be important in cyanobacterial growth and toxin production include light intensity, dissolved inorganic carbon, zooplankton grazing, iron, turbidity, pH, salinity and dissolved silica (Paerl, 1988; Utkilen and Gjølme, 1992; Carmichael, 1994; Chorus and Bartram, 1999; Jacoby et al., 2000; Kaebernick and Neilan, 2001; Gupta et al., 2003; Domingues et al., 2007; Moisander et al., 2009a; Paerl et al., 2011; Paerl and Otten, 2013).

In this study, we document the spatial and temporal extent of *Microcystis* blooms in the Southern Bay-Delta Estuary (south Delta), an area further southeast and upstream of the San Francisco Estuary than previously studied. Copepod, rotifer, cladoceran and total zooplankton abundance was examined in comparison to the spatial distribution of microcystin. The environmental factors associated with cyanobacterial blooms in the south Delta were investigated.

Methods

Sample sites

Ten sites, located in the Stockton Deep Water Ship Channel (DWSC) and nearby tributaries, were sampled in the summer of 2009 for cyanobacteria, zooplankton and water quality. In 2011 and 2012, sampling was extended to include 17 sites in the DWSC and nearby tributaries. Samples were collected between May and December. The DWSC is located directly upstream of the confluence of the Sacramento and San Joaquin Rivers, and is tidally influenced with a tidal excursion of approximately 2 km. Net flows can be zero during summer months. A map of sampling sites is shown in Figure 1.

Water quality sampling and analysis

In the field, a calibrated YSI 6600 Sonde (YSI Incorporated, Yellow Springs, OH) was deployed to measure the following water quality parameters (mid-depth of water column): temperature (°C), specific conductance (µS cm^{-1}), total dissolved solids (g L^{-1}), dissolved oxygen (mg L^{-1} and % of saturation), depth (ft), pH, turbidity (NTU), chlorophyll fluorescence) (µg L^{-1}), and phycocyanin fluorescence (estimating cyanobacteria cell density) (cells mL^{-1}).

Laboratory analyses were conducted on grab samples taken at each site from mid-depth in the water column. Samples were stored in the dark at 4°C until analyzed or processed and preserved. Water samples were collected in either glass or HDPE Trace-Clean 250 mL or 1,000 mL bottles (VWR International). Samples were collected, preserved, stored, and analyzed following Standard Methods (SM) (APHA, 2005) except where noted. The following

parameters were measured: total, mineral, and volatile suspended solids (TSS/MSS/VSS) SM 2540 D and E; dissolved orthophosphate as phosphorus (PO_4-P) SM 4500-P.D, total phosphorus (TP) was determined on unfiltered sample by persulfate digestion (Yu et al., 1994) and followed by SM 4500-P.D; dissolved organic carbon (DOC) and inorganic carbon (IC) by SM 5310 B; dissolved silicate as silicon (SiO_2-Si) by modified SM 4500-SiO2 D; biochemical oxygen demand (BOD), carbonaceous BOD (CBOD), and nitrogenous BOD (NBOD) follows SM 5210 B (APHA, 2005) with a modification for measurement of oxygen demand at ten days rather than five days; total ammonia + ammonium nitrogen (TAN), dissolved nitrate + nitrite as nitrogen (NO_3-N), and total nitrogen (TN) by (Carlson, 1978; Carlson, 1986; Carlson et al., 1990) and digestion of TN sample by (Yu et al., 1994); specific ultraviolet absorbance (SUVA, L mg^{-1}) was calculated according to SM 5910B; alkalinity by SM 2320B; Chloride (Cl) by EPA method 9212; and chlorophyll and pheophytin pigments according to SM 10200 H.

For zooplankton, cyanobacteria, and microcystin analysis, samples were collected from surface water and concentrated from a 28 L bucket sampler or a 36 L Schindler Patalas Trap (Wildco, Yulee, FL) to 250 mL using a 63 µm plankton net. After thorough mixing, concentrated samples were divided, with approximately 5 mL stored in the dark at -20°C, and analyzed in the laboratory for microcystin content using an ELISA kit (Abraxis, Warminster, PA). The remaining concentrated sample was preserved with 1 mL Lugol's solution, 5 mL M-3 fixative (SM 10200, APHA, 2005) or a 30 mL buffered formalin sucrose mixture (SM 10200, APHA, 2005), depending on microscopic needs, and stored in amber bottles at room temperature for later identification of cyanobacteria and zooplankton by microscopy.

Microscopy of Cyanobacteria

Microscopic measurements of cyanobacteria were made in 2009. Cyanobacteria were identified, counted, and measured with a Leica DM IL inverted microscope (Leica Microsystems, Wetzlar, Germany) and a 1 mL standard Utermöhl chamber (Aquatic Research Instruments, Hope, ID) with magnifications up to 400x. Species identifications followed (Prescott, 1962; Komarek and Komarkova, 2002).

Subsamples of 10 mL were taken from each sample bottle with a Hensen-Stempel pipette after thorough mixing by inverting the sample bottle approximately 30 times. Subsamples were sedimented overnight prior to microscopic examination, resulting in a final volume of 1 mL. For each sample, four subsamples were prepared and analyzed. For all species, cell density (cell mL^{-1}) was measured, but the method varied with the colony type.

Cell density for irregularly-shaped colonies was estimated by first counting the number of cells in a 625 µm² area at 400x magnification, which involved focusing through the colonies' thickness. In the case of small colonies with uniform thickness, only one cell count estimate was made; however, for larger colonies of irregular thickness, three regions of the colony were counted for cell density. Cell density counts were made on the first 10 colonies encountered in the sample, and the average density was used to estimate cell densities for the remaining colonies in the sample. For estimating colony area, the first 100 colonies encountered in a sample were measured for area by determining how many 625 µm² square would cover the colony. In cases of greater than 100 colonies in a sample, the mean colony area of the first 100 colonies was

applied to the additional colonies. Total cell density for each colony was the product of the mean cell density in 625 μm² and colony area.

For filamentous species, the first 20 trichomes from each sample were measured for length and diameter, and cells if they could be seen. The number of trichomes beyond 20 was counted, and the mean cell density from the first 20 trichomes was used to estimate the total cell density for these remaining trichomes.

Regularly coiled trichomes were counted by diameter of one coil and number of coils to the closest ½ coil. The diameter of one coil was determined by measuring the distance from the outside of one coil to the inside of the next coil for the first 20 coils found in the sample. If possible, the number of cells per coil was also counted. The number of coils was counted from the first 100 colonies seen in a sample, and all additional were counted and then the size was estimated from the mean of the first 100 coils. Cell diameter (and length if the cell was not spherical) were measured when possible, otherwise literature values for cell size were used (Prescott, 1962). To calculate the mass of cyanobacterial cells, the cell size of each taxa was measured and was combined with cell counts to calculate volume. To calculate mass, the density of cells was assumed to be the same as water.

Microscopy of Zooplankton

Zooplankton analysis follows U.S. Environmental Protection Agency (EPA) protocol LG403. Briefly, zooplankton samples are thoroughly mixed by inversion and a 5 – 20 mL subsample is taken from each using a Stempel pipette (volume adjusted for sediment amount in sample). The subsamples are added to a settling apparatus, and settled for 5 – 20 hours, depending on volume. Prior to settling, 100 μL of 1% rose Bengal dye is added to facilitate counting of zooplankton.

Additional Data Sets

Hourly weather data was downloaded from the California Irrigation Management Information System (CIMIS) webpage http://wwwcimis.water.ca.gov/cimis/welcome.jsp on January 7th, 2013 from the Manteca station 70, for 2009, 2011, and 2012. Sunrise and sunset data was acquired from the webpage www.sunrisesunset.com using Stockton, CA as the representative location for all sites. Tide data was acquired through the National Oceanic and Atmospheric Administration (NOAA) webpage www.tidesandcurrents.noaa.gov on December 17th, 2012. The Stockton, California site was used to represent the DWSC and nearby tributaries. Flow and water temperature data for Brandt Bridge and Rough and Ready Island in Stockton, CA was acquired through the California Data Exchange Center (CDEC) on January 7th, 2013.

Water quality data collected bimonthly at three sites in the south Delta by the Department of Water Resources (DWR) was provided by Carol for comparison to water quality data measured by the Ecological Engineering Research Program (EERP) in this study.

Data Analysis

Data was plotted in Grapher (Golden Software, Inc.) and geospatially visualized using ArcGIS 10.0 (Esri, Redlands, CA). Statistical analyses were performed using JMP 9.0 software (SAS, Cary, NC).

Results and Discussion

Microcystis and microcystin

The distribution of cyanobacteria and zooplankton was determined in the south Delta in 2009, 2011, and 2012 (Figure 1). Cyanobacteria were common in the south Delta and *Microcystis* was the most common cyanobacteria. The distribution of cyanobacteria was widespread, and cyanobacteria were present at all sites sampled. The dominant genus found was *Microcystis*, which morphologically was identified as *Microcystis aeruginosa* (Figure 2). *Microcystis* was found at least once at every site sampled. *Aphanizomenon floss aquae* was the second most prominent cyanobacteria and was found in most samples, but in smaller cell numbers and biomass concentrations than *Microcystis*. *Anabaena*, *Oscillatoria*, and *Planktothrix* were also present, but not in all samples and generally in low quantities. *Merismopedia* and five other cyanobacteria genera (*Chroococcus, Coelosphaerium, Snowella* and *Phormidium*) were found occasionally, but did not significantly contribute to the standing cyanobacterial biomass values.

There was a strong relationship between cyanobacteria biomass and microcystin concentration (r^2=0.74, Figure 3). Low concentration of microcystin were detected in 2009 and 2011, with maximum observed microcystin concentrations of 44 and 78 ng L^{-1} and average summer concentrations of 9 and <5 ng L^{-1}, for 2009 and 2011 respectively. In contrast, a large, persistent *Microcystis* bloom was observed in the summer of 2012 (Figure 4) and measured July and August microcystin concentration *averaged* 257 ng L^{-1}. In July and August of 2012, microcystin concentrations were measured above California EPA recreational advisory limits of 800 ng L^{-1} and the World Health Organization drinking water limit of 1,000 ng L^{-1}, with a maximum observed concentration of 2,140 ng L^{-1}. These concentrations are in line with microcystin concentrations measured in other parts of the Sacramento-San Joaquin Delta (Lehman et al., 2005; Lehman et al., 2010; Lehman et al., 2013). The microcystin concentrations observed in 2012 are high enough to raise concerns for direct contact exposure as well as food-web effects.

Microcystin and zooplankton

Zooplankton were measured and compared to the concentration of microcystin in the south Delta in all years (2009, 2011, and 2012). Samples that had quantifiable concentrations of microcystin (>5 ng L^{-1}) during peak boom months (July and August) had lower mass of total zooplankton (alpha = 0.1). Waters with microcystin concentrations less than 20 ng L^{-1} (four times quantifiable concentrations) in July and August harbored significantly lower densities and masses of all categories of zooplankton, except *P. Forbesi + E. Affinis* (alpha = 0.05), (Table 1). High densities and masses of zooplankton were never observed when high concentrations of toxin were measured. These results indicate that *Microcystis* and possibly other algae that produce microcystin may be having a negative effect on the south Delta food-web.

Spatial and Temporal Distribution of Microcystin

The highest concentrations of cyanobacteria were observed in July and August (Table 2). Cyanobacterial blooms in previous studies of the Bay-Delta and other parts of California have been observed during late summer and fall months (Lehman, 2008; Kann and Corum, 2009; Lehman et al., 2010; Miller et al., 2010). *In situ* vertical profiling studies of the DWSC in the summer of 2012 indicate that cyanobacteria as measured by phycocyanin was concentrated near the surface (Figure 5). In the turning basin, phycocyanin concentration was elevated at depths of up to 3.5m (Figure 5). A map of the distribution of maximum microcystin concentrations for July and August of 2012 is shown in Figure 6. In this figure, large red circles represent concentrations above recreational swimming action levels (800 ng L^{-1}), medium size orange circles represent concentrations below recreational action levels but above concentrations associated with low zooplankton mass and densities (20-800 ng L^{-1}), and small green circles represent concentrations below 20 ng L^{-1} microcystin.

The spatial distribution of microcystin and *Microcystis* was examined within the study area. The sites located in or near the DWSC, were compared to tributaries north of the DWSC and to water coming from the upstream SJR (Figure 1). The possibility of *Microcystis* growing in the tributaries or in the SJR upstream of the DWSC and being exported to the DWSC was investigated. In July and August of all years, the mean microcystin concentration in the DWSC sites, was 140 ± 374 ng L^{-1} in comparison with 16 ± 65 ng L^{-1} in the tributaries and 17 $\pm$ 43 ng L^{-1} in the upstream sties (Table 3). The DWSC sites were found to have significantly more microcystin and microcystin chlophyll-a^{-1} than either the upstream or tributary sites (pairwise t-test, $p<0.05$). The low concentration of *Microcystis* in the upstream and tributary sites indicates that *Microcystis* is likely growing locally in the DWSC rather than being imported from another source.

Environmental drivers

An analysis of water quality and environmental parameters was conducted to identify factors or conditions that correspond to the occurrence of the 2012 cyanobacterial bloom. Previous studies have identified warm water temperatures , high nutrient concentrations, low N:P ratios, low flows, and low mixing as factors contributing to *Microcystis* blooms (Robarts and Zohary, 1987; Paerl, 1988; Carmichael, 1994; Chorus and Bartram, 1999; Jacoby et al., 2000; Downing et al., 2001; Kaebernick and Neilan, 2001; Briand et al., 2003; Gupta et al., 2003; Husisman et al., 2004; Lehman, 2008; Moisander et al., 2009b; Zillen and Conley, 2010; Butler et al., 2012; Paerl and Otten, 2013). *Microcystis* ecology is complex and a preliminary analysis suggests there are no simple answers for the cause of the 2012 bloom. Flows in 2011 were high, and similar to previous studies in the Sacramento-San Joaquin Delta, these high flows were associated with low microcystin (Lehman et al., 2013). Yearly flows were approximately the same in 2009, a year without a bloom, and 2012, when the large bloom occurred (Table 4). Flows in July and August were also similar in those years, however timing of spring flows differed (Table 4, Figure 7).

High flows bringing in nutrients in the cool season, followed by low flows and high residence times during warm seasons have been found to promote cyanobacterial blooms in some water

bodies (Paerl and Otten, 2013). In the south Delta, the timing and flow path of spring flows are highly manipulated by management actions. In 2009, a dry year, the head of Old River barrier was not installed, and instead a bubble barrier was installed, allowing flow to be diverted from the San Joaquin River before reaching the DWSC (U.S. Fish and Wildlife Service, 2012). Unlike most dry years, additional water exports were not required under the Vernalis Adaptive Management Program (VAMP) in 2009 due to regulations concerning sequential dry years (San Joaquin River Group Authority, 2011). In 2012, the head of Old River barrier was installed and flow pulse releases occurred as required for fish outmigration (U.S. Fish and Wildlife Service, 2012; San Joaquin River Restoration Program, 2013).

Climate factors did not show a clear correspondence to *Microcystis* blooms, but higher soil temperatures in 2012 indicate that year had more consecutive days of high temperatures than 2009 or 2011 (Table 4). In July and August of 2012, there were 15 consecutive days with maximum daily temperatures over 32°C (90°F) and in 2009 and 2011, there were only 8 and 7 consecutive days with maximum daily temperatures over 32°C respectively. Samples with quantifiable concentrations of microcystin (>5 ng L^{-1}) in July and August came from warmer water than samples <5 ng L^{-1} microcystin (Table 5). Higher nutrient concentrations, especially phosphorous, were observed in 2012 (Tables 4 and 5). Elevated phosphorous levels in 2012, compared to 2009 and 2011, were confirmed with independent data collected by DWR in the same region.

Several water quality parameters were statistically different in samples with >5 ng L^{-1} than in sites with <5 ng L^{-1} microcystin in July and August including warmer water temperature, higher specific conductivity, chloride, pH, alkalinity, soluble and total P, and lower turbidity and suspended solids (Table 5). Although these preliminary analyses suggest hypotheses to investigate, the cause and effect between climate, environment, and *Microcystis* blooms cannot be determined without further investigation.

Conclusions

A bloom of *Microcystis*, a toxin-producing cyanobacteria, was observed in the southern area of the Sacramento-San Joaquin Delta near Stockton, CA. This bloom occurred in the summer of 2012, but not in either 2009 or 2011. In July and August of 2012, microcystin toxin concentrations in the southern area of the Delta were measured above California EPA recreational advisory limits of 800 ng L^{-1} and World Health Organization drinking water limit of 1,000 ng L^{-1}, with a maximum observed concentration of 2,140 ng L^{-1}. This bloom was associated with lower density and mass of zooplankton, including species which are important to a functioning food-web in the Delta. The cause of the 2012 bloom could not be attributed to any specific environmental condition, but timing of Spring flows, consecutive days of high temperature, and high nutrient concentrations might be contributing factors. Continued study of *Microcystis*, microcystin, and zooplankton in the southern Delta is needed to determine the causes of cyanobacterial blooms in the region and to further understand the effect of these blooms on the aquatic food-web

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Table 1. Zooplankton density and mass concentrations in south Delta waters in relation to microcystin (MC) toxin concentrations above and below 20 ng L^{-1} and above and below detection limits (5 ng L^{-1}) including July and August samples only. Significance of the difference between the two groups was determined using a single-sided t-test.

Taxa Mass is (µg L^{-1}) Density is (count L^{-1})	MC < 20 ng L^{-1} (n = 83) Mean	 (SD)	MC > 20 ng L^{-1} (n = 21) Mean	 (SD)	Statistically different at α = 0.05	[a]Statistically lower for MC > 5 ng L^{-1} α = 0.1
Total zooplankton density	93	(121)	33	(29)	Yes	No
Total zooplankton mass	16	(19)	9.4	(7.3)	Yes	Yes
Rotifer density	60	(108)	11	(22)	Yes	No
Rotifer mass	1.8	(3.6)	0.3	(0.5)	Yes	Yes
Total Copepod density	31	(38)	21	(17)	Yes	Yes
Total Copepod mass	13	(18)	8.7	(7.0)	Yes	Yes
***P. Forbesi* + *E. Affinis* density**	3.0	(5.8)	3.3	(4.0)	No	No
***P. Forbesi* + *E. Affinis* mass**	2.2	(7.4)	2.0	(2.5)	No	No
Cladoceran density (count L^{-1})	2.2	(5.5)	0.7	(1.0)	Yes	Yes
Cladoceran mass (µg L^{-1})	1.1	(2.7)	0.4	(0.7)	Yes	Yes

[a]The last column indicates which types of zooplankton were statistically lower in samples with quantifiable (>5 ng L^{-1}) microcystin.

Table 2. Fraction of samples with microcystin concentrations above detectable levels (>5 ng L^{-1}) in the south Delta in each month of the study.

Year	Month	n samples	% of samples with detectable microcystin	Maximum microcystin (ng L^{-1})
2009	July	10	50.0 %	21
2009	August	28	35.7 %	44
2011	April	0	-	-
2011	May	8	0.0 %	-
2011	June	29	11.7 %	78
2011	July	20	7.1 %	20
2011	August	21	3.4 %	13
2011	September	16	0.0 %	-
2011	October	25	0.0 %	-
2011	November	16	0.0 %	-
2011	December	15	0.0 %	-
2012	April	20	0.0 %	-
2012	May	17	6.4 %	34
2012	June	16	20.8 %	49
2012	July	15	72.7 %	2,140
2012	August	15	52.4 %	990
2012	September	7	7.1 %	36
2012	October	16	0.0 %	-
2012	November	16	0.0 %	-
2012	December	16	0.0 %	-

Table 3. Microcystin concentration in July and August in the Deepwater Ship Channel (DWSC), Tributaries, and the Upstream Sites.

Site Type	Microcystin Concentration (ng/L)		Pairwise t-test
	mean (SD)	Min - Max	
DWSC	140 (374)	0 - 2,140	A
Tributaries	16 (65)	0 - 407	B
Upstream	17 (43)	0 - 174	B

Table 4. Yearly flow and July-August flow, nutrients, and climate data for 2009, 2011 and 2012 are compared. Hourly temperature data was acquired from the CIMIS for the Manteca station. Water temperature and flow data was from CDEC for the Brandt Bridge station (BDT). Nutrient data was collected by EERP and includes data from July and August only. In the pairwise comparisons using student's t test, A's are the highest followed by B's and C's. If years are significantly different from each other (alpha = 0.05) this difference is indicated by a different letter.

Year	Analysis	Mean	(SD)	Pairwise t-test Results
2009	Yearly Flow (cfs)	293	-1,490	C
2011	Yearly Flow (cfs)	4,030	-2,820	A
2012	Yearly Flow (cfs)	558	-1,520	B
2009	July/Aug Flow (cfs)	-114	-1,490	C
2011	July/Aug Flow (cfs)	3,430	-1,530	A
2012	July/Aug Flow (cfs)	-51	-1,540	B
2009	Soil Temp (°C)	20.4	-0.54	B
2011	Soil Temp (°C)	19.7	-0.46	C
2012	Soil Temp (°C)	20.9	-0.79	A
2009	Air Temp (°C)	22.6	-6.3	A, B
2011	Air Temp (°C)	22.2	-6.2	B
2012	Air Temp (°C)	22.8	-6.5	A
2009	Water Temp (°C)	25.9	-0.71	A
2011	Water Temp (°C)	19.7	-1	C
2012	Water Temp (°C)	24.61	-2.4	B
2009	Wind Direction (0-360)	235	-94	B
2011	Wind Direction (0-360)	227	-102	C
2012	Wind Direction (0-360)	239	-98	A
2009	Specific Conductance ($\mu S\ cm^{-1}$)	377	-171	A
2011	Specific Conductance ($\mu S\ cm^{-1}$)	251	-87	B
2012	Specific Conductance ($\mu S\ cm^{-1}$)	447	-229	A

Table 4. Cont.

Year	Analysis	Mean	(SD)	Pairwise t-test Results
2009	pH	7.6	-0.49	A, B
2011	pH	7.4	-0.4	B
2012	pH	7.8	-0.57	A
2009	Turbidity (NTU)	9.2	-7.9	A
2011	Turbidity (NTU)	15	-9.2	A
2012	Turbidity (NTU)	12	-10	A
2009	T SS (mg L^{-1})	11	-5.6	A
2011	T SS (mg L^{-1})	23	-19	A
2012	T SS (mg L^{-1})	17	-15	A
2009	MSS (mg L^{-1})	8.8	-4.8	A
2011	MSS (mg L^{-1})	20	-18	A
2012	MSS (mg L^{-1})	13	-12	A
2009	Chlorophyll-a (µg L^{-1})	14	-30	B
2011	Chlorophyll-a (µg L^{-1})	9.5	-8.9	B
2012	Chlorophyll-a (µg L^{-1})	18	-25	A
2009	Soluble Phosphate as P (mg L^{-1})	0.1	-0.05	B
2011	Soluble Phosphate as P (mg L^{-1})	0.08	-0.03	B
2012	Soluble Phosphate as P (mg L^{-1})	0.17	-0.11	A
2011	Total Phosphorus as P (mg L^{-1})	0.14	-0.06	B
2012	Total Phosphorus as P (mg L^{-1})	0.24	-0.14	A
2009	Total Ammonia-N (µg L^{-1})	33	-40	A
2011	Total Ammonia-N (µg L^{-1})	29	-21	A
2012	Total Ammonia-N (µg L^{-1})	40	-48	A
2009	Dissolved Nitrate-N (µg L^{-1})	367	-369	B
2011	Dissolved Nitrate-N (µg L^{-1})	462	-298	A, B
2012	Dissolved Nitrate-N (µg L^{-1})	601	-651	A
2009	Total Nitrogen (µg L^{-1})	854	-486	B
2011	Total Nitrogen (µg L^{-1})	820	-292	B
2012	Total Nitrogen (µg L^{-1})	1100	-792	A
2009	Dissolved Silica-Si (mg L^{-1})	5.2	-1.6	A
2011	Dissolved Silica-Si (mg L^{-1})	5.5	-0.7	A
2012	Dissolved Silica-Si (mg L^{-1})	5.6	-1.6	A

Table 4. Cont.

Year	Analysis	Mean	(SD)	Pairwise t-test Results
2009	Inorganic Carbon (mg L^{-1})	17	-4.2	A
2011	Inorganic Carbon (mg L^{-1})	13	-2.7	B
2012	Inorganic Carbon (mg L^{-1})	17	-3.4	A
2011	Alkalinity (mg L^{-1})	50	-11	B
2012	Alkalinity (mg L^{-1})	82	-19	A
2011	Chloride (mg L^{-1})	31	-20	B
2012	Chloride (mg L^{-1})	72	-54	A
2009	Dissolved N:Dissolved P	9.1	-7.9	A, B
2011	Dissolved N:Dissolved P	15	-9.5	A
2012	Dissolved N:Dissolved P	8.8	-10	B
2009	Dissolved Si:Dissolved P	32	-16	A, B
2011	Dissolved Si:Dissolved P	43	-28	A
2012	Dissolved Si:Dissolved P	28	-25	B
2009	Inorganic C:Dissolved P	559	-282	A, B
2011	Inorganic C:Dissolved P	511	-326	A
2012	Inorganic C:Dissolved P	434	-366	B
2009	Inorganic C:Dissolved N	200	-303	A
2011	Inorganic C:Dissolved N	145	-345	A
2012	Inorganic C:Dissolved N	187	-290	A

Table 5. Water quality and nutrients in July and August of all study years in the south Delta when microcystin (MC) was measured above or below quantifiable levels (5 ng L^{-1}). Significance of the difference between the two groups was determined using a single-sided t-test. Nutrient ratios reported as a molar ratio.

Parameter	MC < 5 ng L^{-1} (n = 330) Mean	(SD)	MC > 5 ng L^{-1} (n = 57) Mean	(SD)	Statistically Different at α = 0.05
Water Temp (°C)	23.7	(2.1)	24.5	(1.3)	Yes
Specific Cond. (µS cm^{-1})	334	(173)	404	(193)	Yes
pH	7.55	(0.47)	7.71	(0.55)	No[a]
Turbidity (NTU)	13	(9.6)	8.2	(6.9)	Yes
TSS (mg L^{-1})	23	(19)	15	(12)	Yes
MSS (mg L^{-1})	19	(17)	11	(8.4)	Yes
Chlorophyll-a (µg L^{-1})	13	(18)	20	(28)	No
Soluble PO_4-P (mg L^{-1})	0.09	(0.06)	0.14	(0.10)	Yes
Total P (mg L^{-1})	0.16	(0.09)	0.27	(0.15)	Yes
Ammonia-N (µg L^{-1})	29	(36)	38	(39)	No
Dissolved Nitrate-N (µg L^{-1})	462	(405)	509	(589)	No
Total N (µg L^{-1})	871	(458)	966	(747)	No
Dissolved Silica-Si (mg L^{-1})	5.4	(1.3)	5.5	(1.3)	No
Inorganic Carbon (mg L^{-1})	16	(4.5)	16	3.3	No
Alkalinity (mg L^{-1})	61	(23)	77	(18)	Yes
Chloride (mg L^{-1})	40	(34)	87	(53)	Yes
Dissolved N:Dissolved P	12	(9.7)	8.2	(8.1)	Yes
Dissolved Si:Dissolved P	82	(47)	63	(38)	Yes
Inorganic C:Dissolved P	548	(300)	411	(264)	Yes
Inorganic C:Dissolved N	190	(297)	101	(192)	Yes

[a]Significant at α = 0.1

Figure 1. Sampling locations in the southern Delta, near the city of Stockton.

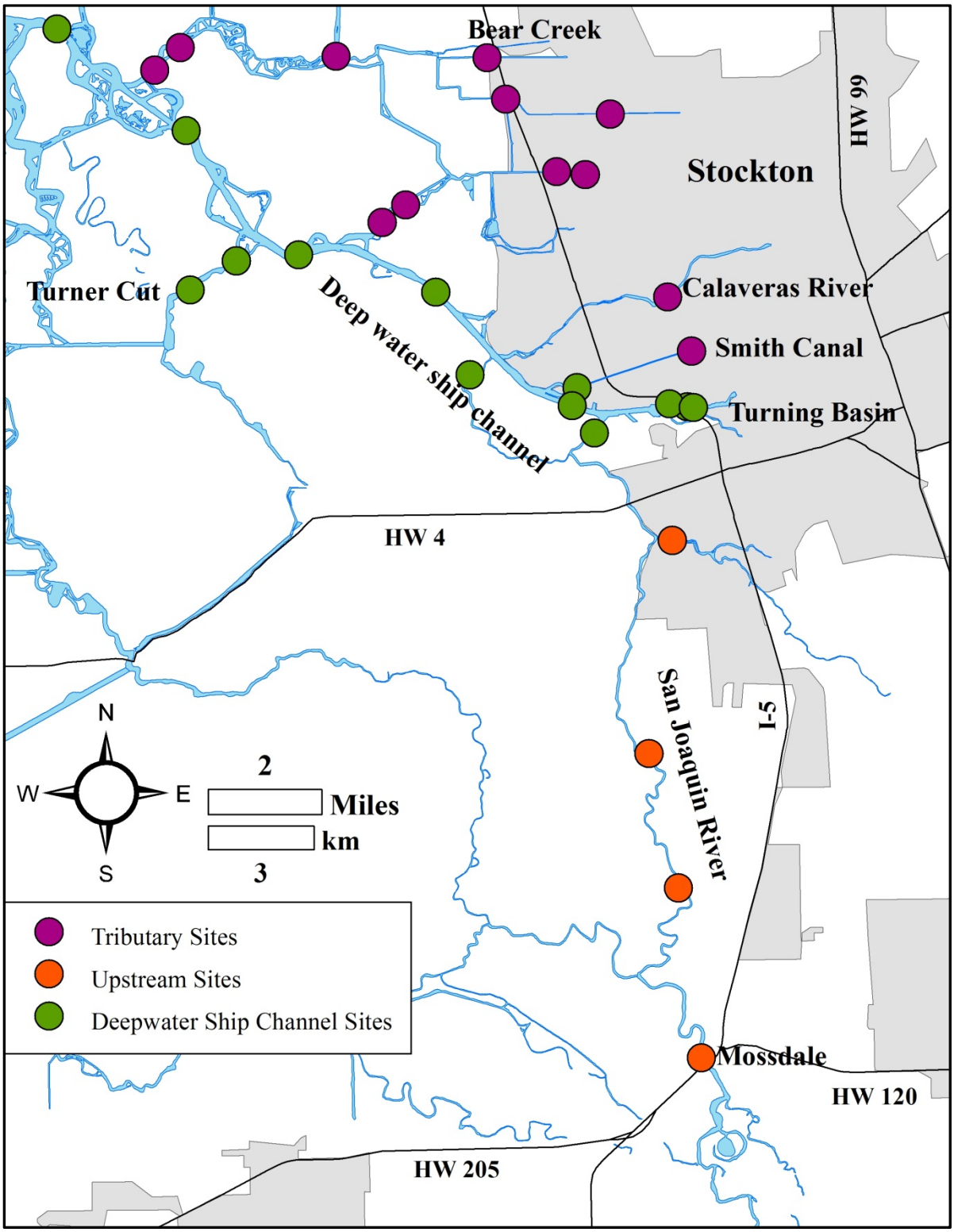


Figure 2. Microscopic image of *Microcystis* sp. collected 8/16/12 (100x magnification).

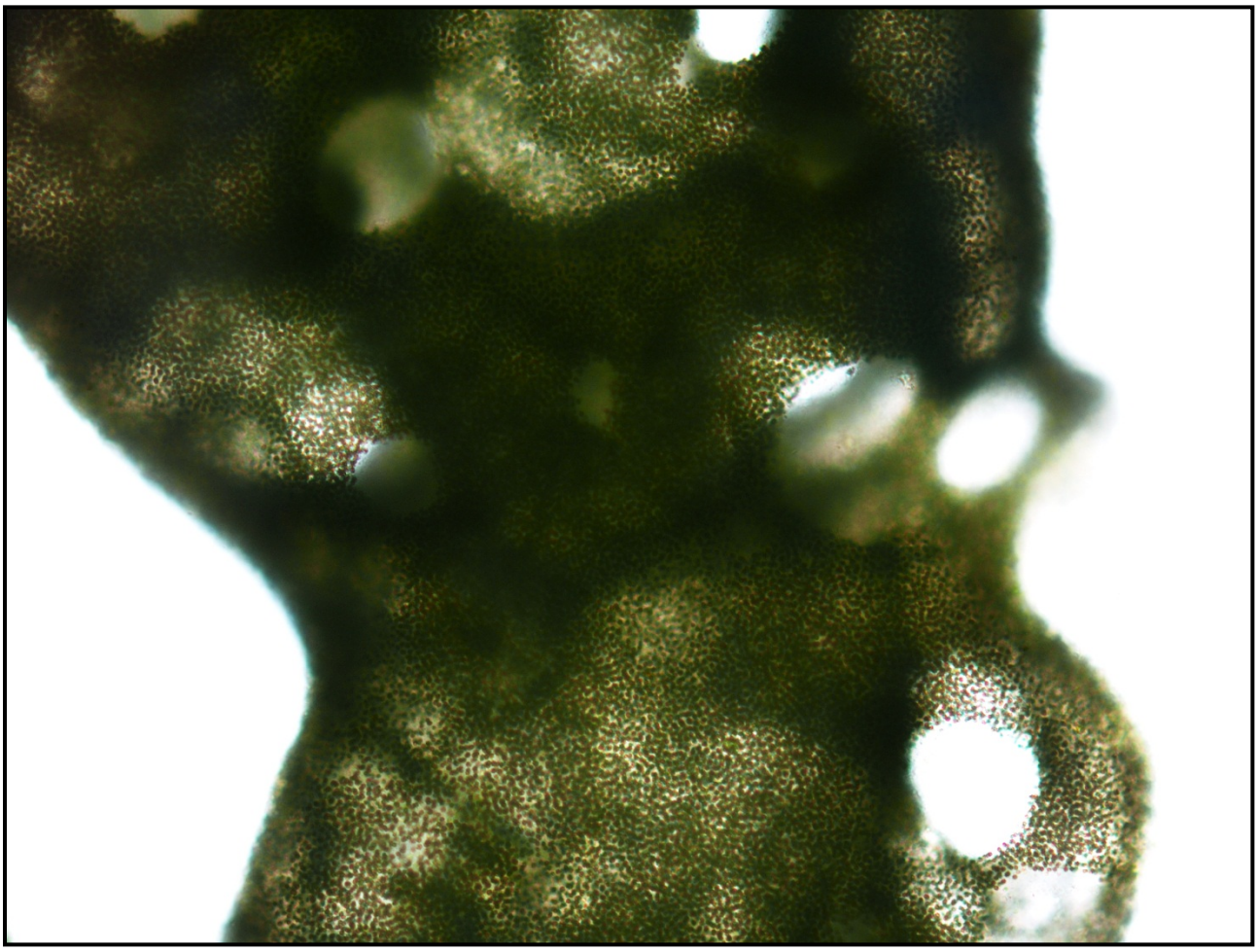


Figure 3. Relationship between cyanobacteria biomass and the toxin microcystin. Microcystin toxin was associated with the presence of cyanobacteria, which were predominately *Microcystis* sp.

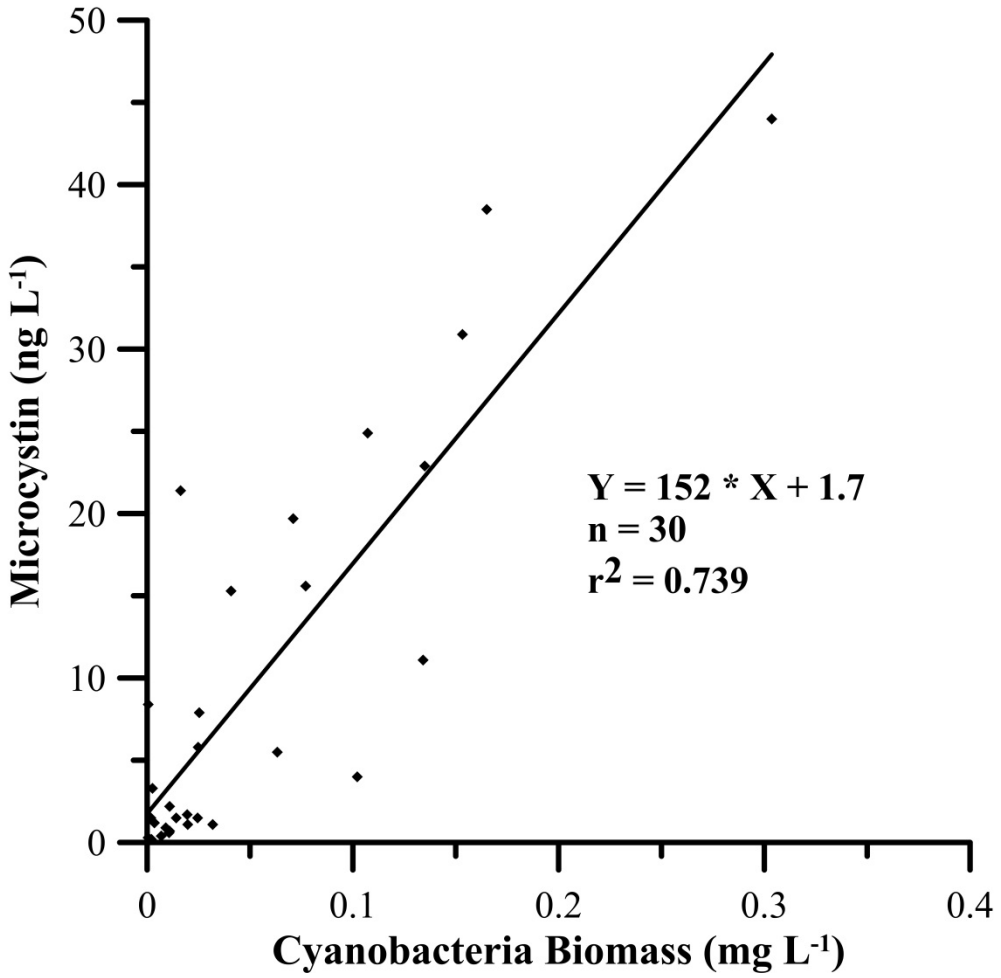


Figure 4. A) Microcystin concentration (ng MC L^{-1}) in July and August of each study year. B) Microcystin as a fraction of Chlorophyll-a (ng MC µg Chl-a^{-1}) in July and August of each study year. Note break in scale on y-axis.

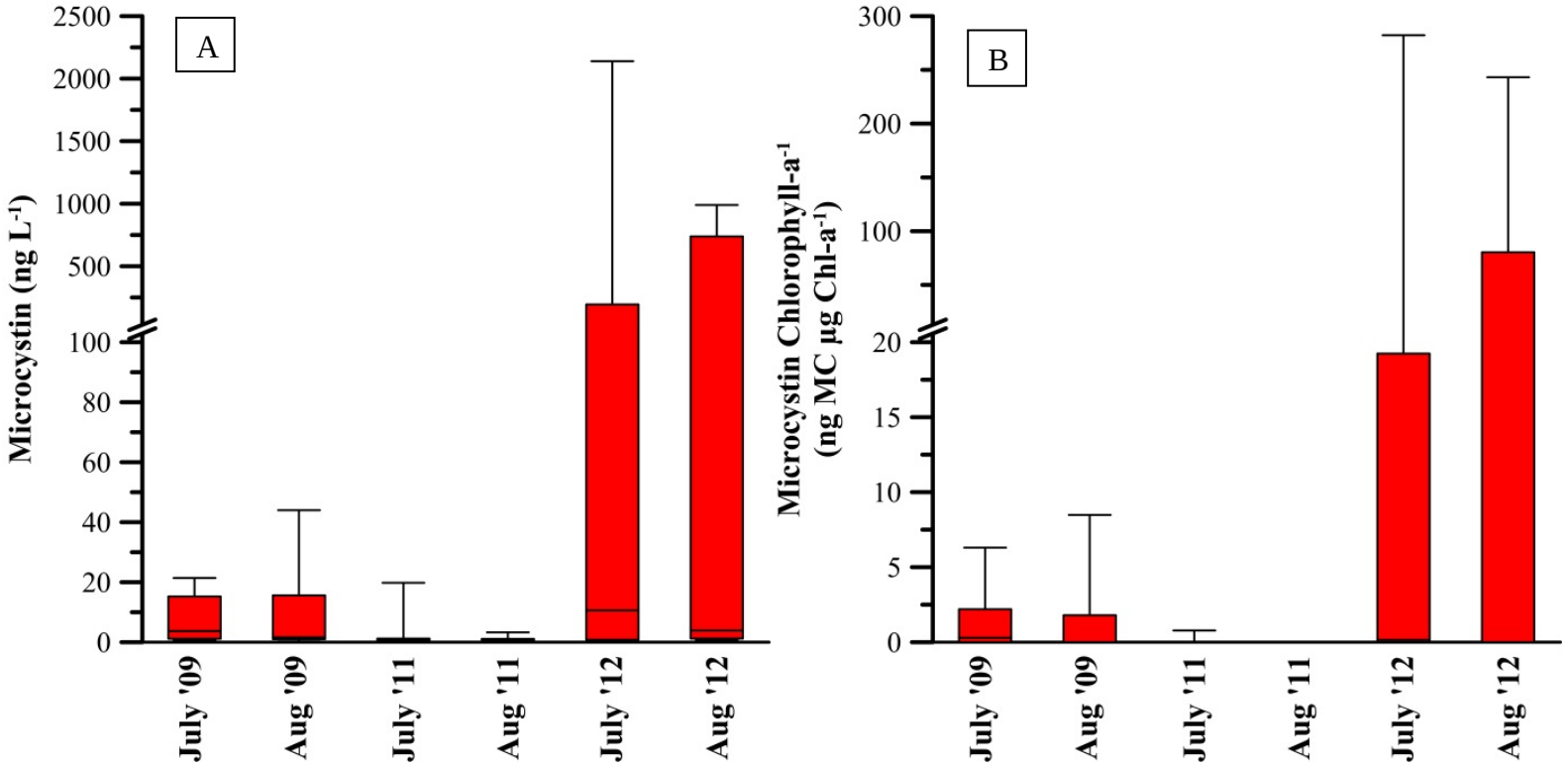
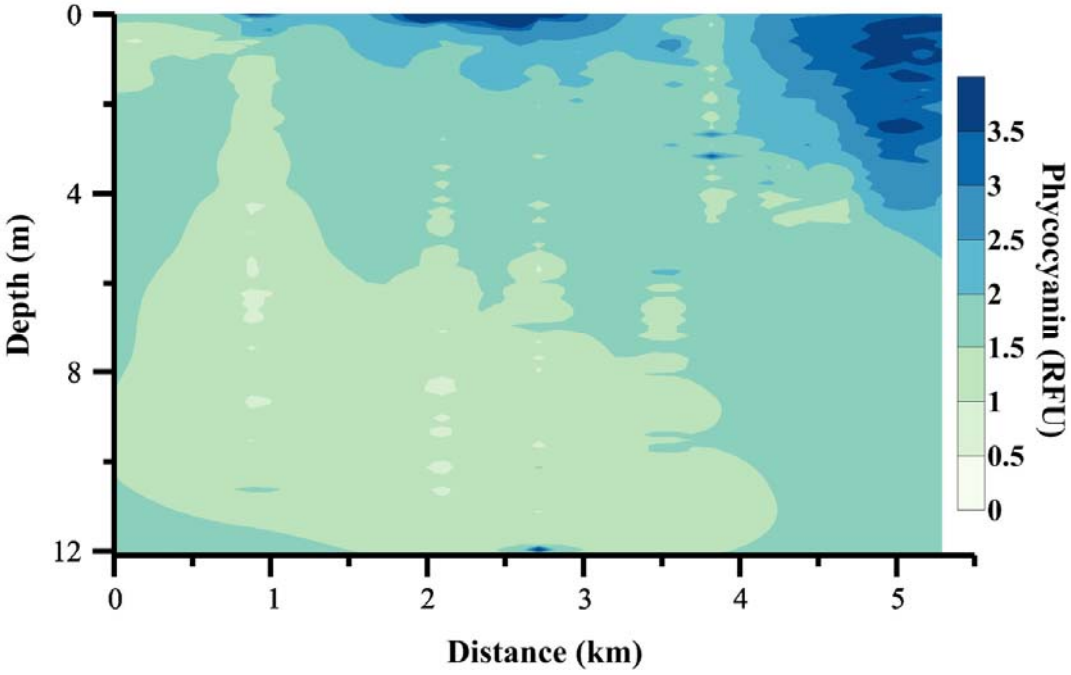


Figure 5. Distribution of phycocyanin fluorescence, a substitute measure of cyanobacteria, in Stockton's Deep water ship channel. Measurements were made on August 2, 2012 during a vertical profiling study. Figure (A) shows phycocyanin in the water column. Kilometer zero starts just west of RRI and kilometer 5 is located in the Turning Basin. Figure (B) shows the same data from a bird's eye view. Phycocyanin is most concentrated near the surface and in the Turning Basin.

(A)



(B)

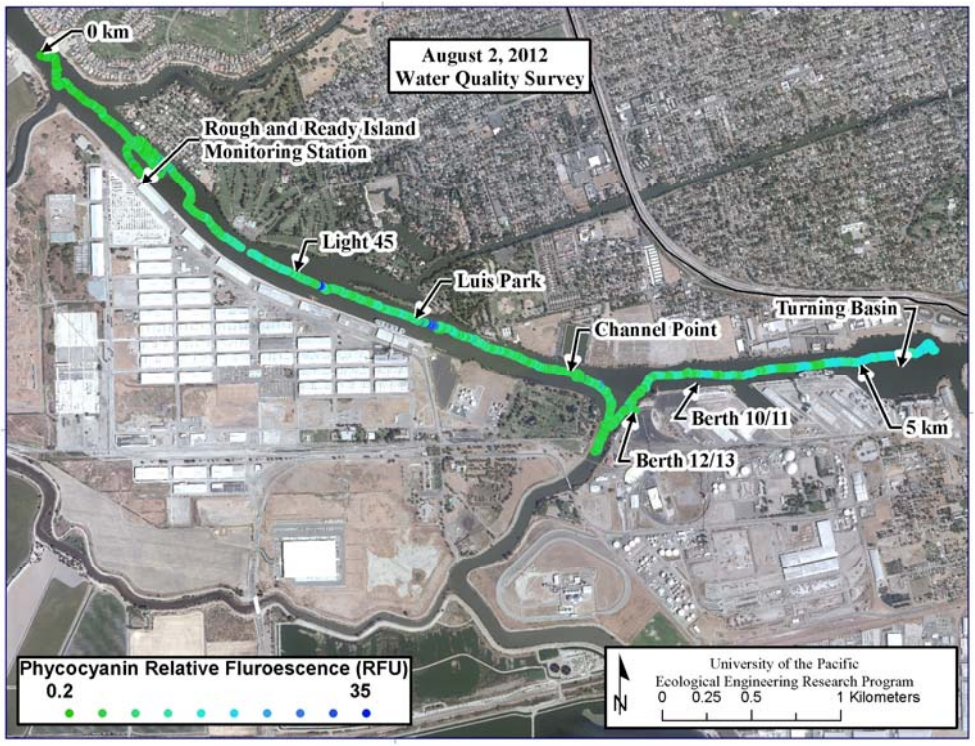


Figure 6. Maximum microcystin concentrations in the south Delta in July and August of 2012.

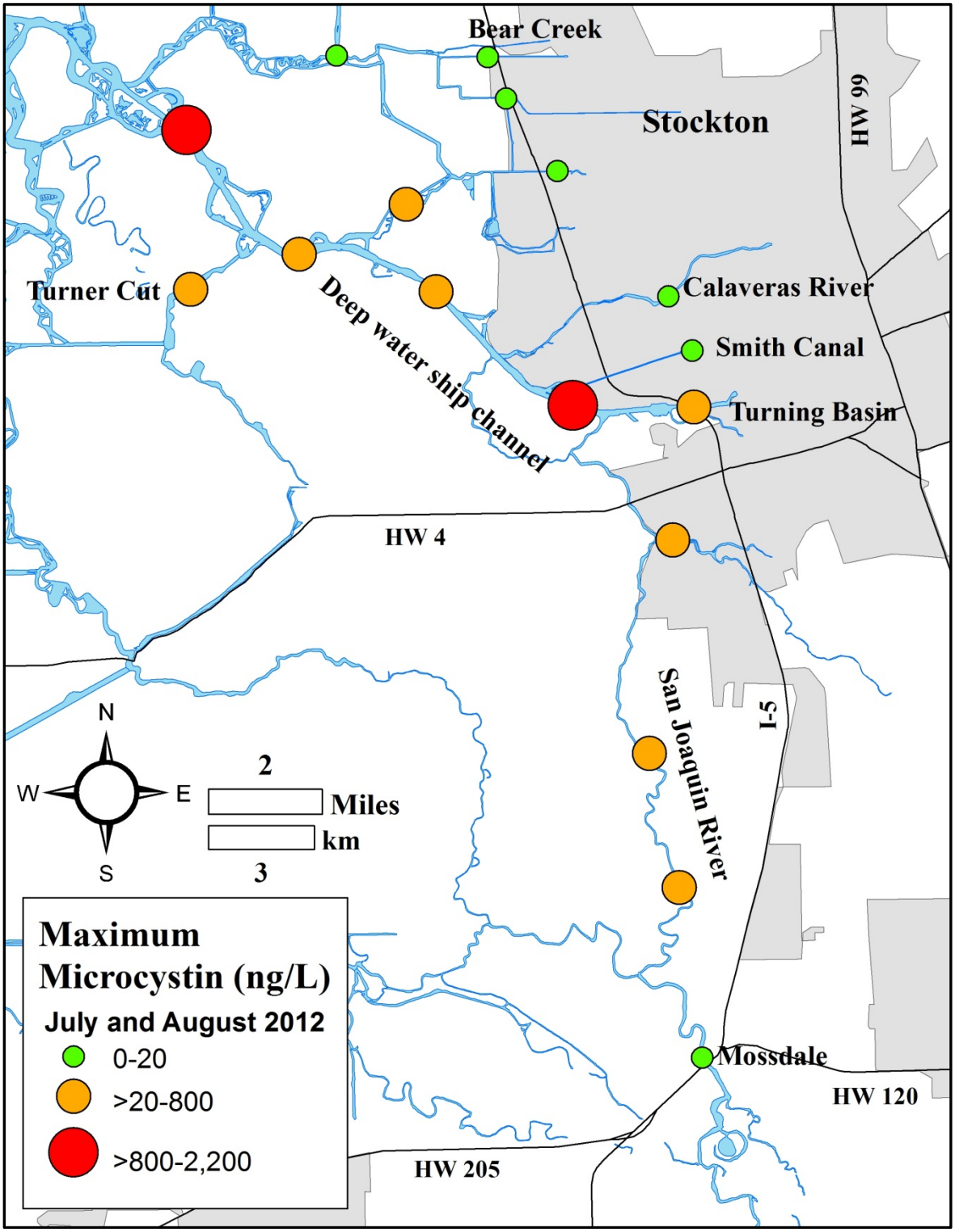


Figure 7. Comparison of flows at Brandt Bridge by month for 2009 and 2012. A large *Microcystis* bloom was observed in 2012, but not 2009. On average, flows were similar between years, but 2012 was characterized by high spring flows. Net negative flows in July and August are due to tidal action and upstream pumping.