2005 Pelagic Organism Decline Program Progress Report:

Microcystis biomass and toxicity

P. W. Lehman[1], G. Boyer[2], S. Teh[3], E. Bass[1] and C. Hogel[1]

[1]Division of Environmental Studies, California Department of Water Resources, 3251 S Street, Sacramento, CA**,** [2] College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210 and [3]Department of Veterinary Medicine, One Shields Avenue, University of California, Davis, CA 95616

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This is a draft work in progress subject to review and revision as information becomes available.

Summary

- The cyanobacteria *Microcystis aeruginosa* bloomed throughout the central, southern and western delta and lower Suisun Bay in 2005 and is the 7th consecutive year of the bloom
- *Microcystis* biomass was highest in the central delta and western delta
- Bloom biomass reached higher values in the central delta during 2005 than 2004
- The bloom contained microcystins which suggested it was toxic as in previous years
- Bloom toxicity was highest in the central and western delta, particularly in the lower San Joaquin River, Antioch and Collinsville
- The range of toxicity values was similar to those measured in previous years
- The high bloom biomass in the central delta was associated with higher water temperature and lower turbidity, chloride and nutrient concentrations than the other regions of the delta

I. Introduction

This progress report briefly describes the current status and preliminary data from a field study described in the 2005 Pelagic Organism Decline (POD) work plan titled "Field survey of *Microcystis aeruginosa* bloom biomass and toxicity". The goal of this project was to measure the biomass and toxicity of *Microcystis aeruginosa* in the estuary and determine if it was a potential contributing factor to the poor health and survival of pelagic organisms in the Delta measured since 2002. *Microcystis* is a cyanobacterium that has bloomed in the Delta since 1999. This organism is considered a harmful algal bloom (HAB) because it contains microcystins that can cause cancer in humans and wildlife and its presence alone is known to impact aquatic ecosystem structure and function (Lehman et al. 2005).

II. Status by task

Task 1. The goal of Task 1 was to determine the distribution and biomass of *Microcystis* at ten summer tow net survey stations and the associated water quality conditions during August and September 2005.

- Completed a two month biweekly sampling program at ten townet survey stations
- Completed laboratory analysis of *Microcystis* biomass (size fraction > 75 um) estimated by chlorophyll *a* concentration through September 23, 2005.
- Completed laboratory analysis of water quality variables at all sampling stations including nutrient concentration, total suspended solids and total and dissolved organic carbon through September 15, 2005.
- Completed vertical profiles of water quality conditions at all sampling stations

Task 2. The goal of Task 2 was to determine the toxicity of *Microcystis* algal tissue at ten summer tow net survey stations during August and September 2005.

- Completed a two month biweekly sampling program at ten townet survey stations for algal tissue microcystins content and dissolved microcystins in the water column
- Completed analysis of total microcystins concentration in algal tissue through September 15, 2005.

Task 3. The goal of Task 3 was to determine the microcystins content of the animal tissue in the target fish species striped bass, delta smelt and inland silversides and their prey zooplankton and amphipods.

- Completed a two month biweekly sampling program at ten townet survey stations for zooplankton, amphipods and fish muscle and liver tissue

III. Methods

Field sampling – Algal and animal tissue and water quality variables were collected biweekly at ten tow net survey stations between August 2 and September 27, 2005. Sample stations were selected that represented different regions of the delta: the Central delta (stations 902, 910 and 915), the western delta (stations 704, 711 and 804), Suisun Bay (stations 504 and 508), Suisun Marsh (station 609) and a control station at Napa (station 340) (Fig. 1).

Large *Microcystis* colonies > 75 µm diameter were collected by horizontal surface tows of a 0.72 m diameter plankton net fitted with a 75 µm mesh screen on the cod end (Fig. 1). Sampling a large size fraction assured that the sample primarily contained *M. aeruginosa*. Net tows were conducted at the center of the channel at a speed of 60 m min^{-1} for 1 minute. Horizontal net tows were used to obtain a quantitative and integrated sample of the bloom which had a patchy distribution. Total volume of the sample was determined from an attached General Oceanics 2030R flow meter.

Water samples containing algal biomass were stored at 4^{o}C and filtered within 2 hours onto GF/F glass fiber filters. Filters for total microcystins analysis were wrapped in aluminum foil and frozen until laboratory analysis (see below). Filters for chlorophyll *a* analysis were treated with magnesium carbonate as a preservative and frozen until laboratory analysis (U.S. EPA 1983).

Zooplankton were sampled by a 3 minute diagonal net tow from the bottom to the surface of the water column with a 0.7 m diameter plankton net fitted with a 150 µm mesh. Zooplankton were kept at 4^{o}C and separated by hand from *Microcystis* in the water sample within 48 hours. The final zooplankton sample was rinsed in distilled water and frozen until analysis.

Fish were sampled by beach seine at shallow water beaches or by hook and line. Fish muscle and liver tissue were immediately dissected from the fish, placed in aluminum foil and snap frozen with liquid nitrogen until analysis. Only live fish were used for the analysis.

Water quality conditions associated with the bloom were also measured at each station. Vertical profiles of water temperature, pH, turbidity, dissolved oxygen, fluorescence and specific conductance were conducted using a YSI 6600 sonde. In addition, water samples for nitrate, phosphate, silica, total and volatile suspended solids and total and dissolved organic carbon were collected at the surface using a van Dorn water sampler. Water samples for laboratory analyses were stored at 4^{o}C and processed as appropriate (U.S. EPA 1983; APHA 1998).

Toxicity analysis – Filters for toxin analysis were extracted by sonication with 10 ml of 50% methanol containing 1 % acetic acid, clarified by centrifugation, and the extract used for analysis of the different toxins. Total microcystins concentration in algal tissue was assessed using the protein phosphatase inhibition assay (PPIA) technique. Assays were run in 96-well plates containing 0.1 mU enzyme (recombinant protein phosphatase 1A, catalytic subunit, Roche Applied Science), 1.05 mg para-nitrophenyl phosphate (Sigma Biochemical) and 10 µl of sample or microcystin-LR (Sigma Biochemical) using the method of Carmichael & An (1999). The rate of phosphate hydrolysis was calculated from the change in absorbance at 405 nm over 1 hour and compared to the control (no added microcystin-LR) and standards containing between 6 and 40 µg l^{-1} microcystin-LR. Blanks (no enzyme, no toxin), unknowns, standards, and controls were all run in duplicate.

IV. Initial Findings

Biomass - Microcystis bloomed throughout much of the Delta and lower Suisun Bay in 2005 and was observed at eight stations of the ten sampled (Fig. 1). *Microcystis* colonies were not observed at the control station 340 at Napa or the Suisun Marsh station 609. *Microcystis* has bloomed continuously in the Delta region during the summer since the bloom began in 1999. This year marks the 7th year of the bloom. High streamflow associated with an unusual cool and wet spring this year delayed the onset of the bloom from July to August.

The highest chlorophyll *a* concentration of the large *Microcystis* colonies (> 75 µm diameter size fraction) occurred in the central delta where median chlorophyll *a* concentration was at least an order of magnitude higher than other stations except Antioch (Table 1). Although the *Microcystis* biomass was highest in the central delta, biomass was widely distributed and reached well into the western Delta and lower Suisun Bay (Fig. 1). The highest *Microcystis* biomass also occurred in the central delta in 2003 and 2004 (Lehman et al. 2005; Table 2). Median chlorophyll *a* concentration was higher at Old River and similar for Franks Tract and the San Joaquin River in 2005 compared with 2004 during August and September. However, the 95th percentile values were twice as high for the San Joaquin and Old rivers in 2005 (compare Table 1 and 2). Median chlorophyll *a* concentrations in the central delta during August and September 2005 were within the range of single day values measured in October 2003 of 40 to 75 ng l^{-1}, but maximum values were four times higher. No statistical significance levels were computed among station or region values.

Chlorophyll *a* concentration of the > 75 µm diameter size fraction was only an index of the population and does not represent the total mass of the bloom which is unknown. Ambient chlorophyll *a* concentration was 1-2 µg l^{-1}. An attempt to obtain a larger portion of the *Microcystis* biomass by sampling with a 40 µm net was unsuccessful due to high sediment.

Toxicity – The presence of microcystins in *Microcystis* tissue well above the median of 0.02 ng l^{-1} at the control station at Napa suggested the bloom was toxic at most stations except the Suisun Marsh (Table 1). Previous research indicated the bloom contained the highly toxic hepatotoxin microcystin-LR (Lehman et al. 2005). Both volumetric (µg l^{-1}) and chlorophyll *a*-specific toxicity (µg total microcystins (µg chlorophyll *a*)$^{-1}$) were highest at stations in the central and western delta and similar to those measured in 2004 (compare Tables 1 and 2). No statistical significance levels were computed among station or region values.

Environmental conditions – The high *Microcystis* biomass in the central delta was associated with low chloride, nitrate + nitrite, total phosphorus, total suspended solids concentrations and nitrate to phosphate ratios (Table 3). High *Microcystis* biomass in the central delta was also associated with higher water temperature and lower specific conductance and turbidity than the western delta and Suisun Bay (Table 4). No statistical significance levels were computed among station or region values.

Vertical profiles suggested *Microcystis* biomass was associated with water column stratification (Fig. 2). Vertical profiles for the central delta station 902 on August 2, 2005 suggested high algal biomass at the surface was associated with somewhat lower turbidity and higher water temperature. The coincident high dissolved oxygen concentration and pH near the surface were probably produced by photosynthesis. Otherwise the water column was not stratified. A surface response in the central delta during the bloom was not always present as demonstrated on August 30, 2005. Stratification was also not associated with the bloom downstream in Suisun Bay at station 504 (Fig. 2) where water temperature and specific conductance were similar throughout the water column. Slight changes in turbidity may have been important to bloom development because chlorophyll *a* concentration was inversely associated with turbidity. No statistical significance levels were computed in association with these data.

V. Citations

American Public Health Association, American Water Works Association and Water Environment Association. 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. American Public Health Association, Washington, D. C.

Carmichael, W. W. & J. An, 1999. Using an enzyme linked immunosorbent assay (ELISA) and a protein phosphatase inhibition assay (PP1A) for the detection of microcystins and nodularins. Natural Toxins 7: 377-385.

Lehman, P. W., G. Boyer, C. Hall, S. Waller and K. Gehrts. 2005. Distribution and toxicity of a new colonial *Microcystis aeruginosa* bloom in the San Francisco Bay Estuary, California. Hydrobiologia 541:87-99.

United States Environmental Protection Agency (US EPA), 1983. Methods for Chemical Analysis of Water and Wastes. Washington, DC. Technical Report EPA-600/4-79-020.

Fig. 1. Map showing the sampling stations and the presence or absence of *Microcystis* in 2005.

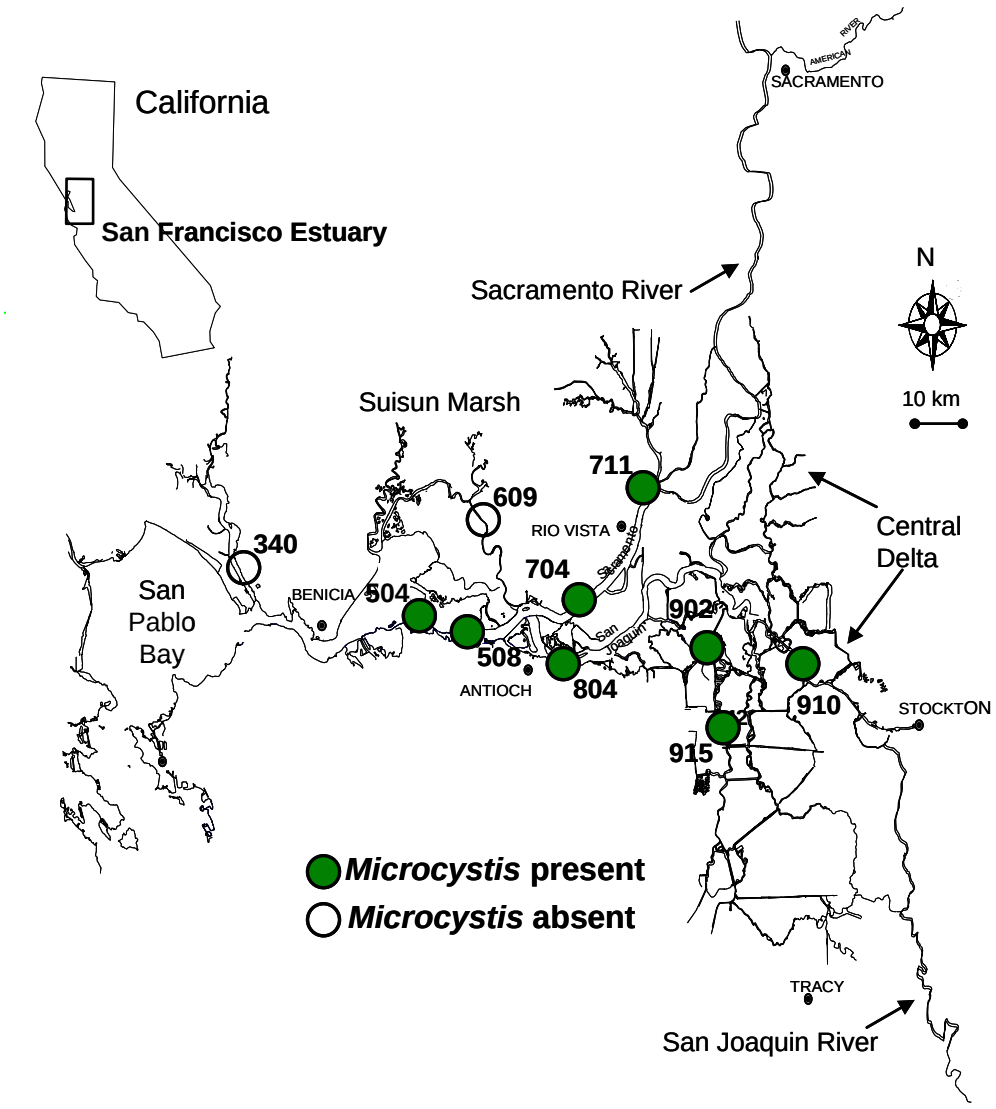
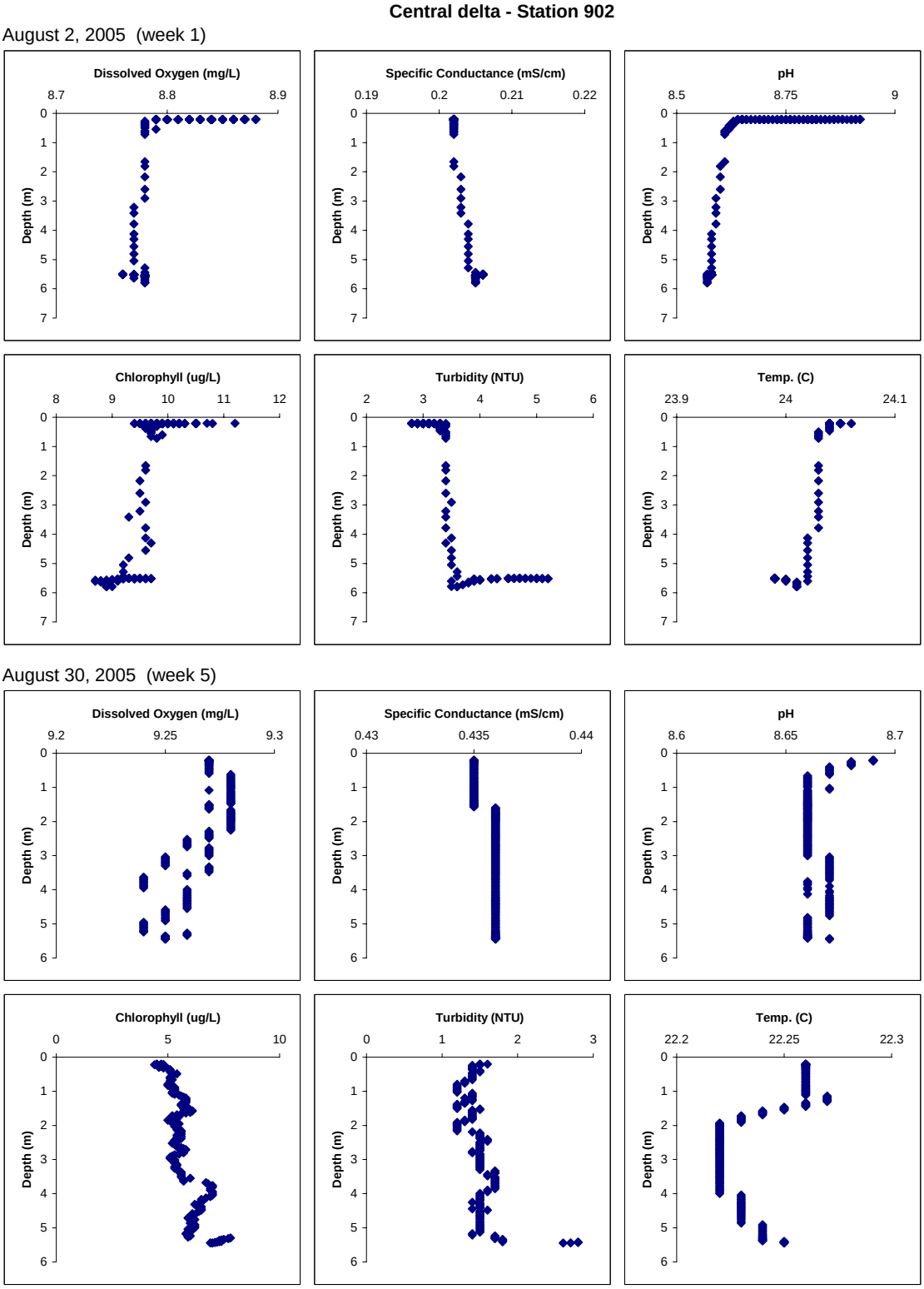
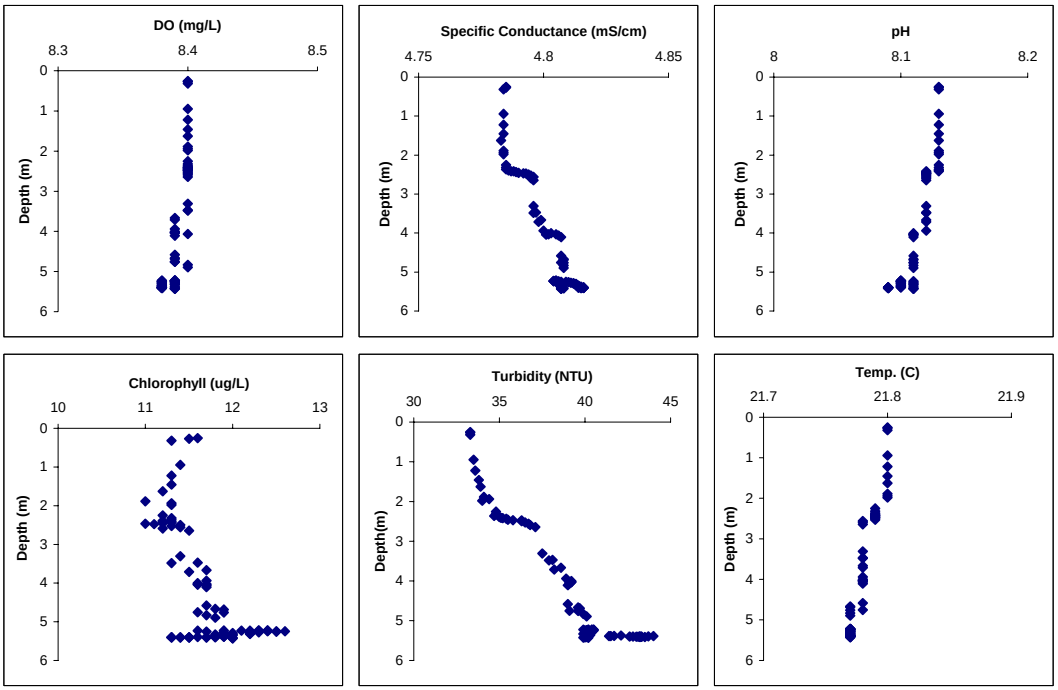


Fig. 2. Vertical profiles of water quality conditions in the central delta station 902 and Suisun Bay station 504 and in early August and September 2005.



Suisun Bay - Station 504

August 4, 2005 (week 1)



September 1, 2005 (week 5)

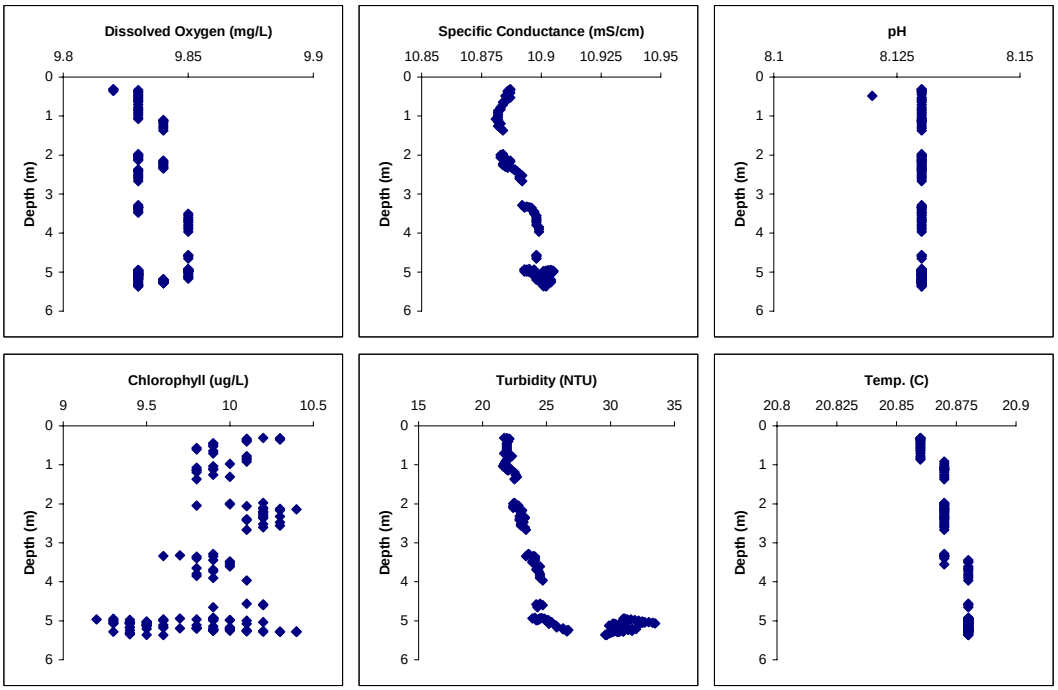


Table 1. Median and 0.05 and 0.95 percentile values for chlorophyll *a* concentration (> 75 µm diameter size fraction), total microcystins measured as microcystin-LR equivalents and the ratio of total microcystins to chlorophyll *a* concentration measured bi-monthly between August 2 and September 22, **2005** in the upper San Francisco Estuary. N=4

Region	Descrition	Station	chlorophyll *a* ng l $^{-1}$	total microcystins in microcystin LR equivalents ng l $^{-1}$	ug total microcystins (ug chlorophyll *a*) $^{-1}$ ratio
Control	Napa	340	1.27 (0.81, 1.40)	0.02 (0.01, 0.03)	0.02 (0.01, 0.02)
Suisun Bay	Suisun Bay at Chipps Island	508	2.86 (2.35, 6.32)	0.20 (0.05, 0.35)	0.06 (0.02, 0.10)
	Suisun Bay at Middle Ground	504	4.35 (2.02, 8.34)	0.42 (0.15, 1.76)	0.10 (0.08, 0.20)
Suisun Marsh	Suisun Marsh	609	6.27 (1.36, 8.41)	0.04 (0.04, 0.32)	0.01 (0.00, 0.37)
Western Delta	Antioch	804	52.07 (21.91, 63.28)	10.10 (4.79, 22.30)	0.25 (0.16, 0.42)
	Collinsville	704	2.56 (0.73, 6.97)	0.13 (0.05, 0.39)	0.19 (0.03, 0.27)
	Sacramento R. at Cache Slough	711	4.35 (2.35, 7.02)	0.09 (0.02, 0.16)	0.02 (0.01, 0.04)
Central Delta	Franks Tract	902	57.01 (36.71, 102.17)	9.75 (1.86, 11.98)	0.09 (0.03, 0.15)
	San Joaquin River	910	72.67 (49.62, 224.0)	17.24 (3.25, 58.67)	0.23 (0.06, 0.24)
	Old River	915	80.69 (33.97, 231.89)	15.49 (3.14, 18.57)	0.07 (0.06, 0.13)

Table 2. Median and 0.05 and 0.95 percentile values for chlorophyll *a* concentration (> 75 µm diameter size fraction), total microcystins measured as microcystin-LR equivalents and the ratio of total microcystins to chlorophyll *a* concentration measured biweekly between August September **2004** in the upper San Francisco Estuary. N=4

Region	Descrition	Station	chlorophyll *a* ng l^{-1}	total microcystins in microcystin LR equivalents ng l^{-1}	ug total microcystins (ug chlorophyll *a* $)^{-1}$ ratio
Suisun Bay	Suisun Bay at Chipps Island	508	9.46 (1.72, 25.53)	0.90 (0.48, 6.48)	0.14 (0.05, 0.55)
Western Delta	Collinsville	704	3.68 (0.44, 17.40)	1.38 (0.15, 3.87)	0.34 (0.27, 0.40)
Central Delta	Franks Tract	902	52.46 (25.55, 161.71)	7.96 (3.23, 26.18)	0.18 (0.16, 0.23)
	San Joaquin River	910	93.89 (61.08, 168.40)	22.78 (3.08, 39.10)	0.20 (0.11, 0.34)
	Old River	915	37.90 (15.75, 105.13)	5.71 (0.04, 17.26)	0.24 (0.01, 0.33)

Table 3. Median and .05 and .95 percentile values for water quality conditions associated with the *Microcystis aeruginosa* bloom at regions throughout the upper estuary in 2005.

	Napa (control)	Suisun Bay	Suisun Marsh	Western Delta	Central Delta
	Median Percentile (0.05, 0.95)	Median Percentile (0.05, 0.95)	Median Percentile (0.05, 0.95)	Median Percentile (0.05, 0.95)	Median Percentile (0.05, 0.95)
Ammonia mg/L	0.01 (0.01, 0.01)	0.02 (0.02, 0.03)	0.02 (0.01, 0.03)	0.04 (0.02, 0.11)	0.02 (0.01, 0.03)
Chloride mg/L	6430 (5002, 7662)	2310 (1578, 3742)	1880 (1224, 2722)	316 (7, 625)	38 (14, 97)
Nitrite + Nitrate mg/L	0.01 (0.01, 0.01)	0.32 (0.31, 0.34)	0.21 (0.17, 0.28)	0.26 (0.19, 0.29)	0.14 (0.09, 0.27)
Dissolved Organic Carbon mg/L	2.8 (2.6, 3.1)	1.7 (1.5, 1.8)	4.2 (3.9, 4.9)	1.8 (1.4, 2.1)	2.0 (1.6, 2.2)
Ortho-phosphate mg/L	0.03 (0.02, 0.03)	0.07 (0.07, 0.08)	0.06 (0.05, 0.06)	0.06 (0.05, 0.06)	0.05 (0.04, 0.06)
Silica mg/L	10.0 (6.5, 122.2)	14.8 (13.8, 15.2)	14.2 (13.8, 14.9)	15.4 (13.7, 16.8)	13.1 (11.6, 14.0)
pH	7.3 (7.0, 7.5)	6.8 (6.8, 7.0)	6.8 (6.6, 6.9)	6.7 (6.5, 6.8)	6.7 (6.5, 7.3)
Alkalinity mg/L	121 (120, 122)	67 (64, 72)	81 (73, 85)	68 (62, 74)	62 (56, 71)
Kjeldahl Nitrogen mg/L	0.6 (0.4, 1.0)	0.2 (0.2, 0.5)	0.5 (0.4, 0.8)	0.2 (0.1, 0.3)	0.2 (0.1, 0.4)
Total Organic Carbon mg/L	3.2 (2.8, 3.4)	1.9 (1.5, 1.9)	4.4 (4.1, 5.4)	1.9 (1.7, 2.0)	2.0 (1.6, 2.3)
Phosphorus mg/L	0.08 (0.06, 0.13)	0.11 (0.10, 0.18)	0.13 (0.11, 0.17)	0.10 (0.07, 0.11)	0.07 (0.06, 0.09)
Total Suspended Solids mg/L	14 (4, 35)	36 (21, 93)	33 (27, 67)	19 (7, 49)	2 (1, 6)
Volatile Suspended Solids mg/L	3 (2, 5)	6 (2, 11)	3 (1, 10)	2 (1, 5)	2 (1, 2)

Table 4. Median and .05 and .95 percentile values of field conditions associated with the *Microcystis aeruginosa* bloom in 2005. Field conditions were computed from vertical profiles collected by a YSI 6600 water quality sonde.

	Napa (control)	Suisun Bay	Suisun Marsh	Western Delta	Central Delta
	Median Percentile (0.05,0.95)	Median Percentile (0.05,0.95)	Median Percentile (0.05,0.95)	Median Percentile (0.05,0.95)	Median Percentile (0.05,0.95)
Temperature °C	21.16 (19.73, 22.68)	20.63 (18.62, 21.51)	21.38 (19.65, 23.54)	21.11 (18.74, 22.63)	22.99 (21.04, 24.77)
Specific Conductance mS/cm	18.94 (14.72, 22.67)	7.53 (4.94, 10.56)	5.65 (3.79, 7.80)	1.18 (0.15, 2.15)	0.20 (0.10, 0.46)
Dissolved Oxygen mg/L	7.80 (6.83, 8.32)	8.89 (8.47, 9.75)	8.06 (7.63, 8.29)	9.01 (8.04, 9.36)	8.27 (6.94, 9.48)
pH	7.70 (7.61, 7.76)	8.11 (8.07, 8.25)	8.02 (7.96, 8.16)	8.03 (7.85, 8.47)	8.15 (7.71, 8.68)
Turbidity NTU	7.36 (4.54, 12.32)	37.92 (15.17, 87.37)	42.47 (37.30, 61.01)	21.94 (9.61, 46.59)	2.68 (1.50, 3.93)