

# The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in San Francisco Estuary

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**Abstract** A bloom of the cyanobacteria *Microcystis aeruginosa* was sampled over the summer and fall in order to determine if the spatial and temporal patterns in cell density, chlorophyll *a* (chl *a*) concentration, total microcystins concentration, and percent microcystins composition varied with environmental conditions in San Francisco Estuary. It was hypothesized that the seasonal variation in *Microcystis* cell density and microcystin concentration was ecologically important because it could influence the transfer of toxic microcystins into the aquatic food web. Sampling for *Microcystis* cell density, chl *a* concentration, total microcystins concentration and a suite of environmental conditions was conducted biweekly at nine stations throughout the freshwater tidal and brackish water regions of the estuary between July and November 2004. Total microcystins in zooplankton and clam tissue was also sampled in August and October. *Microcystis* cell density, chl *a* concentration and total microcystins concentration varied by an order of magnitude and peaked during August and

September when  $P_m^B$  and  $\alpha^B$  were high. Low stream-flow and high water temperature were strongly correlated with the seasonal variation of *Microcystis* cell density, total microcystins concentration ( $\text{cell}^{-1}$ ) and total microcystins concentration (chl *a*) $^{-1}$  in canonical correlation analyses. Nutrient concentrations and ratios were of secondary importance in the analysis and may be of lesser importance to seasonal variation of the bloom in this nutrient rich estuary. The seasonal variation of *Microcystis* density and biomass was potentially important for the structure and function of the estuarine aquatic food web, because total microcystins concentration was high at the base of the food web in mesozooplankton, amphipod, clam, and worm tissue during the peak of the bloom.

**Keywords** *Microcystis* · Estuary · Microcystins · Food web · Seasonal variation

## Introduction

*Microcystis aeruginosa* (*Microcystis*) is a common freshwater cyanobacterium in freshwater lakes and reservoirs worldwide (Federal Environmental Agency, 2005). It also occurs in rivers that form estuaries including the Potomac River and the Neuse River in the USA, the Swan River in Australia and the Guadiana River in Spain and Portugal (Sellner et al., 1988; Pearl, 1988; Rocha et al., 2002; Orr et al., 2004).

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*Microcystis* is considered a cyanobacterial harmful algal bloom (CHAB) species because it produces surface scums that impede recreation sports, reduce aesthetics, lower dissolved oxygen concentration and cause taste and odor problems in drinking water (Carmichael, 1995). *Microcystis* also produces toxic microcystins that are powerful hepatotoxins associated with liver cancer and tumors in humans and wildlife (Carmichael, 1995).

The toxicity of *Microcystis* blooms negatively impact phytoplankton, zooplankton, and fish production directly or indirectly through the transfer or accumulation of toxins in the food web (Kotak et al., 1996; Ibelings et al., 2005; Sedmak & Elerseck, 2005; Malbrouck & Kestemont, 2006). *Microcystis* also affects aquatic community structure and function by impacts on feeding success or food quality for zooplankton and fish (Rohrlack et al., 2005; Malbrouck & Kestemont, 2006). The abundance of cyanobacteria further affects total carbon production by causing a shift from large to small zooplankton species (Fulton & Pearl, 1987; Smith & Gilbert, 1995).

*Microcystis* blooms vary over the summer and fall in response to environmental factors that influence bloom initiation and those that sustain bloom growth. Since *Microcystis* does not contain heterocysts that produce nitrate from atmospheric nitrogen, both high nitrogen, and phosphorus are needed for blooms to develop (Pearl et al., 2001). Bloom initiation requires water temperature above 20°C (Jacoby et al., 2000), but accumulation of high biomass, requires long residence time for this slow growing species (Reynolds, 1997). Blooms also develop faster in vertically stable environments that allow the buoyant *Microcystis* colonies to rise to the surface of the water column where they out compete other phytoplankton for light (Huisman et al., 2004). Other factors such as high pH and turbidity or low carbon dioxide concentration enhance growth of *Microcystis* over other phytoplankton once the bloom is established, but are not required for bloom initiation or growth (Shapiro, 1990). Most of the information on the importance of environmental factors for *Microcystis* bloom development and persistence is obtained from freshwater lakes and reservoirs, less is known about the relative importance of environmental factors in estuaries, particularly nutrient-rich estuaries like San Francisco Estuary (SFE).

The cause of *Microcystis* blooms and their potential impact on estuarine productivity is an important concern for SFE where a bloom of *Microcystis* first appeared in 1999 (Lehman et al., 2005). Little is known about the seasonal variation of *Microcystis* cell density, biomass and toxic microcystin concentration, the environmental factors that affect the seasonal variation of the bloom or its impact on the structure and function of the estuarine food web. Data from a single sampling day in October 2003 indicated *Microcystis* was widely distributed across the freshwater to brackish water reaches of the estuary and contained the hepatotoxic microcystin-LR (Lehman et al., 2005). The bloom was associated with high and non-limiting nitrogen and phosphorus concentration, high water temperature and high water transparency but values from this single sampling day were not sufficient to assess the importance of these variables. The presence of total microcystins in zooplankton and clam tissue also suggested the toxins in the bloom might impact the aquatic food web.

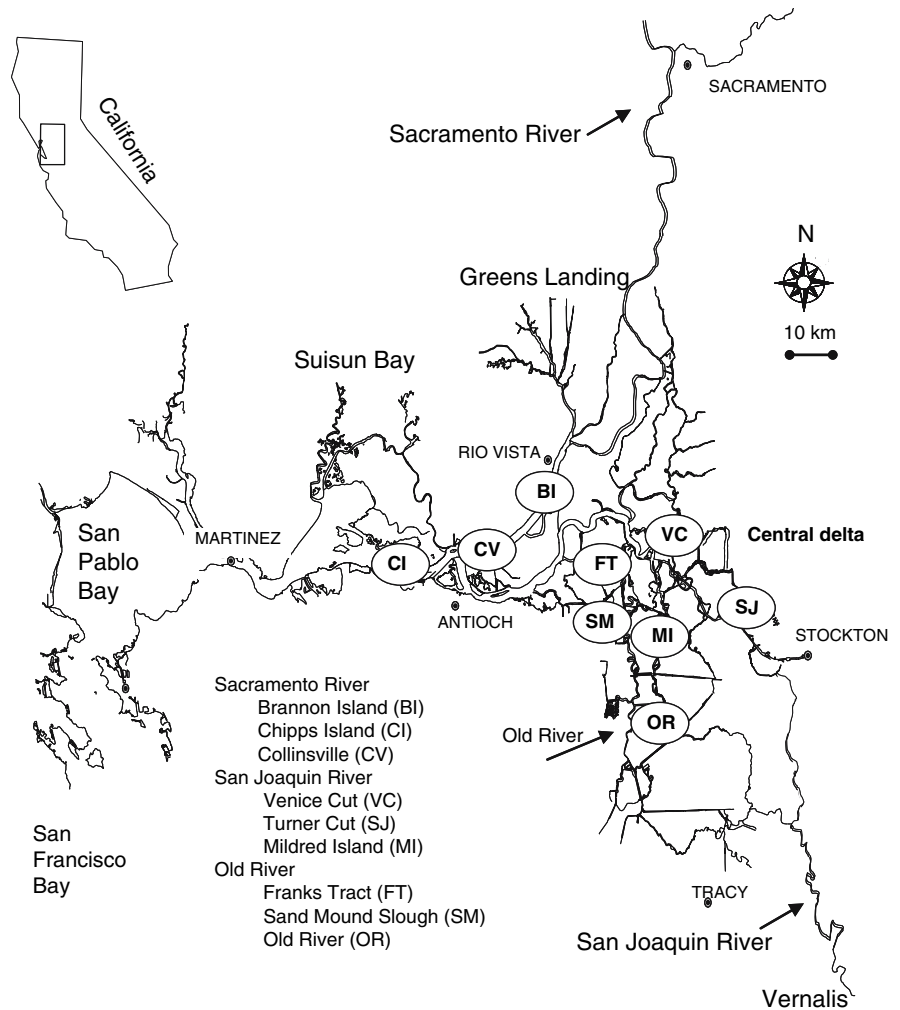
The purpose of this study was to quantify the seasonal variation of *Microcystis* cell density, chlorophyll *a* (chl *a*) concentration, microcystins concentration and the presence of microcystins in the tissues of lower aquatic food web organisms and to determine how these variables are influenced by environmental conditions in SFE. Understanding the influence of environmental conditions on the seasonal variation of the *Microcystis* bloom and its associated microcystins concentration is potentially important for management of fishery production in SFE, where the food web is dependent on phytoplankton and zooplankton production and characterized by a long-term decrease in fish, zooplankton and diatom carbon (Lehman, 2004; Sommer et al., 2007).

## Materials and methods

### Study area

SFE consists of an inland delta that flows into a chain of downstream marine bays—Suisun, San Pablo and San Francisco—and creates one of the largest estuaries on the west coast of North America (Fig. 1). The inland delta formed by the Sacramento River (SAC) on the north and the San Joaquin River (SJR)

**Fig. 1** Map of San Francisco Estuary showing location of sampling stations



on the south contains 200 km<sup>2</sup> of waterways. SAC is the largest of the rivers with an average discharge of 4795 m<sup>3</sup> s<sup>-1</sup> compared with 400 m<sup>3</sup> s<sup>-1</sup> for SJR over the July through October period of this study. Other rivers influence streamflow in the delta including the Mokelumne (MOKE) and Cosumnes (CSR) Rivers with average discharge of 21 m<sup>3</sup> s<sup>-1</sup> and 6 m<sup>3</sup> s<sup>-1</sup>, respectively. An important feature of the delta is the large amount of water removed for agriculture that causes average reverse streamflow of 1578 m<sup>3</sup> s<sup>-1</sup> in Old River (ODR) and 1339 m<sup>3</sup> s<sup>-1</sup> in SJR during August and September (<http://www.waterdata.usgs.gov/nwis>). Depth varies in the delta from a few meters in the flooded islands in the center of the delta to 13 m in the main river channels. Tides in the delta reach 2 m in height with tidal velocities up to 30 cm s<sup>-1</sup> and tidal excursions of 10 km.

#### Field and laboratory sampling

Chl *a* concentration, *Microcystis* cell density and microcystin (total and individual) concentration were sampled biweekly between July 13 and November 3, 2004 at nine stations throughout the freshwater to brackish water reaches of SFE. Selected stations represented different habitat types or beneficial use including recreational swimming (BI), shallow water habitat (MI and FT), deep river channel (CV, VC and SM), native fish habitat (SJ and CI) and agricultural and drinking water supply (OR; Fig. 1). *Microcystis* colonies were sampled by horizontal surface tows of a 0.72 m diameter plankton net fitted with a 75- $\mu$ m mesh screen (Lehman et al., 2005). Use of a smaller mesh net (40  $\mu$ m) was not possible because the net became clogged with heavy sediment. Water

temperature, specific conductance, pH, dissolved oxygen, and turbidity were also measured at each station using a Yellow Springs Instrument (YSI) 6600 sonde. Depth of the euphotic zone was estimated from Secchi disk depth. Photosynthetically active surface irradiance (PAR) was measured at 15 min intervals in Langleys at Antioch, CA using an Eppley phyroheliumeter (<http://www.iep.water.ca.gov>). Langleys were converted to mole quanta using linear correlation with LiCOR quantum sensor values ( $r^2 = 0.91$ ;  $P < 0.01$ ).

Surface water samples were collected by van Dorn water sampler and immediately stored at 4°C. Algal biomass was filtered within 2 h onto Millipore APFF glass fiber filters (0.7 µm pore diameter). Filters for microcystins analysis were folded, wrapped in aluminum foil and frozen at -80°C until analysis. Filters for chl *a* (corrected for phaeophytin) and phaeophytin analysis were treated with 1 ml of saturated magnesium carbonate solution as a preservative and frozen at -14°C until analysis (method 10200H, APHA et al., 1998). Phytoplankton for identification and enumeration were preserved and stained with Lugol's iodine solution and species were counted at 700X using the inverted microscope technique (Utermöhl, 1958). Sample replication was 10%.

Water samples for dissolved ammonium, nitrate-plus-nitrite, soluble reactive phosphorus, and silicate concentration were filtered through 0.45 µm pore size Millipore HATF04700 nucleopore filters. Filtered samples plus raw water samples for total phosphorus were stored at -14°C until analysis by colorimetric techniques (US EPA, 1983; USGS, 1985). Total suspended solids concentration was determined by standard methods (APHA et al., 1998). Daily average streamflow, air temperature, and water temperature were obtained from hourly data collected by the Interagency Ecological Program (<http://www.iep.water.ca.gov>).

Net primary productivity and community respiration (phytoplankton and bacteria) were measured for a single station each sampling day by 4–6 h incubations at 0.075 m depth near Antioch, CA (Fig. 1) using the dissolved oxygen light and dark bottle incubation technique (Vollenweider, 1974). Values obtained from incubating bottles in a light gradient were used to compute the photosynthetic capacity from the chl *a* specific light saturated rate of photosynthesis ( $P_m^B$ , mg C

(mg chl *a*)<sup>-1</sup> h<sup>-1</sup>), the photosynthetic efficiency from the chl *a* specific initial slope ( $\alpha^B$ ; mg C (mg chl *a*)<sup>-1</sup> (mole quanta m<sup>-2</sup>)<sup>-1</sup>) and the photoinhibition parameter from the chl *a* specific negative slope of the P-I curve above light saturation ( $\beta^B$ , mg C (mg chl *a*)<sup>-1</sup> (mole quanta m<sup>-2</sup>)<sup>-1</sup>; Lehman et al., 2007). These parameters were used to compute integrated gross ( $GP_{ez}$ , mg C m<sup>-2</sup> h<sup>-1</sup>) and areal ( $GP_{ez}$ ) primary productivity of the euphotic zone.

Zooplankton including mesozooplankton, amphipods, worms, and jellyfish were sampled at CV, SM, SJ, and MI by horizontal tows of a 0.7 m diameter plankton net fitted with a 150 µm mesh. Zooplankton tissue was kept at 4°C and separated by pipette from *Microcystis* in the water sample using a dissecting microscope within 48 h of sampling. The final zooplankton tissue sample was rinsed in distilled water and frozen at -80°C until analysis. Clams were collected using a ponar dredge. The muscle tissue was removed from the shell, rinsed in distilled water and frozen at -80°C until analysis.

#### Microcystin analysis

Filters and animal tissue for microcystin analysis were extracted and assessed for total microcystins using the protein phosphate inhibition assay (PPIA). Samples with high levels of total microcystins were further analyzed by high pressure liquid chromatography (HPLC) to identify the specific microcystins in the sample (Lehman et al., 2005).

#### Statistical analysis

Statistical analyses included correlation and single and multiple comparisons using analysis of variance. Kruskal-Wallis nonparametric analysis of variance was used when the assumptions of the analysis (normally distributed data and homogeneity of variance) were not met. Canonical correlation analysis was computed using log-transformed values in order to minimize differences in variance produced by differences in absolute size and adjust for nonhomogeneity of variance among variables. All statistical analyses were computed using Statistical Analysis System software (SAS Institute Inc., 2004).

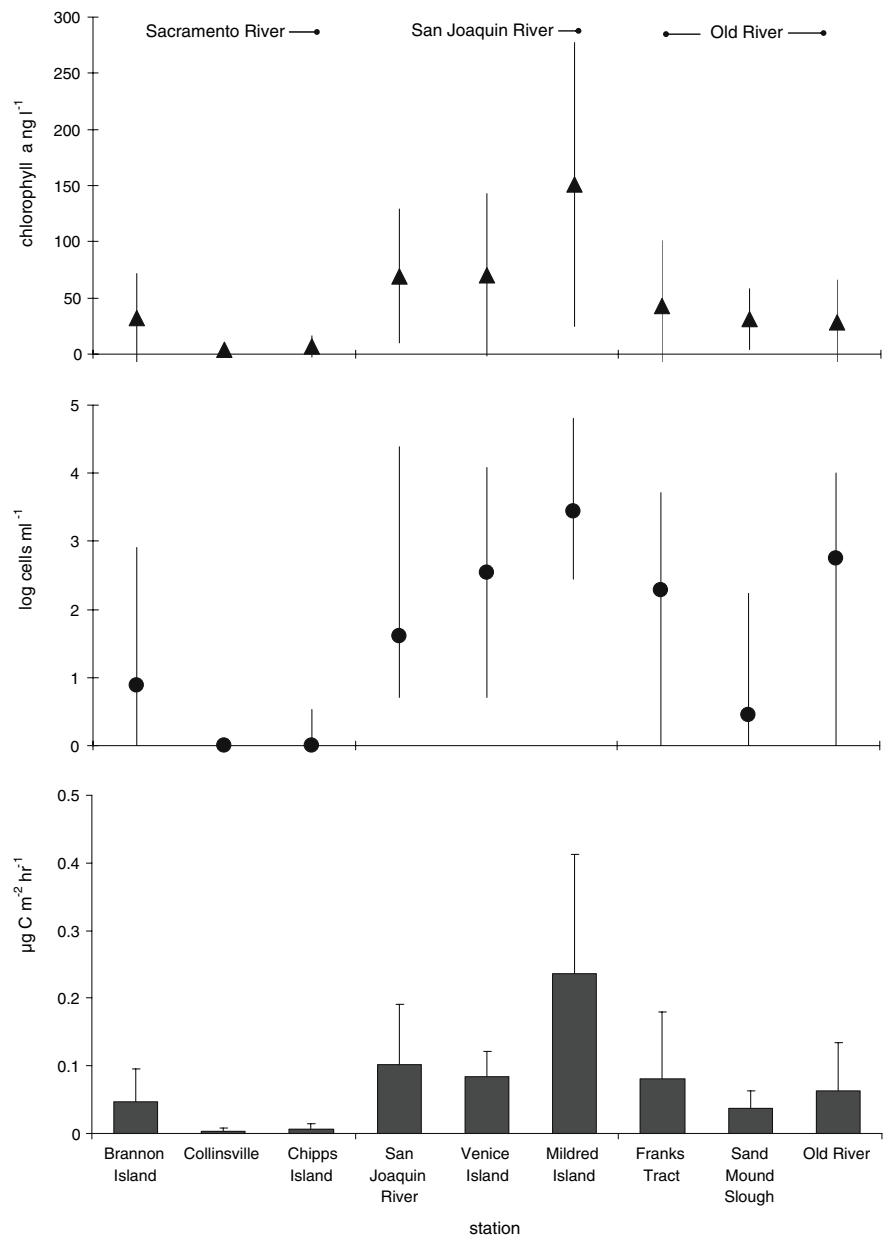
## Results

### *Microcystis* spatial and temporal variation

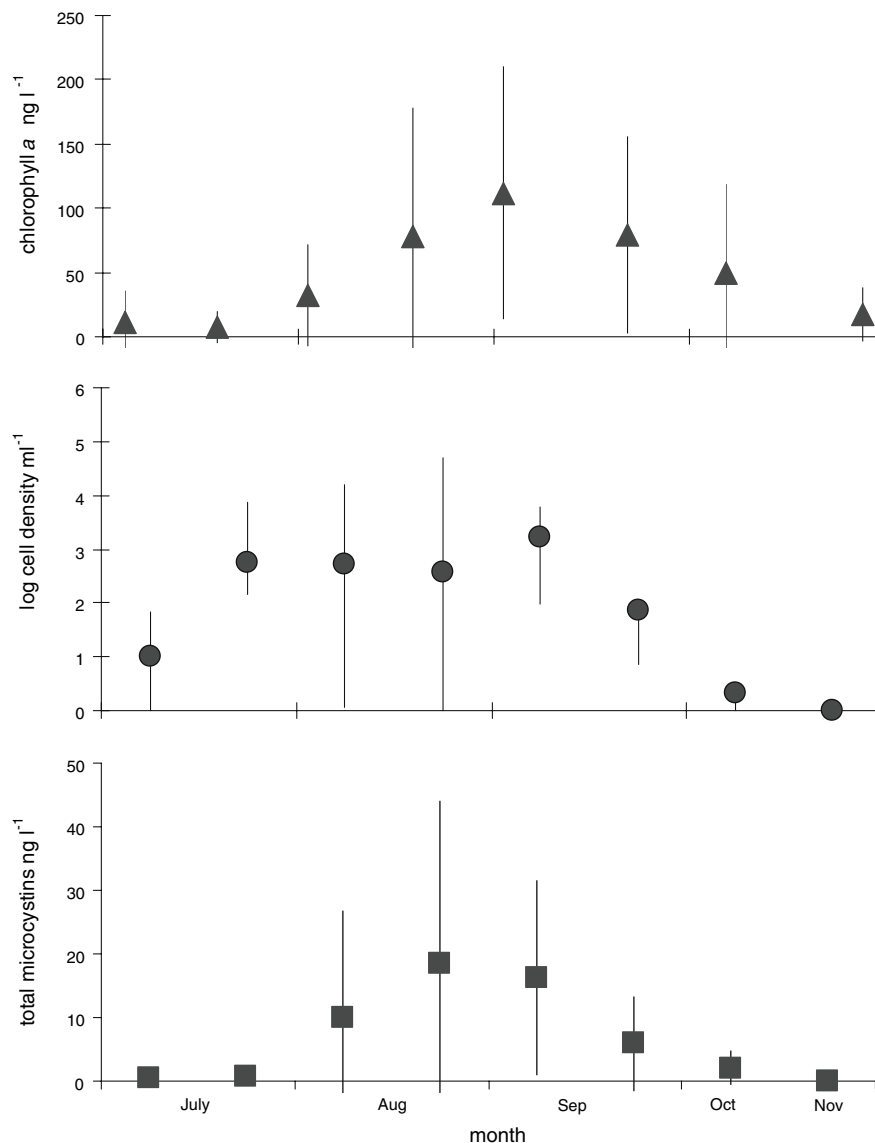
Chl *a* concentration and *Microcystis* cell density in the net tow samples were greatest in SJR (Fig. 2). Average chl *a* concentration was 7-fold greater ( $P < 0.05$ ) in SJR compared with SAC ( $P < 0.05$ ; mean  $97 \pm 70$ ,  $34 \pm 36$ , and  $14 \pm 18$  ng l<sup>-1</sup> for SJR, ORD and SAC, respectively). Among stations,

chl *a* concentration was greatest ( $P < 0.05$ ) at the shallow flooded island and slow moving river channel stations MI and SJ in SJR and lowest at the fast flowing river channels CI and CV in SAC. *Microcystis* cell density varied in a similar fashion to chl *a* concentration among rivers and was greatest ( $P < 0.05$ ) in SJR followed by ODR and SAC (Fig. 2). Cell densities were low in the net tows and exceeded 20,000 cells ml<sup>-1</sup> only three times at MI, two times at SJ and VC in SJR and once at OR and

**Fig. 2** Mean and standard deviation (vertical bar) of chlorophyll *a* concentration in the >75  $\mu$ m algal size fraction, log *Microcystis* cell density and areal gross primary productivity in the euphotic zone at stations in San Francisco Estuary between July and October 2004



**Fig. 3** Mean and standard deviation (vertical bar) of chlorophyll *a* concentration in the >75  $\mu\text{m}$  algal size fraction, log *Microcystis* cell density  $\text{ml}^{-1}$  and total microcystins concentration among months between July and November 2004 in San Francisco Estuary



FT in ODR. The maximum cell density was  $22,480,000 \text{ cells ml}^{-1}$  at MI.

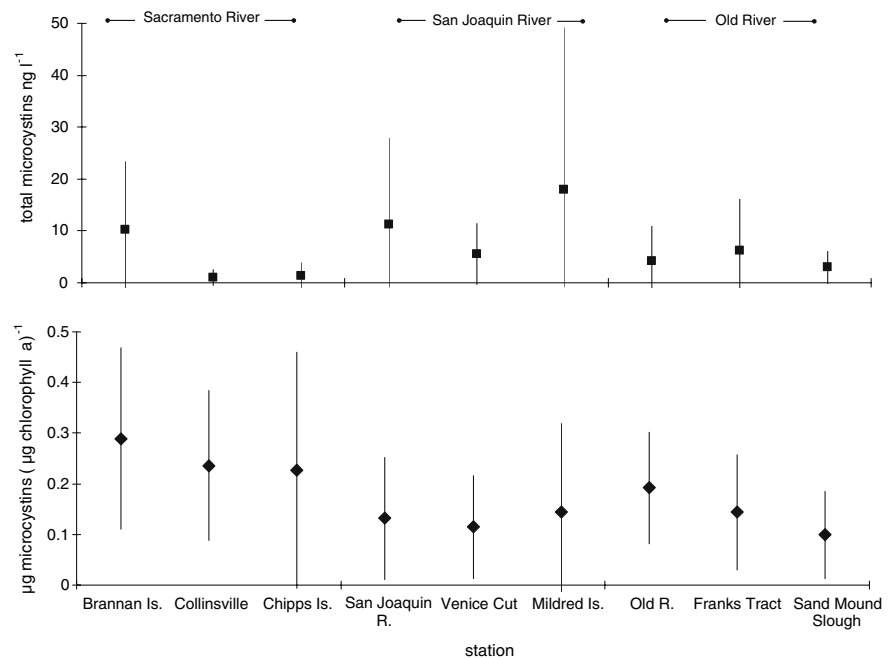
Average chl *a* concentration was 3-fold greater ( $P < 0.05$ ) in August and September than July, October and November ( $75 \pm 66 \text{ ng l}^{-1}$  and  $22 \pm 29 \text{ ng l}^{-1}$ , respectively; Fig. 3). This pattern differed somewhat among rivers, with peak chl *a* concentration occurring earlier ( $P < 0.05$ ) in SJR (August) than SAC (September). Chl *a* concentration was equally high in August and September for ODR. Chl *a* concentration did not vary with *Microcystis* cell density which was consistently high ( $P < 0.05$ ) between late July and early September (Fig. 3). *Microcystis* cell density also did

not have a strong seasonal pattern among rivers except in SJR where cell density was greatest ( $P < 0.05$ ) in August and September.

Areal  $\text{GP}_{\text{ez}}$  was greater ( $P < 0.01$ ) at stations in SJR and ODR than SAC ( $141 \pm 70$ ,  $67 \pm 26$  and  $19 \pm 24 \text{ ng C m}^{-2} \text{ h}^{-1}$ , respectively; Fig. 2) but did not differ among rivers when normalized to chl *a* concentration. Areal  $\text{GP}_{\text{ez}}$  and  $\text{GP}_{\text{ez}}$  normalized to chl *a* concentration were also greatest ( $P < 0.01$ ) in September even though respiration ( $\text{chl } a)^{-1}$  was highest that month. The seasonal variation of  $\text{GP}_{\text{ez}}$  mirrored changes in the photosynthetic parameters  $P_{\text{m}}^{\text{B}}$ ,  $\alpha^{\text{B}}$  and  $\beta^{\text{B}}$  which were high in August and

**Table 1** Photosynthetic parameters and respiration normalized to chlorophyll *a* concentration computed from the photosynthesis-irradiance curve and light and dark bottle dissolved oxygen incubations for three stations sampled between August and October, 2004

Date	Sampling station	$P_m^B$ mg C (mg chl <i>a</i> ) <sup>-1</sup> hr <sup>-1</sup>	$\alpha^B$ mg C (mg chl <i>a</i> ) <sup>-1</sup> (mole quanta m <sup>-2</sup> ) <sup>-1</sup>	$\beta^B$ mg C (mg chl <i>a</i> ) <sup>-1</sup> (mole quanta m <sup>-2</sup> ) <sup>-1</sup>	Respiration mg C (mg chl <i>a</i> ) <sup>-1</sup> hr <sup>-1</sup>
August 27	Franks Tract	1.15 ± 0.12	2.52 ± 0.15	0.11 ± 0.07	-0.73 ± 0.06
September 9	Old River	2.38 ± 0.33	1.54 ± 0.26	0.11 ± 0.11	-1.76 ± 0.07
September 28	Mildred Island	2.13 ± 0.50	0.88 ± 0.37	–	-0.88 ± 0.03
October 18	Old River	0.36 ± 0.03	0.42 ± 0.10	0.06 ± 0.01	-0.10 ± 0.07

**Fig. 4** Mean and standard deviation (vertical bar) of total microcystins concentration and total microcystins (chl *a*)<sup>-1</sup> for stations in the San Francisco Estuary between July and November 2004

September (Table 1). However, the seasonal variation in primary productivity and the photosynthetic parameters was large. Areal  $GP_{cz}$  varied by two orders of magnitude while  $P_m^B$  and  $\alpha^B$  varied 6-fold (Fig. 4; Table 1). By comparison, there was a little seasonal variation in  $\beta^B$  which varied by a factor of 2.

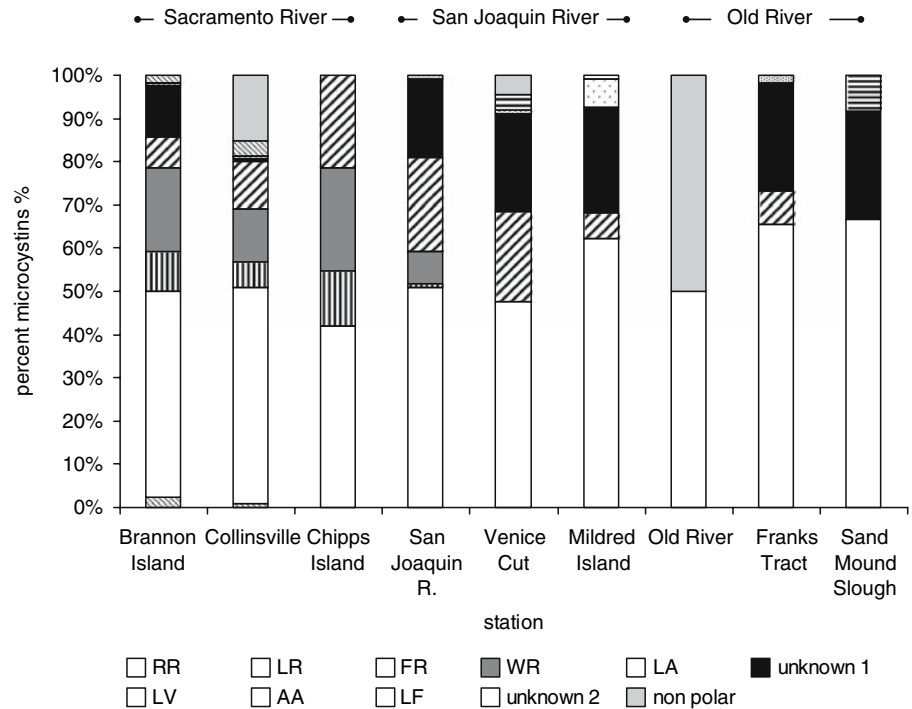
#### Microcystins concentration

Total microcystins concentration ranged from 0.01 ng l<sup>-1</sup> to 81 ng l<sup>-1</sup> in net tows and was 2-fold greater ( $P < 0.05$ ) in SJR than the other rivers (Fig. 4). This contrasted with total microcystins concentration (chl *a*)<sup>-1</sup> which was 2-fold greater ( $P < 0.05$ ) in SAC compared with SJR. Among

months, average total microcystins concentration was an order of magnitude greater ( $P < 0.05$ ) in August and September for the whole estuary ( $12.64 \pm 17.46$  ng l<sup>-1</sup> and  $0.85 \pm 1.56$  ng l<sup>-1</sup>, respectively; Fig. 3), but the monthly pattern differed among rivers. Total microcystins concentration was greatest ( $P < 0.05$ ) in August for SJR and September for SAC and was equally high in August and September for ODR. Total microcystins (chl *a*)<sup>-1</sup> did not differ among months for SAC and ODR but was greater ( $P < 0.05$ ) in August for SJR.

A suite of 11 microcystins contributed to the spatial and temporal variation in total microcystins concentration (Fig. 5). Microcystin-LR comprised the greatest percent (54%) of the total microcystins at all stations followed by microcystin-unknown 1 (14%) and microcystin-LA (11%). The percent

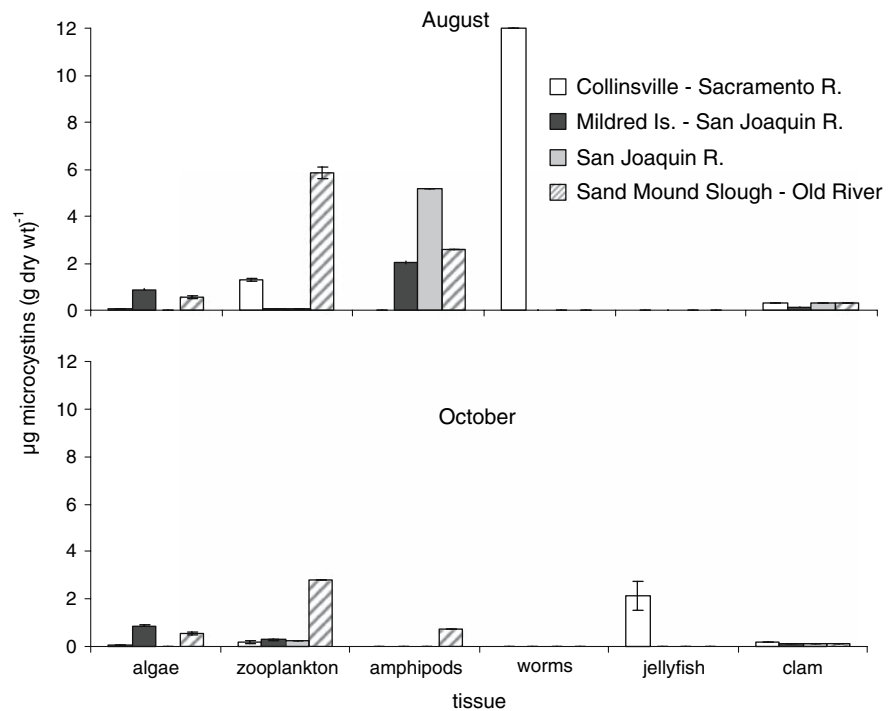
**Fig. 5** Average percent composition of microcystin congeners in algal tissue between July and November 2004 in San Francisco Estuary



composition of most microcystins did not differ significantly among rivers, except for microcystin-FR and microcystin-WR which were an order of

magnitude greater ( $P < 0.01$ ) in SAC and microcystin-LA which was at least 2-fold greater ( $P < 0.05$ ) in SJR than ODR.

**Fig. 6** Total microcystins concentration in algal and animal tissue at four stations in the San Francisco Estuary during August and October 2004





The microcystin composition was also seasonally variable among rivers. Microcystin-LA was greatest ( $P < 0.05$ ) in July for SJR. Both microcystin-FR and microcystin-WR were greatest ( $P < 0.05$ ) in October for SAC while microcystin-LR was greatest ( $P < 0.05$ ) in October for SJR and ODR. Further, the number of microcystins present at the stations varied over the season with a larger average number ( $P < 0.01$ ) of microcystins occurring between September and November ( $2.0 \pm 1.4$ ) than between July and August ( $1.3 \pm 1.8$ ).

The total microcystins in the tissues of lower food web animals was generally greater in August during the peak of the bloom and lowest in October during the decline of the bloom (Fig. 6). Total microcystins in animal tissue also varied widely among animals and was often higher in worms and amphipods than mesozooplankton ( $12 \pm 0.00$ ,  $2.62 \pm 1.88$ ,  $1.34 \pm 2.05$   $\mu\text{g}$  microcystins (g dry wt.)<sup>-1</sup>, respectively). However, average total microcystins concentration in mesozooplankton tissue (e.g. *Eurytemora affinis* and *Pseudodiaptomus forbesii*) was still 3–6 fold greater than in the algae (*Microcystis* and surface algae) and clam tissue ( $0.50 \pm 0.37$  and  $0.21 \pm 0.10$   $\mu\text{g}$  microcystins (g dry wt.)<sup>-1</sup>, respectively). Among rivers, total microcystins concentration in mesozooplankton tissue appeared to be greater in ODR than SAC and SJR ( $5.8 \pm 1.70$ ,  $0.18 \pm .65$ ,  $0.15 \pm 11$   $\mu\text{g}$  microcystins (g dry wt.)<sup>-1</sup>, respectively). A more quantitative statistical comparison of the spatial and

temporal variation in total microcystins content in animal tissue was precluded by small sample size; two samples per animal type per station.

#### Environmental factors

Chl *a* concentration and *Microcystis* cell density varied with physical and chemical conditions among rivers. The greatest chl *a* concentration and cell density occurred in SJR which had the lowest chloride, low total suspended solids and soluble reactive phosphorus concentration and high nitrate concentration (Table 2). The second highest chl *a* concentration and cell density occurred in ODR which like SJR had low chloride and total suspended solids concentration, but also had relatively low nitrogen and phosphorus concentration and high specific conductance. SAC with the greatest total microcystins concentration (chl *a*)<sup>-1</sup> had the highest chloride, total suspended solids and dissolved nitrogen and phosphorus concentration and the lowest specific conductance among rivers. These differences in chemical conditions were accompanied by differences in streamflow which was an order of magnitude greater ( $P < 0.05$ ) for SAC than SJR. Water temperature was not significantly different among rivers.

*Microcystis* growth rate in the euphotic zone also varied with physical and chemical conditions. GP<sub>ez</sub>

**Table 2** Mean and standard deviation of physical and chemical variables measured for the Sacramento, San Joaquin and Old Rivers between July and November 2004

Variable	Sacramento River	San Joaquin River	Old River	Significance at $P < 0.05$ level
Chloride (mg l <sup>-1</sup> )	1259.81 ± 1175.95	34.91 ± 21.21	121.54 ± 48.64	1, 2, 3
Specific conductance (μS cm <sup>-1</sup> )	4.18 ± 3.72	66.25 ± 126.24	123.92 ± 261.17	1&2, 2&3
Secchi disk depth (cm)	60.67 ± 20.50	129.37 ± 37.66	139.73 ± 26.29	1, 2, 3
Total suspended solids (mg l <sup>-1</sup> )	20.60 ± 11.14	3.80 ± 2.06	3.79 ± 1.75	1&2, 1&3
Water temperature (°C)	19.84 ± 2.83	21.03 ± 3.68	20.95 ± 3.52	None
Nitrate (mg l <sup>-1</sup> )	0.32 ± 0.07	0.36 ± 0.22	0.19 ± 0.10	1&3, 2&3
Ammonia (mg l <sup>-1</sup> )	0.06 ± 0.03	0.05 ± 0.04	0.02 ± 0.02	1, 2, 3
Total phosphorus (mg l <sup>-1</sup> )	0.10 ± 0.02	0.08 ± 0.03	0.06 ± 0.01	1, 2, 3
Soluble reactive phosphorus (mg l <sup>-1</sup> )	0.08 ± 0.09	0.05 ± 0.01	0.05 ± 0.01	1&2, 1&3
N:P molar ratio	12.19 ± 3.63	15.97 ± 7.49	9.25 ± 4.59	1&3, 2&3
S:N molar ratio	20.76 ± 4.88	24.07 ± 10.59	35.93 ± 13.89	1&3, 2&3

Significant differences between rivers at the  $P < 0.05$  level are indicated by a comma

increased with water temperature ( $r = 0.56$ ,  $P < 0.01$ ) and decreased with specific conductance and chloride ( $r = -0.49$ ,  $P < 0.01$  and  $r = -0.68$ ,  $P < 0.01$ , respectively).  $GP_{ez}$  was also correlated with streamflow but the direction of the correlation differed among rivers with a positive correlation for SAC ( $r = 0.46$ ,  $P < 0.01$ ) and a negative correlation for SJR ( $r = -0.51$ ,  $P < 0.01$ ). The difference in these correlations may be due to the correlation between streamflow, dissolved salts, and water temperature. Streamflow was positively correlated with water temperature and negatively correlated with specific conductance and chloride ( $r = 0.70$ ,  $P < 0.01$ ;  $r = -0.45$ ,  $P < 0.01$ ;  $r = -0.30$ ,  $P < 0.01$ , respectively) in SAC. In contrast, streamflow was negatively correlated with water temperature and positively correlated with specific conductance in SJR ( $r = -0.74$ ,  $P < 0.01$  and  $r = 0.32$ ,  $P < 0.01$ ). The streamflow pattern also differed between SAC and SJR with consistently low streamflow ( $P < 0.05$ ) in SJR, but a gradual decrease in streamflow ( $P < 0.05$ ) over the bloom season in SAC.  $GP_{ez}$  was also negatively correlated with total irradiance in the euphotic zone as suggested by the negative correlation between  $GP_{ez}$  and Secchi disk depth ( $r = -0.24$ ,  $P < 0.05$ ). This contrasted with the negative correlation between total suspended solids or dissolved solids and  $GP_{ez}$  ( $r = -0.35$ ,  $P < 0.01$  and  $r = -0.67$ ,  $P < 0.01$ , respectively).  $GP_{ez}$  was also negatively correlated ( $P < 0.05$ ) with ammonium, nitrate and soluble reactive phosphorus concentration and the N:P ratio. Nutrient concentration (Table 2) remained above limiting values for dissolved inorganic nitrogen, soluble reactive phosphorus and silica of  $0.02 \text{ mg l}^{-1}$ ,  $0.002 \text{ mg l}^{-1}$  and  $0.15 \text{ mg l}^{-1}$ , respectively (Jassby, 2005). Average N:P ratios were also less than 16 between July and November and less than 10 during the peak of the bloom in August and September.

#### Canonical correlation analysis

*Microcystis* cell density was strongly correlated with streamflow and water temperature in canonical correlation analysis. Eleven water quality and seven streamflow variables that were significantly ( $P < 0.05$ ) correlated with *Microcystis* cell density were included in the canonical correlation analysis

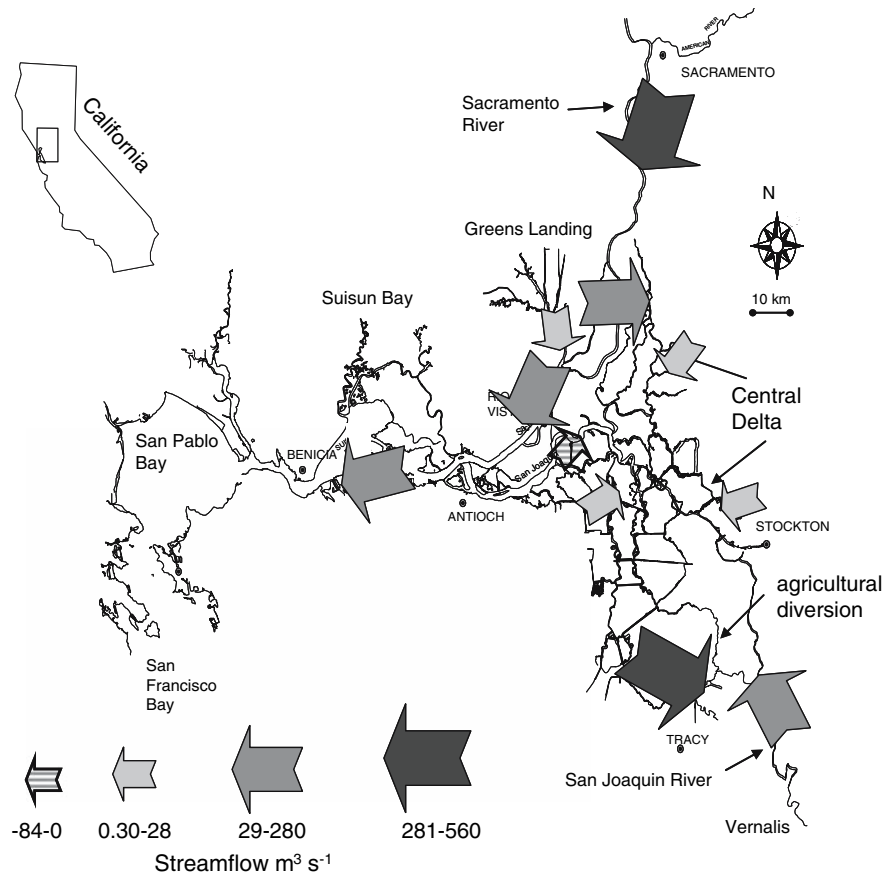
**Table 3** Standardized coefficients for variables on the first significant canonical environmental variable computed by canonical correlation analysis to describe the variability of *Microcystis* cell density for data collected between July and November 2004

Variable	Standardized coefficient
<i>Microcystis cell density</i>	
East side streamflow	4.77
Contra Costa Canal pumping	1.31
Water temperature	1.04
Total dissolved solids	0.92
Silica:phosphorus molar ratio	0.48
Old River agricultural diversion	0.46
Total phosphorus	-0.05
Nitrate	-0.12
Ammonia	-0.17
Specific conductance	-0.21
N:P molar ratio	-0.43
Total suspended solids	-0.48
Miscellaneous agricultural diversions	-0.54
Chloride	-0.73
Si:N molar ratio	-0.78
Mokelumne River streamflow	-1.99
Sacramento River streamflow	-2.20
San Joaquin River streamflow	-3.03
Variance explained	59%

(Table 3). The canonical environmental variable created from these variables was significant and described 59% ( $P < 0.01$ ) of the variation in *Microcystis* cell density between July and November. Standardized coefficients for each variable suggested high streamflow in the eastern delta, low streamflow in the SJR, SAC and MOKE, high water diversion near the city of Contra Costa and high water temperature accounted for most of the variability in cell density. However, correlation between individual environmental variables and the canonical environmental variable suggested water temperature ( $r = 0.56$ ,  $P < 0.01$ ), Si:N ratio ( $r = -0.52$ ,  $P < 0.01$ ), ammonium concentration ( $r = -0.51$ ,  $P < 0.01$ ) and streamflow in the MOKE and SJR ( $r = -0.50$ ,  $P < 0.01$  and  $r = -0.45$ ,  $P < 0.01$ , respectively) contributed to the variance described by the canonical environmental variable.

The streamflow variables in the canonical analysis were correlated ( $P < 0.05$ ) with a larger set of measured and computed streamflow variables

**Fig. 7** Map of San Francisco Estuary indicating the mean of selected streamflow variables for August and September 2004



available for the estuary including the Sacramento, Cosumnes, Mokelumne, and San Joaquin Rivers flow, east side tributary flow, Contra Costa, State Water Project and Central Valley Project water diversion flow and streamflow past Jersey Point, and Rio Vista (<http://www.iep.water.ca.gov>). When these streamflow variables were averaged over August and September, high *Microcystis* cell density coincided with relatively low streamflow in the central delta, high streamflow in SAC, moderate streamflow in SJR and reversed (upstream arrow) streamflow in the southern delta produced by high diversion flow (Fig. 7).

The correlation between environmental conditions and *Microcystis* cell density varied among rivers. In SJR, *Microcystis* cell density was positively correlated with MOKE streamflow ( $r = 0.52$ ,  $P < 0.01$ ) and negatively correlated with agricultural diversion near the city of Tracy and the N:P ratio ( $r = -0.67$ ,  $P < 0.01$ ;  $r = -0.47$ ,  $P < 0.05$ ). In ODR, *Microcystis* cell density was negatively correlated with

streamflow in the MOKE ( $r = -0.52$ ,  $P < 0.01$ ) and SJR ( $r = -0.52$ ,  $P < 0.01$ ) and positively correlated with water temperature ( $r = 0.52$ ,  $P < 0.01$ ) and Secchi disk depth ( $r = 0.42$ ,  $P < 0.05$ ). In SAC, *Microcystis* cell density was positively correlated with both water temperature ( $r = 0.65$ ,  $P < 0.01$ ) and Secchi disk depth ( $r = 0.47$ ,  $P < 0.05$ ).

*Microcystis* also occurred within a narrow range of environmental conditions. *Microcystis* cells first appeared when water temperature reached 20°C. *Microcystis* cells were present at total suspended solids concentrations between 100 mg l<sup>-1</sup> and 500 mg l<sup>-1</sup>, specific conductance between 0.1 mS cm<sup>-1</sup> and 0.3 mS cm<sup>-1</sup>, Si:N ratios between 20 and 50 and ammonium concentration between 0.01 mg l<sup>-1</sup> and 0.03 mg l<sup>-1</sup>. *Microcystis* cells also occurred, when streamflow was 28.32–35.40 m<sup>3</sup> s<sup>-1</sup> in SJR and 0.85–1.13 m<sup>3</sup> s<sup>-1</sup> in MOKE.

Total microcystins concentration (cell)<sup>-1</sup> and total microcystins (chl *a*)<sup>-1</sup> were also strongly correlated with streamflow in separate canonical correlation

**Table 4** Standardized coefficients for variables on the first significant canonical environmental variable computed by canonical correlation analysis to describe the variability of total microcystins ( $\text{cell}^{-1}$ ) and total microcystins (chlorophyll  $a$ ) $^{-1}$  for data collected between July and November 2004

	Standardized coefficient
<i>Microcystins (cell)<math>^{-1}</math></i>	
East side streamflow	4.77
Contra Costa Canal pumping	1.31
Water temperature	1.04
Total dissolved solids	0.92
Silica:phosphate molar ratio	0.48
Old River agricultural diversion	0.46
Total phosphorus	-0.05
Nitrate	-0.12
Ammonia	-0.17
Variance explained	59%
<i>Microcystins (chlorophyll <math>a</math>)<math>^{-1}</math></i>	
Specific conductance	-0.21
Nitrogen:phosphate molar ratio	-0.43
Total suspended solids	-0.48
Miscellaneous agricultural diversions	-0.54
Chloride	-0.73
Silica:nitrogen molar ratio	-0.78
Mokelumne River streamflow	-1.99
Sacramento River streamflow	-2.20
San Joaquin River streamflow	-3.03
Variance explained	32%

analyses (Table 4). Nine water quality and streamflow variables that were significantly ( $P < 0.05$ ) correlated with total microcystins concentration ( $\text{cell}^{-1}$ ) produced a significant ( $P < 0.01$ ) canonical environmental variable that described 32% ( $P < 0.01$ ) of the variation in total microcystins ( $\text{cell}^{-1}$ ). Large standardized coefficients within the canonical environmental variable suggested east side streamflow, municipal water diversion at the city of Contra Costa and water temperature were positively correlated with microcystins concentration ( $\text{cell}^{-1}$ ). A somewhat different set of nine water quality and streamflow variables were correlated with total microcystins (chl  $a$ ) $^{-1}$ . These variables described 59% ( $P < 0.01$ ) of the variance in total microcystins concentration (chl  $a$ ) $^{-1}$ . Large standardized coefficients on the significant canonical environmental variable indicated total microcystins concentration (chl  $a$ ) $^{-1}$  was greater at low streamflow in SJR, SAC, and MOKE.

#### Environmental constancy

Environmental variability may further influence the seasonal variation of *Microcystis* cell density and total microcystins concentration. *Microcystis* cell density was greater in August and September when the variance in daily streamflow was low ( $P < 0.05$ ; Table 5). The greatest number of microcystins occurred in September and October ( $P < 0.01$ )

**Table 5** Coefficients of variation computed for daily streamflow and water temperature between July and October for locations throughout the estuary

	Coefficient of variation			
	July (%)	August (%)	September (%)	October (%)
<i>Streamflow</i>				
San Joaquin River	9	10	6	36
Sacramento River	5	6	10	18
Mokelumne River	64	16	9	23
East streamflow	8	8	6	35
Agricultural diversion	5	6	10	17
Total agricultural export	12	5	8	21
Significantly different	*			*
<i>Water temperature</i>				
San Joaquin River	2	2	5	9
Sacramento River	2	2	5	9
Significantly different			*	*

Significant difference in variance at the  $P < 0.01$  level are indicated by a star (\*)

during the decline of the bloom when the variance in daily water temperature was highest ( $P < 0.01$ ). The variance in daily water temperature was influenced by daily air temperature which were correlated at Stockton on the San Joaquin River ( $r = 0.50$ ,  $P < 0.01$ ,  $n = 96$ ) and Rio Vista on the Sacramento River ( $r = 0.56$ ,  $P < 0.01$ ,  $n = 97$ ) between July and November 2004.

## Discussion

### Distribution

*Microcystis* occurred throughout SFE from freshwater habitats in SJR and ODR to brackish water habitats in SAC during the summer and fall of 2004. *Microcystis* was probably more widely distributed than the 2004 study suggests because *Microcystis* cells were found as far seaward as Martinez in 2003 (see Fig. 1 for location; Lehman et al., 2005). The consistently higher *Microcystis* cell density in SJR and ODR compared with SAC suggests optimum conditions for *Microcystis* growth occurred in the central delta. It is unlikely that *Microcystis* grew outside of the freshwater habitats in the central delta where salinities are commonly less than 5 ppt (Lehman et al., 2005) because *Microcystis* does not grow at salinities above 7 ppt (Robson & Hamilton, 2003). Instead, *Microcystis* cells were probably transported from the central delta with streamflow, wind and tide to more brackish water habitats downstream where they might survive, but not grow (Pickney et al., 1997). Low cell density in SAC was probably a combination of dilution and cell death at high chloride. High salinity conditions encountered during seaward transport could cause *Microcystis* colonies to lyse, aggregate, and settle to the bottom in Chesapeake Bay (Sellner et al., 1988; Orr et al., 2004).

*Microcystis* cell density and chl *a* concentration peaked during the summer and fall between August and September in 2004. *Microcystis* cell density and biomass commonly peak during the summer and fall when they occur in freshwater lakes and reservoirs (Watson et al., 1997). *Microcystis* also occurs during the summer and fall in the low salinity regions of some estuaries including the Swan River estuary, Australia, the Los Platos Estuary, Brazil, and the Potomac and Neuse River estuaries in the USA

(Pearl, 1988; Robson & Hamilton 2003; Sellner et al., 1993; Yunes et al., 1996). In SFE, peak *Microcystis* chl *a* concentration and cell density in August and September were associated with high  $GP_{ez}$  and characterized by high  $P_m^B$ ,  $\alpha^B$  and low  $\beta^B$ . Warm water temperature during August and September may have contributed to high  $P_m^B$  which is correlated with high water temperature for *Microcystis* populations in lakes (Robarts & Zachary 1987). August and September are also characterized by high streamflow in SAC and low streamflow in SJR that promote the warm water temperature, low salinity and low specific conductance conditions associated with high  $GP_{ez}$ .

Total microcystins concentration was highest in SJR during August and September when *Microcystis* cell density and chl *a* concentration were high, but was poorly correlated with either. Total microcystins concentration and chl *a* concentration were also poorly correlated for the single-day survey conducted in SFE during October 2003 (Lehman et al., 2005). The lack of a correlation between total microcystins concentration and chl *a* concentration is common because cellular microcystins content is uncoupled from growth rate (Utkilen & Gjølme, 1992). Total microcystins concentration was probably influenced by the relative growth of *Microcystis* strains or “genotypes” that contain different kinds of microcystins as well as the direct influence of environmental conditions on microcystin formation in *Microcystis* cells or “chemotypes” (Ouellette et al., 2006). Significant differences in the microcystins composition in SJR and SAC suggest there were at least two different genotypes or chemotypes contributing to the total microcystins concentration.

The variation of total microcystins concentration suggested the potential toxicity of *Microcystis* was variable. Eleven microcystins varied by eight orders of magnitude during the bloom in SFE. This level of variation might not be unusual because it was similar to the variation measured for *Microcystis* blooms in German lakes and reservoirs where 14 microcystins varied by four orders of magnitude (Fastner et al., 1999). The potential toxicity of the *Microcystis* bloom in SFE was strongly influenced by the presence of the hepatotoxic microcystin-LR which comprised about 54% of the total microcystins. However, the full toxicity of the bloom depends on the remaining 46% of the microcystins for which a

little is known (Zurawell et al., 2004). It is likely that the potential toxicity of the microcystins in SAC was higher than the other rivers because it had the highest total microcystins ( $\text{chl } a$ )<sup>-1</sup>.

### Environmental factors

Streamflow was a major factor controlling *Microcystis* cell density in SFE and probably influenced development of the *Microcystis* bloom directly and indirectly through a suite of environmental conditions. Since *Microcystis* has a relatively slow growth rate, long water residence time is needed for biomass to accumulate (Reynolds, 1997). Low streamflow in the central delta region coupled with high  $\text{GP}_{\text{ez}}$ ,  $\text{P}_m^{\text{B}}$ , and  $\alpha^{\text{B}}$  in August and September probably facilitated accumulation of *Microcystis* cells in SFE. Accumulation rather than growth was supported by the similar  $\text{GP}_{\text{ez}}$  ( $\text{chl } a$ )<sup>-1</sup> among rivers. Flushing rate was also a key factor affecting the seasonal variation of *Microcystis* blooms in the Swan River Estuary and in the Neuse River estuary where *Microcystis* blooms only develop when streamflow is below 13–15  $\text{m}^3 \text{s}^{-1}$  (Christian et al., 1986; Robson & Hamilton, 2003). A streamflow threshold was similarly suggested for SFE where *Microcystis* only occurred when SJR streamflow was 28–32  $\text{m}^3 \text{s}^{-1}$ .

*Microcystis* probably grew well in the shallow-flooded island habitats in the central delta region of SFE where low streamflow helps to keep vertical mixing low (Jacoby et al., 2000). Low vertical mixing enables *Microcystis* colonies to float to the surface of the water column where they out compete other phytoplankton for light (Huisman et al., 2004). Such an adaptation was probably important in SFE, where phytoplankton growth is light limited due to high suspended sediment concentration (Jassby et al., 2002) and may partially explain the negative correlation between *Microcystis* cell density and Secchi disk depth. Low vertical mixing in the central delta region may also enhance phytoplankton metabolic activity and cell viability which are reduced at high mixing rates (Huisman et al., 2004; Regel et al., 2004). Low vertical mixing in the central delta was suggested by abundant large 2–3 cm wide colonies in MI, a shallow-flooded island in the center of the delta and small 1-cm wide colonies in the middle of the fast

flowing and turbulent river channels where *Microcystis* cell density was low (Lehman, personal observation). Large colonies were shown to rapidly break apart under turbulent conditions in laboratory tests (O'Brien et al. 2004).

*Microcystis* cell density was also positively correlated with water temperature in SFE. *Microcystis* growth begins in early summer, when water temperature above 20°C stimulates esterase activity in vegetative cells on the surface of the sediment and ceases in the fall when water temperature declines to below 20°C (Latour et al., 2004). Water temperature similarly contributed to the seasonal pattern in *Microcystis* cell density in SFE where *Microcystis* cells only occurred above 20°C. Maximum  $\text{chl } a$  concentration occurred during mid-summer when water temperature reached 25°C, but this may not represent maximum growth rate of *Microcystis* which was higher at 29–32°C in laboratory studies (Robarts & Zohary, 1987). Water temperature probably influenced the spatial and temporal variation in *Microcystis* cell density among rivers because it reached 20°C sooner in SJR and ODR than SAC.

The importance of water temperature for microcystin development was suggested by the large coefficient for water temperature on the canonical environmental variable in canonical correlation analysis for total microcystins ( $\text{cell}$ )<sup>-1</sup>. In lab studies, total microcystins concentration varied more with water temperature than irradiance and was highest at 20–24°C (Van der Wethuizen & Eloff, 1985; Wiedner et al., 2003); water temperatures similar to those measured in SFE during mid-summer. Water temperature primarily influences total microcystins concentration through its impact on growth rate, because cellular microcystins are only produced during log-phase growth (Lyck, 2004). The greater total microcystins concentration during mid-summer in SFE may be influenced by the high  $\text{P}_m^{\text{B}}$  and  $\alpha^{\text{B}}$  during this time.

It is possible environmental variability contributed to the seasonal variation in *Microcystis* cell density and the quantity and quality of microcystins in SFE.  $\text{Chl } a$  and total microcystins concentration peaked in August and September when the variance in streamflow was low. Low daily variance in streamflow may promote the accumulation of *Microcystis* cells and the growth of relatively few *Microcystis* genotypes. In contrast, the high daily variance of water

temperature in September and October may contribute to the increased number of microcystins in these months through differential growth and survival of *Microcystis* genotypes or chemotypes (Ouellette et al. 2006). Daily water temperature is linked to seasonal changes in air temperature with streamflow dominating water temperature early in the season at high streamflow and air temperature dominating water temperature late in the season at low streamflow. This impact is supported by decadal change in water temperature in SFE that was inversely correlated with streamflow and positively correlated with air temperature in SJR and SAC (Lehman, 2004).

Nutrient concentration was not a driving force for variation of the *Microcystis* bloom in SFE. The high nutrient concentrations in SFE were a necessary condition for initiation of the *Microcystis* bloom because *Microcystis* requires both high nitrogen and phosphorus concentration for growth (Paerl et al., 2001). However, the persistence and variation of the bloom was not nutrient driven because nutrient concentrations were consistently an order of magnitude greater than limiting values throughout the water column in SFE (Jassby 2005). Nutrient ratios are generally important for cyanobacterial bloom formation (Paerl et al., 2001) with *Microcystis* blooms occurring at an N:P ratio <15 (Jacoby et al. 2000). The average N:P ratio of 10 (range 6–10) in August and September was favorable for *Microcystis* growth in SFE. The lesser influence of nutrients on *Microcystis* cell density and total microcystins concentration was supported by the low coefficients for nutrient concentration and nutrient ratios in the canonical correlation analyses.

#### Food web impact

The spatial and temporal variation of *Microcystis* cells might affect the presence of toxic microcystins in the estuarine food web in SFE. The high concentration of total microcystins in lower food web organisms during the peak of the *Microcystis* bloom suggested there was a direct link between microcystins in algal tissue and microcystins in the tissue of aquatic animals. Microcystins concentration was also high in the tissue of food web animals during the peak of the bloom in central Alberta Lakes, Canada

(Kotak et al., 1996). Microcystins in zooplankton and other lower food web animals can occur from active and passive ingestion of algal tissue, even though *Microcystis* may not be selectively grazed (DeBernardi & Giussani, 1990; DeMott & Moxter, 1991; Sellner et al., 1993).

The greater microcystins concentration in animal than algal tissue suggested microcystins were transferred and perhaps biomagnified through the aquatic food web in SFE. Microcystins were also transferred through food web organisms in the Alberta Lakes, Canada, Lake IJsselmeer, the Netherlands and Lakes Rotoiti and Rotoehu in the Czech Republic (Kotak et al., 1996; Ibelings et al., 2005; Wood et al., 2006). Detritus feeders may be an important transfer agent of microcystins into the SFE food web because total microcystins concentrations were high in amphipod and worm tissue. Detrital grazers were also thought to be the primary pathway for the transfer of microcystins into the food web in Alberta lakes (Kotak et al., 1996). Unexpectedly, clams which fed directly on phytoplankton may not be an important source of microcystins to the food web in SFE. Clam tissue had the lowest total microcystins content among the animals tested in 2004 and low microcystins content compared with zooplankton tissue in 2003 (Lehman et al., 2005). Mollusks could accumulate microcystins, but tissue content is often low due to the rejection of *Microcystis* colonies or rapid depuration of toxins from tissue (Prepas et al. 1997).

The *Microcystis* bloom probably did not cause acute toxicity to aquatic food web organisms in SFE. Total microcystins concentration in zooplankton tissue was below the value of 10–18  $\mu\text{g (g dry wt.)}^{-1}$  associated with acute death in *Daphnia* during laboratory feeding studies (Rohrlack et al., 2005). However, even at low concentrations, *Microcystis* can affect zooplankton community structure and function by sublethal toxicity or non-toxin related factors such as feeding inhibition or providing phytoplankton food of poor quality or low digestibility (DeMott & Mueller-Navarra, 1997; Rohrlack et al., 2005). Further, dissolved microcystins released from lysed *Microcystis* cells at the end of the bloom are toxic and can reduce feeding success for zooplankton (Pietsch et al., 2001). Large zooplankton such as *Daphnia* are sensitive to dissolved microcystins and demonstrate reduced growth and fecundity in

the presence of *Microcystis* (Reinikainen et al., 1999). More information on these potential impacts are needed for SFE.

### Management strategies

The worldwide impact of *Microcystis* blooms on ecosystem structure and function and human health through drinking water and recreation suggests the potential need for management of *Microcystis* populations in SFE (White et al., 2005). Because the spatial and temporal variability of *Microcystis* cell density and total microcystins concentration is high in SFE, management might require consideration of physical, chemical, and biological factors at both large and small spatial and temporal scales (Donaghay & Osborn, 1997). Although there are many management strategies for control of *Microcystis* and its toxins (Pearl et al., 2001), regulation of streamflow may be the most important for SFE. High streamflow would prevent accumulation of *Microcystis* biomass in stable backwater sloughs or shallow-flooded islands, where residence time is long and vertical mixing is low. High streamflow would also increase vertical mixing which decreases colony viability and the competitive advantage of *Microcystis* colonies to obtain light by floating on the surface of the water column (Huisman et al., 2004). Streamflow could further be managed to influence water quality conditions such as water temperature and salinity that initiate and sustain bloom biomass and affect microcystins concentration (Jacoby et al. 2000). A decline in the density and biomass of fish, zooplankton, mysid shrimp, and diatoms has left the food web in SFE vulnerable to any adverse impact so that even a small change in the impact of *Microcystis* and its associated toxins on the food web may be important for fishery production (Lehman, 2004; Sommer et al. 2007).

### Conclusion

*Microcystis* and its associated toxin microcystin varied spatially and temporally over the bloom season in SFE. Significant differences in cell density and chl *a* concentration were associated with the *Microcystis* bloom among months, rivers and

stations. Differences in *Microcystis* cell density and total microcystins concentration per cell<sup>-1</sup> and microcystins concentration chl *a*<sup>-1</sup> were correlated with environmental conditions, particularly streamflow and water temperature. These environmental conditions were correlated with differences in areal growth rate within the euphotic zone and probably driven by high P<sub>m</sub><sup>B</sup> and α<sup>B</sup> during the peak of the bloom. The variation of the bloom and its associated toxin concentration is potentially important ecologically because total microcystins are present in the tissues of the lower food web animals, mesozooplankton, amphipods, worms, jellyfish and clams. Although the bloom contains hepatotoxic microcystins, the present concentrations are low and probably not acutely toxicity to food web animals. However, the higher concentration of total microcystins in some animals and higher total microcystins concentration in animal than algal tissue suggests biomagnification or accumulation could increase the impact of these toxins on the aquatic community.

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