

Primary Research Paper

## Distribution and toxicity of a new colonial *Microcystis aeruginosa* bloom in the San Francisco Bay Estuary, California

P.W. Lehman<sup>1,\*</sup>, G. Boyer<sup>2</sup>, C. Hall<sup>3</sup>, S. Waller<sup>1</sup> & K. Gehrts<sup>1</sup>

<sup>1</sup>California Department of Water Resources, 3251 S Street, Sacramento, CA 95816, USA

<sup>2</sup>College of Environmental Science and Forestry, State University of New York, Syracuse, NY 13210, USA

<sup>3</sup>Environmental Science and Policy, University of California, Davis, CA 95616, USA

(\*Author for correspondence: plehman@water.ca.gov)

Received 30 June 2004; in revised form 24 September 2004; accepted 11 October 2004

**Key words:** *Microcystis*, cyanobacteria, toxic, estuary, harmful algal bloom

### Abstract

The first distribution, biomass and toxicity study of a newly established bloom of the colonial cyanobacteria *Microcystis aeruginosa* was conducted on October 15, 2003 in the upper San Francisco Bay Estuary. *Microcystis aeruginosa* was widely distributed throughout 180 km of waterways in the upper San Francisco Bay Estuary from freshwater to brackish water environments and contained hepatotoxic microcystins at all stations. Other cyanobacteria toxins were absent or only present in trace amounts. The composition of the microcystins among stations was similar and dominated by demethyl microcystin-LR followed by microcystin-LR. *In situ* toxicity computed for the >75  $\mu\text{m}$  cell diameter size fraction was well below the 1  $\mu\text{g l}^{-1}$  advisory level set by the World Health Organization for water quality, but the toxicity of the full population is unknown. The toxicity may have been greater earlier in the year when biomass was visibly higher. Toxicity was highest at low water temperature, water transparency and salinity. Microcystins from the bloom entered the food web and were present in both total zooplankton and clam tissue. Initial laboratory feeding tests suggested the cyanobacteria was not consumed by the adult copepod *Eurytemora affinis*, an important fishery food source in the estuary.

### Introduction

*Microcystis aeruginosa* is a common freshwater cyanobacterium that blooms in eutrophic lakes and reservoirs throughout the world including the United States, Canada, Australia, New Zealand, South Africa and Japan (Paerl, 1988; Carmichael, 1995; Watanabe, 1995; Downing et al., 2001). It has also been observed in estuaries including the Neuse River estuary (Paerl, 1988) and the Potomac River estuary (Sellner et al., 1993) in the USA, the Swan River estuary in Western Australia (Robson & Hamilton, 2003) and the Patos Lagoon Estuary in Southern Brazil (Yunes et al., 1996). A bloom of the colonial form of *M. aeruginosa* has also been observed in the northern reach of San Francisco Bay Estuary (NSFE), California

between June and November since 1999 (Lehman & Waller, 2003). The single-celled form of *M. aeruginosa* is currently a common cyanobacterium in NSFE but was not identified as a dominant genus in the phytoplankton community between 1975 and 1982 (Lehman & Smith, 1991). Total cyanobacteria biomass has increased throughout the NSFE coincident with a decline in diatom biomass between 1975 and 1993 (Lehman, 2000). The highest total cyanobacteria density occurred in the spring and summer of normal and critically-dry precipitation years (Lehman, 1996).

*M. aeruginosa* is sometimes characterized as a harmful algal bloom (HAB) species. Blooms of these species form surface scums that impede contact recreation sports, reduce aesthetics, lower dissolved oxygen concentration and cause taste and odor

problems in drinking water (Carmichael, 1995). Some of these blooms also contain hepatotoxic microcystins that cause liver tumors and cancer in wildlife and humans (Carmichael, 1995). One surface water sample collected in the NSFE in 2000 contained microcystins concentration above the World Health Organization advisory level of  $1 \mu\text{g l}^{-1}$  (World Health Organization, 1998; Lehman & Waller, 2003). No further information is available on the biomass, toxicity and distribution of the *M. aeruginosa* bloom or its potential impact to human health and ecosystem function in NSFE.

Marine and coastal HABs have occurred more frequently over the past decade worldwide (Anderson & Garrison, 1997; Horner et al., 1997) and have been observed along the coast of California since 1793 (Horner et al., 1997). Most of these blooms were associated with dinoflagellates that produced paralytic shellfish poisoning (PSP) or diatoms that produced domoic acid poisoning (DAP). It is assumed that coastal marine HABs enter estuaries along the west coast of North America although *in situ* growth was identified in Puget Sound (Horner et al., 1997). Coastally derived HABs contrast with the freshwater *M. aeruginosa* blooms that are assumed to enter estuaries from upstream during high streamflow events (Robson & Hamilton, 2003).

The colonial form of *M. aeruginosa* adds to an already extensive list of introduced species in San Francisco Estuary with adverse impacts. Approximately 212 species were introduced into the estuary since 1850 (Cohen & Carlton, 1995).

The previous dominant phytoplankton species were identified as cryptogenic, not clearly native or introduced. The potential adverse impact of this HAB on the estuary is large. Water from the northern region is used directly for drinking water and irrigation and the region is an important recreational area for sport fishing and water contact sports. The estuary is habitat for many anadromous commercial and recreational fish including striped bass and Chinook salmon and is a feeding ground for marine mammals. The estuary also contains many threatened or endangered aquatic organisms including the fish Delta smelt and winter run Chinook salmon and many of these endangered fish species are declining (Bennett & Moyle, 1996; California Bay-Delta Authority, 2000). Some of this decline may be linked to the

quantity and quality of the phytoplankton carbon available at the base of the food web (Lehman, 1992; Mueller-Solger et al., 2002; Feyrer et al., 2003; Lehman, 2004).

The purpose of this study is to develop initial information on the spatial distribution, toxicity, algal biomass and environmental conditions associated with the occurrence of the colonial *M. aeruginosa* bloom in the NSFE. Such information is needed to develop focused research and monitoring programs that evaluate the current and future impact of this bloom on estuarine processes.

#### *Study area*

The NSFE 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. The estuary stretches from the Pacific Ocean in the west to the tidal head at Greens Landing on the Sacramento River and Vernalis on the San Joaquin River. The inland Delta varies between fresh and brackish water conditions with season and water-year type and contains 200 km<sup>2</sup> of waterways formed by the Sacramento River on the north and the San Joaquin River on the south. Together these two rivers drain 47% of the runoff in California. The Sacramento River is the larger of the two rivers with an average runoff of 27 million m<sup>3</sup> compared with 10 million m<sup>3</sup> for the San Joaquin River. Depth varied from a few meters in the Delta to 13 m in the shipping lanes. The tide is semidiurnal and reaches 2 m throughout the region. Tidal velocities can reach 30 cm s<sup>-1</sup> in the Bay and are associated with tidal excursions of 10 km.

#### **Materials and methods**

##### *Field and laboratory sampling*

The spatial distribution of the *M. aeruginosa* bloom was identified by observation of surface waters during mid-day on September 12, 2003. *M. aeruginosa* biomass and toxicity was sampled on October 15, 2003 at 14 stations throughout the same area identified in September. Stations were selected that represented different habitat types or

use including recreational swimming (station 23), shallow water habitat (stations 41 and 42), deep river channel (stations 11–13), anadromous and native fish habitat (stations 11–43) and agricultural and drinking water (stations 43–45; Fig. 1). Colonies were sampled by horizontal surface tows of a 0.72 m diameter plankton net fitted with a 75  $\mu\text{m}$  mesh screen on the cod end. Sampling a large size fraction assured that the sample primarily contained the colonial form of *M. aeruginosa*. Net tows were conducted at the center of the channel at a speed of 60  $\text{m min}^{-1}$  and lasted 1–10 min depending on bloom biomass. 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 flowmeter. Water temperature and specific conductance were also measured at each station using a freshly calibrated YSI 85 sonde. Specific conductance was converted to salinity by first converting the specific conductance values to chloride concentration ( $\text{g l}^{-1}$ ) using station specific regression equations and then converting chloride concentration to salinity (ppt) using the equation:  $\text{salinity} = 1.80655 \times \text{chloride}$  (Unpublished data, California Department of Water Resources; APHA et al., 1998).

Water samples containing algal biomass were stored at 4  $^{\circ}\text{C}$  and filtered within 2 h onto GF/F glass fiber filters. Filters for microcystins analysis were wrapped in aluminum foil and frozen until

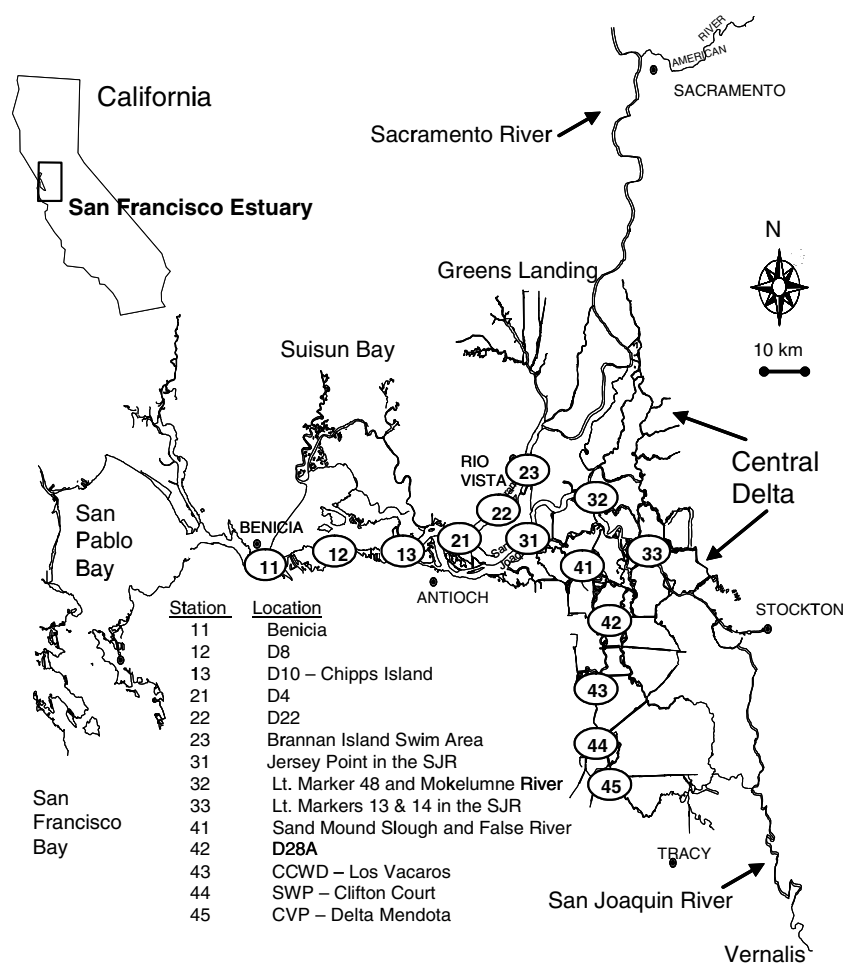


Figure 1. Map of upper San Francisco Bay Estuary with sampling station location.

laboratory analysis. Filters for chlorophyll *a* analysis were treated with magnesium carbonate as a preservative and frozen until laboratory analysis (US EPA, 1983). Sample replication was 20%. In addition, a 50 ml water sample was preserved and stained with Lugol's solution for phytoplankton cell identification with an inverted microscope (Utermohl, 1958).

The presence of microcystins in the food web was assessed by the presence of microcystins in zooplankton and clam tissue. Zooplankton were sampled at 5 stations by horizontal net tows of a 0.7 m diameter plankton net fitted with a 150  $\mu\text{m}$  mesh on the cod end. Zooplankton were kept at 4 °C and were separated by hand from *M. aeruginosa* in the water sample within 48 h using a dissecting microscope. The final zooplankton sample was rinsed in distilled water and frozen until analysis. Clams were obtained using a ponar dredge. The tissue was removed from the clam shell, rinsed in distilled water and frozen until analysis.

An initial study to determine the feeding of the adult copepod *Eurytemora affinis* (Copepoda: Calanoida) on *M. aeruginosa* was evaluated by laboratory feeding tests. Six groups of 20 laboratory grown animals were each placed in a 500 ml flask containing 200 ml of 1 ppt L16 culture medium. This culture medium is a synthetic culture medium modified with vitamin B<sub>12</sub>, biotin vitamins and soil extract that was useful for culturing zooplankton and algae because it had an ionic composition similar to that in many eutrophic lakes (Lindstrom, 1983). Zooplankton were held in the culture medium and not fed for 3.5 h. Previous experiments indicated a 50% reduction in gut chlorophyll *a* within 10 min of being placed in the 1 ppt L16 culture medium (Hall unpublished data). Copepods in three flasks were transferred to GFC glass fiber filters for replicate chlorophyll *a* analysis (Marker et al., 1980). An additional 150 ml of *M. aeruginosa* biomass equivalent to 146  $\mu\text{g l}^{-1}$  of chlorophyll *a* was added to the remaining three flasks. *M. aeruginosa* colonies were obtained from the Delta that day and separated from other phytoplankton by pipette with repeated washes in natural water filtered through a 0.45  $\mu\text{m}$  pore size Nucleopore filter. Treatment flasks were incubated for 24 h at 20 °C and a 16:8 light:dark cycle. At the end of the incubation

period, animals were removed from the media, placed on GFC glass fiber filters and the filters were immediately frozen until analysis for chlorophyll *a* concentration.

#### *Toxicity testing*

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. Zooplankton were lyophilized first then extracted as above. Clams were extracted using a 50% acidified methanol in a Waring Blender. Addition of purified microcystin-LR, microcystin-YR, microcystin-RR, anatoxin-a and saxitoxin to wet filters or lyophilized zooplankton, followed by their extraction as described above recovered greater than 90% of all five toxins. To determine the recovery of microcystin from the clam samples, it was necessary to split the crude homogenate into two parts and add a known amount of microcystin spiked into one fraction. Recoveries of microcystin from these tissues ranged from 50 to 65%. This value was then used to correct the unspiked fraction for loss of microcystins during extraction.

Total microcystins concentration in plant and animal tissue was initially 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  $\mu\text{l}$  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 h and compared to the control (no added microcystin-LR) and standards containing between 6 and 40  $\mu\text{g l}^{-1}$  microcystin-LR. Blanks (no enzyme, no toxin), unknowns, standards, and controls were all run in duplicate.

Samples with the highest levels of total microcystins were further analyzed by high pressure liquid chromatography (HPLC) to identify the specific microcystins in the sample. Samples were separated using a Dupont Ace 4.6  $\times$  250 5 $\mu$  C18 column and a two-step linear gradient of 30–70% acidified acetonitrile to acidified water at 0.8 ml/min (Harada, 1995). Detection was either

mass selective using electrospray ionization (LCMS, Agilent 1100 series MSD) and by UV absorbance using a Waters model 996 photodiode array detector (PDA) between 210 and 300 nm. For LCMS, all ions between 900 and 1250 amu were combined to form the total ion chromatograph and potential microcystins identified on the basis of their molecular ions and retention times. For PDA detection, potential microcystins were identified on the basis of having an absorbance maximum at 239 nm in their UV spectrum and on their retention times.

Anatoxin-a was determined by HPLC after derivatization with 7-fluoro-4-nitro-2,1,3-benzoxadiazole (NBD-F) (James & Sherlock, 1996). The PSP toxins (saxitoxin, neosaxitoxin, and gonyautoxins 1–4) were measured by HPLC with fluorescent detection using the electrochemical oxidation system (Boyer & Goddard, 1999) to form fluorescent derivatives.

#### Analysis

Because of the small sample size, differences in the means for variables among regions were determined using the Kruskal–Wallis nonparametric technique. Multiple comparisons were evaluated using least significant differences and correlation was evaluated using Spearman rank correlation coefficients. All analyses were conducted using Statistical Analysis System software (SAS Institute, Inc., 2004)

## Results

#### Distribution

*M. aeruginosa* colonies  $>75 \mu\text{m}$  diameter were present in surface samples at all stations sampled in October (Fig. 1). The bloom distribution in October was the same as that observed in September. Sampling stations represented a wide range of habitat types from marine water habitat at the western end of Suisun Bay to freshwater habitat upstream in the Sacramento, Old and San Joaquin rivers. Chlorophyll *a* concentration in concentrated surface net tows ranged from 4 to  $554 \mu\text{g l}^{-1}$  and was significantly different among regions ( $p < 0.01$ ; Table 1). The highest chloro-

phyll *a* concentrations ( $p < 0.05$ ) were measured in the San Joaquin and Old rivers compared with the Sacramento River and Suisun Bay. *In situ* chlorophyll *a* concentration associated with the  $>75 \mu\text{m}$  diameter size fraction ranged between 0.7 and  $74.6 \text{ m } \mu\text{g l}^{-1}$  based on an expansion of the net sample concentration to tow volume. How much of the surface chlorophyll *a* concentration was composed of *M. aeruginosa* is unknown but phytoplankton identification samples suggested most of the cells were *M. aeruginosa*. The chlorophyll *a* concentration of all size fractions in a Van Dorn sample taken at 1 m depth was  $1\text{--}3 \mu\text{g l}^{-1}$  throughout the region on October 15 (unpublished data, California Department of Water Resources) and probably represents the background concentration of other algae in the water column because *M. aeruginosa* was near the surface during the day.

The environmental conditions associated with the bloom varied among regions. Water temperature, salinity and Secchi disk depth were all significantly different among regions ( $p < 0.01$ ). The high phytoplankton biomass and microcystins concentration in the San Joaquin and Old rivers were accompanied by a combination of higher ( $p < 0.05$ ) water temperature, lower salinity ( $p < 0.05$ ) and higher ( $p < 0.05$ ) water transparency (Fig. 2) than the Sacramento River and Suisun Bay. Chlorophyll *a* concentration was more closely associated ( $p < 0.01$ ) with warmer water temperature ( $r = 0.66$ ) and higher Secchi disk depth ( $r = 0.70$ ) and lower salinity ( $r = -0.71$ ) than microcystins concentration ( $p < 0.05$ ;  $r = 0.54$ ,  $r = 0.52$  and  $r = -0.52$ , respectively). Nutrient concentrations were high and nonlimiting throughout the area. Median dissolved inorganic nitrogen and soluble reactive phosphorus were 0.41 and  $0.06 \text{ mg l}^{-1}$  respectively (unpublished data, California Department of Water Resources).

#### Toxicity

Microcystins were present at all stations but concentrations differed ( $p < 0.01$ ) among regions. Both the San Joaquin and Old rivers had higher ( $p < 0.05$ ) microcystins concentration than the Suisun Bay region downstream. The microcystins

Table 1. Comparison of chlorophyll *a*, microcystin, anatoxin-a (ATX) and PSP concentration in concentrated net tow samples normalized to a 1 min tow for the >75  $\mu\text{m}$  cell diameter size fraction of the *Microcystis aeruginosa* bloom and microcystin concentration in zooplankton and clam tissue at sampling stations in the northern San Francisco Bay Estuary

Station	Location & CA DWR code	Chlorophyll <i>a</i> ( $\mu\text{g l}^{-1}$ )	Microcystin tissue		ATX or PSP	Zooplankton tissue		Clam tissue	
			Microcystin-LR equivalent	CV (%)		Microcystin-LR equivalent $\mu\text{g g}^{-1}$ dwt	%CV	Microcystin-LR equivalent $\mu\text{g g}^{-1}$ dwt	%CV
<i>San Francisco Bay</i>									
11	Benicia-D6	41.6	$0.02 \pm 0.002$	8	ND				
12	Suisun Bay-D8	9.6	$0.29 \pm 0.05$	17	ND				
13	Chips Island-D10	5.6	$0.93 \pm 0.16$	17	ND				
<i>Sacramento River</i>									
21	Sacramento R.-D4	26.6	$4.31 \pm 0.54$	13	ND				
22	Horseshoe Bend-D22	3.6	$5.42 \pm 0.57$	10	ND				
23	Brannon Island	67.1	$27.54 \pm 1.84$	7	ND				
<i>San Joaquin River</i>									
31	Jersey Point-D16	301.0	$118.60 \pm 9.4$	8	trace, ND				
32	Mouth of Mokelumne R.	401.0	$45.30 \pm 6.6$	14	ND	$1.02 \pm 0.2$	18		
33	Navigation marker 13	553.5	$115.10 \pm 7.95$	7	trace, ND				
<i>Old River</i>									
41	San Mound Slough	194.0	$42.40 \pm 5.0$	14	ND	$2.9 \pm 0.6$	24	0.02	NA
42	Old River - D28A	254.0	$45.00 \pm 6.4$	15	ND	$3.3 \pm 0.9$	28		
43	Los Vaqueros Reservoir	89.8	$19.20 \pm 2.7$	14	ND				
44	SWP- Clifton Court-C9	96.2	$14.60 \pm 2.4$	16	ND	$0.7 \pm 0.2$	22		
45	CVP-Delta Mendota Canal	107.0	$12.20 \pm 1.2$	10	ND	$3.5 \pm 0.6$	16		

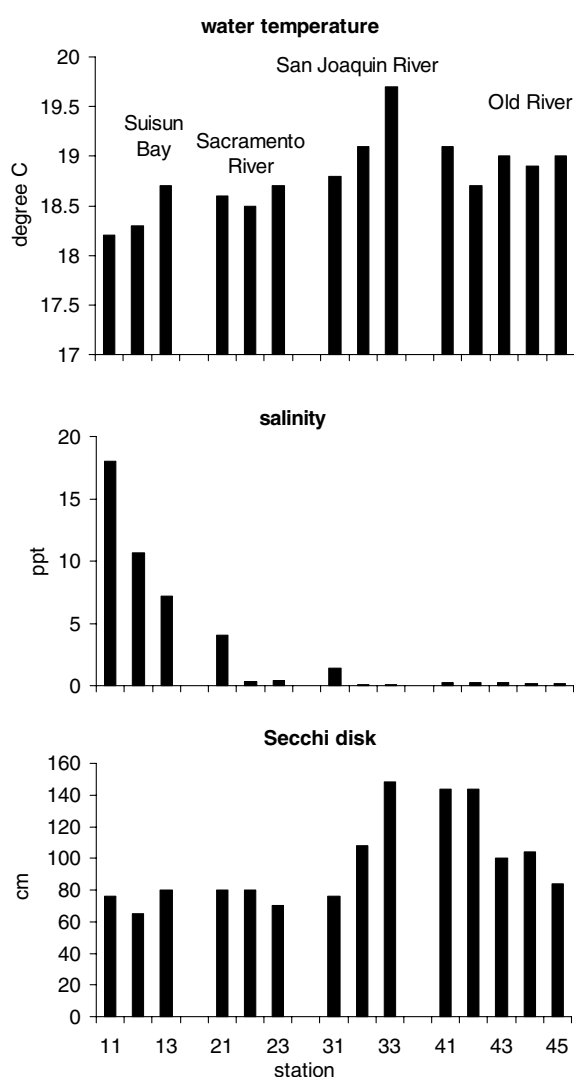


Figure 2. Environmental conditions in surface water measured at sampling stations throughout the upper San Francisco Bay Estuary on October 15, 2003.

were dominated by a demethyl microcystin-LR that comprised between 47 and 66% of the microcystins followed by microcystin-LR that comprised 9 to 23% (Table 2). *In situ* microcystins concentration based on net tow volume was much lower than the concentrated net samples (compare Fig. 3 and Table 1). Similar net tow volume computed from net size, time and tow speed and those computed from the current meter values taken during the tow suggested the volume sampled was a good estimate of total sample volume.

Toxicity generally increased with chlorophyll *a* concentration but the association was not linear (Table 1). The microcystins to chlorophyll *a* and the microcystins to total pigment ratio differed widely among stations (Fig. 4) and was high when water temperature, water transparency and salinity were comparatively low (Fig. 5). The lowest microcystins to chlorophyll *a* ratios occurred at salinities greater than 5 ppt and suggested salinity was an important factor controlling toxicity. The highest toxicity was measured near the transition zone between fresh and brackish water at station 22 on the Sacramento River (Fig. 4).

The algal tissue samples were also tested for the cyanobacterial neurotoxins, anatoxin-a and PSP toxins such as saxitoxin. PSP toxins were not detected at measurable concentrations in any of the samples. Anatoxin-a was not detected or occurred in trace amounts in concentrated 1 min net tow samples at stations 31 ( $0.1 \text{ g l}^{-1}$ ) and 33 ( $0.4 \text{ } \mu\text{g l}^{-1}$ ; Table 1).

#### Food web impact

The animal tissue of lower food web organisms contained small amounts of microcystins. Microcystins concentration ranged from 1 to  $3.5 \text{ } \mu\text{g}$  microcystins ( $\text{g dry weight}^{-1}$ ) in zooplankton tissue and was  $0.02 \text{ } \mu\text{g}$  microcystins ( $\text{g dry weight}^{-1}$ ) in clam tissue (Table 1). The concentration in animal tissue was not a function of the microcystins concentration per unit chlorophyll *a* measured at the station (compare Table 1 and Fig. 4).

*M. aeruginosa* was not eaten by the adult copepod *E. affinis* in initial laboratory feeding studies. The chlorophyll *a* content of initial animals was  $0.07 \pm 0.01 \text{ } \mu\text{g}$  ( $100 \text{ animals}^{-1}$ ) and was not different from the  $0.05 \pm 0.01 \text{ } \mu\text{g}$  ( $100 \text{ animals}^{-1}$ ) of incubated animals. *E. affinis* was observed in the zooplankton samples used for toxicity assays.

## Discussion

### Distribution

The colonial form of *M. aeruginosa* occurred throughout the NSFE at salinities from 0.1 to 18 ppt and habitats from shallow flooded islands

Table 2. Percent composition of microcystins in selected *Microcystis aeruginosa* tissue samples

Congener	Sacramento River		San Joaquin River	
	Station 23 Brannon Island %	Station 31 Jersey Point %	Station 32 Mouth of Mokelumne R. %	Station 33 Navigation marker 13 %
Demethyl	47	51	66	53
Microcystin-LR	20	23	9	16
Microcystin-WR	11	7	13	10
Microcystin-FR	6	5	6	8
Microcystin-RR	4	0	0	4
Unknown	12	14	6	9

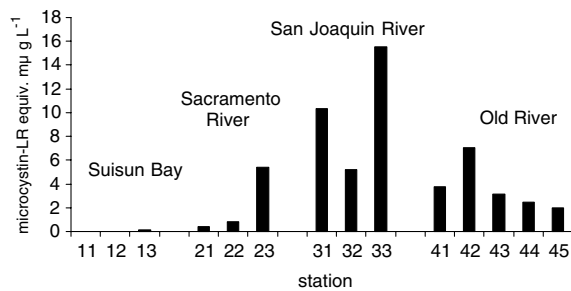


Figure 3. Surface microcystins equivalent concentration in the >75 μm cell diameter size fraction.

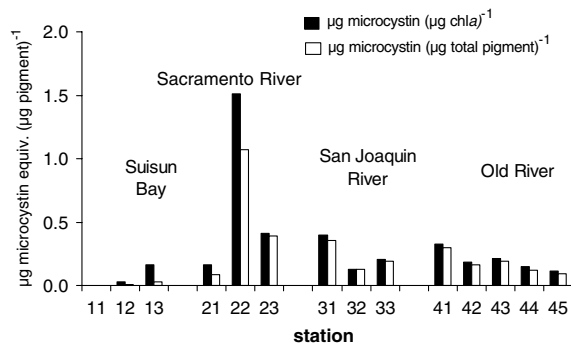


Figure 4. Concentration of total microcystin-LR equivalents per unit chlorophyll a and total pigment concentration in the >75 μm size fraction.

to deep shipping channels in the summer and fall of 2003. *M. aeruginosa* is a common cyanobacterium worldwide but its growth is restricted to salinity less than 7 ppt (Robson & Hamilton,

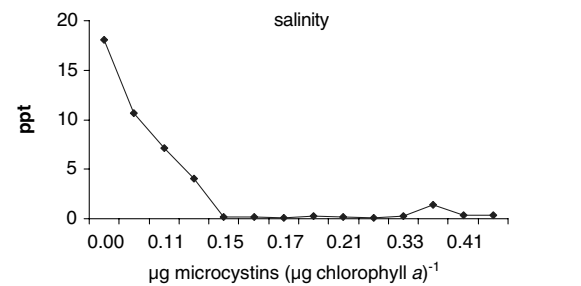
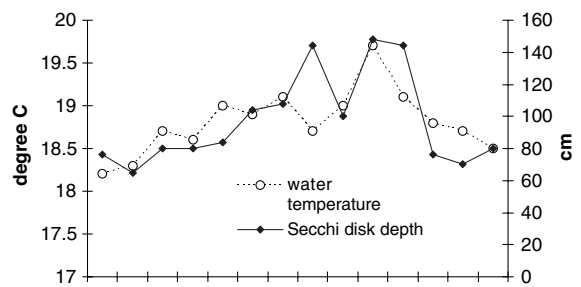


Figure 5. Variation of salinity, water temperature and Secchi disk depth with the microcystins to chlorophyll a ratio.

2003). Growth at low salinities may partially explain the higher biomass measured in the Delta portion of the estuary where salinity was less than 5 ppt. The absence of a visible *M. aeruginosa* bloom in the tributaries upstream suggested growth occurred within the Delta. *M. aeruginosa* blooms also developed within the estuary from cells seeded from upstream during high streamflow in the Swan Estuary in Western Australia (Robson & Hamilton, 2003). *Microcystis* spp. develop



readily from vegetative colonies in shallow water sediments (Brunberg & Blomqvist, 2003) and it is probable the bloom developed in the shallow areas of the Delta from either resident vegetative colonies or colonies seeded from upstream and spread downstream into the deep river channels with the wind and tide. Local biomass peaks in shallow water stations such as Brannon Island was probably produced by aggregation by wind and tide (Paerl, 1988).

The *M. aeruginosa* bloom was associated with high light, warm water temperature, shallow water and eutrophic conditions. This agrees with research in which water column stability, nutrient loading, light availability, water temperature, organic matter and habitat for seeding were identified as conditions needed for development and persistence of *M. aeruginosa* blooms (Paerl, 1988; Reynolds et al., 1981). Water temperature in September and October was somewhat below the optimum water temperature of 28 °C (Christian et al., 1986) but higher water temperature occurred earlier in the season when the bloom was visibly larger (unpublished data, California Department of Water Resources). Surface irradiance of 2000  $\mu\text{Em}^{-2} \text{s}^{-1}$  and Secchi disk depth of 140 cm indicated light in the water column was above the limiting level of 186  $\mu\text{Em}^{-2} \text{s}^{-1}$  (Christian et al., 1986). Median dissolved inorganic nitrogen and soluble reactive phosphorus concentrations were high and nonlimiting. *M. aeruginosa* is associated with eutrophic conditions and appeared to be more closely associated with the total nitrogen and phosphorus concentrations than the nitrogen to phosphorus ratio (Downing et al., 2001).

The colonial *M. aeruginosa* bloom in NSFE was caused by the geographic expansion of a harmful cyanobacteria bloom into a freshwater and brackish water habitat. The recent geographic expansion of harmful diatom and dinoflagellate algal bloom species through both regional spread and the occurrence of new species are well known for marine habitats (Anderson & Garrison, 1997). Many species introductions have occurred in the San Francisco Estuary since the 1850s (Cohen & Carlton, 1995). The colonial form of *M. aeruginosa* is the first recorded toxic phytoplankton bloom in the northern reach of the estuary. It may also be the first clearly known introduced phyto-

plankton bloom species in the San Francisco Estuary because the genus *Microcystis* was not observed in phytoplankton samples between 1975 and 1982 (Lehman & Smith, 1991). Previous phytoplankton bloom species were identified as cryptogenic, not clearly native or introduced (Cohen & Carlton, 1995). It is unknown if the colonial form of *M. aeruginosa* that appeared in 1999 was merely an aggregation of the single-celled form now present in the estuary or a new strain. *M. aeruginosa* colonies can congregate into larger colonies under stable conditions (O'Brien et al., 2004).

#### Toxicity

*M. aeruginosa* was a toxic cyanobacteria strain because it contained hepatotoxic microcystins at all stations. Traces of the neurotoxin anatoxin-a occurred at two stations but are usually not produced by *M. aeruginosa* (Sivonen & Jones, 1999). The trace amount of anatoxin-a at these two stations probably represent the background level in this ecosystem because these stations also contained some of the higher microcystins concentrations. It is unlikely that both the anatoxin-a and microcystins originated in the same cyanobacterium. The presence of both hepatotoxins and anatoxin-a was only reported for a *M. aeruginosa* bloom in Japan in association with the microcystins LR and RR (Park & Watanabe, 1995).

Microcystin toxicity is highly variable but the microcystin-LR found in the *M. aeruginosa* bloom in NSFE is a powerful hepatotoxin associated with both acute and chronic liver damage (Kaya, 1995). The toxicity of the demethyl microcystin-LR in NSFE is unknown. Microcystins are associated with toxicity to birds and fish and are suspected as a cause of human cancer in China and Australia (Carmichael, 1995; Kaya, 1995). The bloom probably originated from a single population of a microcystin-LR producer that spread throughout the region with the tide and wind because microcystins samples had a similar percent microcystin composition.

Acute *in situ* microcystins toxicity was probably low within the  $>75 \mu\text{m}$  cell diameter size fraction for humans and aquatic organisms. Total microcystins concentration in NSFE was well below the Australian and Canadian suggested water quality

standard for humans of 0.5–1  $\mu\text{g l}^{-1}$  (Carmichael, 1995) and the World Health Organization advisory level of 1  $\mu\text{g l}^{-1}$  (World Health Organization, 1998). It was also below the lower limit of the 48 h  $\text{LC}_{50}$  of 450  $\mu\text{g l}^{-1}$  for zooplankton (Hanazato, 1995). However, the *in situ* microcystins concentrations were probably conservative estimates of the bloom toxicity because net tows can underestimate the microcystins content by as much as 10-fold (J. Makarewicz, unpublished observations). This is probably a combination of cells passing through the net and the effect of the frontal boundary causing an overestimate of the amount of water that actually passed through the net. *In situ* toxicity may have been higher at locations where high water residence time, wind, channel morphology and tide aggregated biomass (Paerl, 1988). This was supported by microcystins concentration in concentrated net samples that were orders of magnitude higher than the suggested water quality advisory levels. Toxicity may also be higher earlier in the season when the bloom appears to have larger biomass (S. Waller, personal communication).

A variable microcystins to chlorophyll *a* ratio indicated chlorophyll *a* was not a reliable indicator of microcystin toxicity. The ratios of microcystins to chlorophyll *a* concentration calculated for Suisun Bay and the San Joaquin and Old rivers were within the range of 0.1–0.4  $\mu\text{g}$  microcystins ( $\mu\text{g}$  chlorophyll *a*)<sup>-1</sup> typically measured for *Microcystis* spp. (Sivonen & Jones, 1999). The high  $\mu\text{g}$  microcystins ( $\mu\text{g}$  chlorophyll *a*)<sup>-1</sup> ratio of 1.5 measured at station 22 may be a function of the unique environmental conditions at this station where freshwater and brackish water converge. Toxicity varies with environmental conditions and was highest at intermediate water temperature and light intensity in culture experiments (van der Westhuizen & Eloff, 1985). The highest toxicity in this study occurred at relatively low water temperature, water transparency and salinity and contrasted with the lowest toxicity that occurred at relatively low water temperature and water transparency but high salinity.

#### *Food web impact*

Microcystins entered the base of the food web and were measured in both total zooplankton and

clam tissue. The maximum microcystins concentration of 3.5  $\mu\text{g}$  (g dry wt)<sup>-1</sup> in zooplankton tissue was low compared with the 75–1387  $\mu\text{g}$  (g dry wt)<sup>-1</sup> measured for zooplankton in Lake Kasumigaura (Watanabe et al., 1992). The direct toxicity of *M. aeruginosa* to zooplankton is reduced because it comprises only a small percentage of the zooplankton diet (Sellner et al., 1993). A combination of mechanical interference and feeding selectivity limits its use by zooplankton as a food source (Hanazato, 1995). The limited use of *M. aeruginosa* at the base of the food web in NSFE was suggested by initial laboratory feedings studies for the adult copepod *E. affinis*, but use varies by species, developmental stage, total food availability and structural form of the cyanobacteria (e.g., colonial) (DeMott et al., 1991; Reinikainen et al., 1994; Ghadouani et al., 2004). Even low microcystins concentration at the base of the food web poses a threat to the upper food web because microcystins may bioaccumulate. Tissue of the cladocera *Bosmina* spp. contained microcystins concentration that was 202% higher than in the co-occurring algal tissue (Park & Watanabe, 1995).

The impact of *M. aeruginosa* on the quantity and quality of phytoplankton biomass available to the food web may be a greater threat to the NSFE food web than toxicity. Total phytoplankton biomass is low at the base of the food web in NSFE compared with other estuaries because high turbidity limits phytoplankton growth (Jassby et al., 2002). *M. aeruginosa* blooms can reduce the growth of other phytoplankton because their surface habit limits light transmission into the water column and allows them to out compete other phytoplankton that cannot tolerate high light and water temperatures at the surface (Robarts & Zohary, 1992). Dissolved microcystins associated with the bloom may also inhibit consumption of the available desirable phytoplankton food by zooplankton (DeMott et al., 1991).

Both the quantity and quality of phytoplankton biomass appear to be important for the NSFE food web because they were correlated with long-term changes in zooplankton and *Neomysis mercedis* carbon (Lehman, 1992, 2004) and laboratory growth studies suggested local *Daphnia* grew best on phytoplankton carbon (Mueller-Solger et al., 2002). The loss of phytoplankton

food resources due to the *M. aeruginosa* bloom would add an additional impact to the phytoplankton biomass in the estuary already reduced by grazing of the clam *Potamocorbula amurensis* that was introduced into the estuary in 1987 (Jasby et al., 2002). Fish in the estuary partially adjusted to this loss of phytoplankton biomass by shifting their diet, but these shifts were not sufficient to prevent the decline in many fish species (Bennett & Moyle, 1996; Feyrer et al., 2003).

#### *Beneficial use impact*

The *M. aeruginosa* bloom is a potential threat to beneficial use in NSFE. NSFE provides agricultural and drinking water for local and upstream users. High microcystins concentration occurred in river channels used to divert water into storage reservoirs for the State Water Project and Federal Central Valley Project that supply water throughout California. Diversion of water into these reservoirs may also provide the seed needed to spread *M. aeruginosa* blooms and associated taste and odor problems into drinking water supplies. NSFE is also economically important because of its recreational use. *M. aeruginosa* blooms impact recreation through direct contact and ingestion that can cause skin and eye irritation, hay fever symptoms, dizziness, fatigue and stomach upset (Carmichael, 1995). High exposure water sports in the region include swimming, sail boarding, water skiing and wading. High microcystins concentration was measured at Brannon Island, a popular swimming beach. Sport fishing is also an important economic resource and could be impacted because of the health risk associated with ingestion of concentrated microcystins in animal tissue caused by bioaccumulation (Magalhaes et al., 2003). Wind-concentrated scums often contain microcystins concentrations that are toxic to animals and livestock, an important issue in this agricultural region. In addition, high biomass produced by blooms and the associated decomposition could eventually impact fishery production through its influence on dissolved oxygen concentration. Upstream migration of the threatened species Chinook salmon was blocked by low dissolved oxygen concentration in the San Joaquin River (Hallock et al., 1970) and low dissolved oxygen

concentration adversely impacts the health of aquatic organisms (Breitburg, 2002). High biomass can also enhance trihalomethane production, a cancer causing substance associated with chlorination of drinking water containing organic matter and an important concern in NSFE.

Because of its impact on so many beneficial uses, a regular monitoring will be needed to determine the yearly rate of expansion and toxicity of *M. aeruginosa* and the environmental factors that affect its development. The presence of microcystins in the food web suggested more information is needed on the presence of these toxins in the food web, potential pathways among trophic levels and how these change over time. Such information will be needed to assess the magnitude of the impact of *M. aeruginosa* on beneficial use in the estuary and to assess the need for a long-term management plan to control its development and toxicity.

#### **Conclusion**

This paper documents the first occurrence of a harmful algal bloom of the colonial form of *M. aeruginosa* in San Francisco Estuary. Initial surveys conducted in 2003 indicated this bloom occurred throughout the freshwater to brackish water regions of the estuary and contained hepatotoxic microcystins at all stations sampled. Microcystins were characterized by demethyl microcystin-LR followed by microcystin-LR. *M. aeruginosa* may also be the first known introduced phytoplankton species to the estuary.

The toxicity and widespread distribution of *M. aeruginosa* in NSFE demonstrated the potential of this organism to negatively impact many beneficial uses in NSFE and suggested that an active and long-term monitoring program is needed to assess the potential long-term human and ecological impacts.

#### **Acknowledgements**

This study was funded by the NOAA Coastal Ocean Program (COP) in partnership with the National Office for Marine Biotoxins and Harmful Algal Blooms at Woods Hole Oceanographic

Institution and the COP Monitoring and Event Response for Harmful Algal Blooms (MERHAB) program, the California Department of Water Resources and U.S. Bureau of Reclamation as a part of the San Francisco Bay-Delta Interagency Ecological Program. M. Satachwell at the State University of New York provided the protein phosphatase assays. E. Santos and L. Brenn assisted with monitoring.

## References

- American Public Health Association, American Water Works Association and Water Environment Association. 1998. Standard Methods for the Examination of Water and Wastewater. 20th edn. American Public Health Association, Washington, DC.
- Anderson, D. M. & D. J. Garrison, 1997. The ecology and oceanography of harmful algal blooms. Special issue. *Limnology and Oceanography* 42: 1009–1305.
- Bennett, W. A. & P. B. Moyle, 1996. Where have all the fishes gone? Interactive factors producing fish declines in the Sacramento-San Joaquin Estuary. In J. T. Hollibaugh (ed.), *San Francisco Bay: The Ecosystem*. Pacific Division of the American Association for the Advancement of Science, San Francisco, California: 519–542.
- Brunberg, A. & P. Blomqvist, 2003. Recruitment of *Microcystis* (Cyanophyceae) from lake sediments: The importance of littoral inocula. *Journal of Phycology* 39: 58–63.
- Boyer, G. L. & G. D. Goddard, 1999. High performance liquid chromatography (HPLC) coupled with post-column electrochemical oxidation (ECOS) for the detection of PSP toxins. *Natural Toxins* 7: 353–359.
- Breitburg, D. 2002. Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fishes and fisheries. *Estuaries* 25: 767–781.
- California Bay-Delta Authority, 2000. Programmatic Record of Decision. Technical Report. California Bay-Delta Authority, Sacramento, CA. URL: <http://calwater.ca.gov/Archives/GeneralArchive/rod/ROD8-28-00.pdf>.
- Carmichael, W. W., 1995. Toxic microcystin in the environment. In M. F. Watanabe, K. Harada, W. W. Carmichael & H. Fujiki (eds), *Toxic Microcystis*. CRC Press, New York: 1–12.
- Carmichael, W. W. & J. An, 1999. Using an enzyme linked immunosorbent assay (ELISA) and a protein phosphatase inhibition assay (PPIA) for the detection of microcystins and nodularins. *Natural Toxins* 7: 377–385.
- Christian, R. R., W. L. Bryant Jr. & D. W. Stanley, 1986. The relationship between river flow and *Microcystis aeruginosa* blooms in the Neuse River, North Carolina. *Water Resources Research Institute Report* 223. North Carolina State University.
- Cohen, A. N. & J. T. Carlton, 1995. Indigenous aquatic species in a United States estuary: study of the biological invasions of the San Francisco Bay and Delta. United States Fish and Wildlife Service, Washington, DC and the National Sea Grant College Program, Connecticut Sea Grant.
- DeMott, W. R., Q. Zhang & W. W. Carmichael, 1991. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia*. *Limnology and Oceanography* 36: 1346–1357.
- Downing, J. A., S. B. Watson & E. McCauley, 2001. Predicting cyanobacterial dominance in lakes. *Canadian Journal of Fisheries and Aquatic Science* 58: 1905–1908.
- Feyrer, F., B. Herbold, S. A. Matern & P. Moyle, 2003. Dietary shifts in a stressed fish assemblage: consequences of a bivalve invasion in the San Francisco Estuary. *Environmental Biology of Fishes* 67: 277–288.
- Ghadouani, A., B. Pinel-Alloul, K. Plath, G. A. Codd & W. Lampert, 2004. Effects of *Microcystis aeruginosa* and purified microcystin-LR on the feeding behavior of *Daphnia pulex*. *Limnology and Oceanography* 49: 666–679.
- Hallock, R. J., R. F. Elwell & D. H. Fry, 1970. Migrations of Adult King Salmon *Oncorhynchus tshawytscha* in the San Joaquin Delta, as Demonstrated by the Use of Sonic Tags. Bulletin 151. California Department of Fish and Game Fish, Sacramento, California.
- Harada, K., 1995. Chemistry and detection of microcystins. In M. F. Watanabe, K. Harada, W. W. Carmichael & H. Fujiki (eds), *Toxic Microcystis*. CRC Press, New York: 103–148.
- Hanazato, T., 1995. Toxic cyanobacteria and the zooplankton community. In M. F. Watanabe, K. Harada, W. W. Carmichael & H. Fujiki (eds), *Toxic Microcystis*. CRC Press, New York: 79–102.
- Horner, R. A., D. L. Garrison & F. G. Plumley, 1997. Harmful algal blooms and red tide problems on the US west coast. *Limnology and Oceanography* 42: 1076–1088.
- James, K. J. & I. R. Sherlock, 1996. Determination of the cyanobacterial neurotoxin, anatoxin-a, by derivatization using 7-fluoro-4-nitro-2,1,3-benzoxadiazole (NBD-F) and HPLC analysis with fluorimetric detection. *Biomedical Chromatography* 10: 46–47.
- Jassby, A. D., J. E. Cloern & B.E. Cole, 2002. Annual primary production: patterns and mechanisms of change in a nutrient-rich tidal ecosystem. *Limnology and Oceanography* 47: 698–712.
- Kaya, K., 1995. Toxicology of Microcystins. In M. F. Watanabe, K. Harada, W. W. Carmichael & H. Fujiki (eds), *Toxic Microcystis*. CRC Press, New York: 175–202.
- Lehman, P. W., 1992. Environmental factors associated with long-term changes in chlorophyll concentration in the Sacramento-San Joaquin Delta and Suisun Bay, California. *Estuaries* 15: 335–348.
- Lehman, P. W., 1996. Changes in chlorophyll *a* concentration and phytoplankton community composition with water-year type in the upper San Francisco Bay Estuary. In J. T. Hollibaugh (ed.), *San Francisco Bay: The Ecosystem*. Pacific Division of the American Association for the Advancement of Science. San Francisco, California: 351–374.
- Lehman, P. W., 2000. The influence of climate on phytoplankton community carbon in San Francisco Bay Estuary. *Limnology and Oceanography* 45: 580–590.

- Lehman, P. W., 2004. The influence of climate on mechanistic pathways that impact lower food web production in northern San Francisco Bay estuary. *Estuaries* 27: 311–324.
- Lehman, P. W. & R. W. Smith, 1991. Environmental factors associated with phytoplankton succession for the Sacramento-San Joaquin Delta and Suisun Bay Estuary, California. *Estuarine Coastal and Shelf Science* 32: 105–128.
- Lehman, P. W. & S. Waller, 2003. *Microcystis* blooms in the Delta. Interagency Ecological Program for the San Francisco Estuary Newsletter 16: 18–19.
- Lindstrom, K. 1983. Selenium as a growth factor for plankton algae in laboratory experiments and in some Swedish lakes. *Hydrobiologia* 101: 35–48.
- Magalhaes, V. F., M.M. Marinho, P. Domingos, A. C. Oliveira, S. M. Costa, L. O. Azevedo & S.M. F. O. Azevedo, 2003. Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brazil, RJ). *Toxicon* 42: 289–295.
- Marker, A. F., C. A. Crowther & R. J. M. Gunn, 1980. Methanol and acetone as solvents for estimating chlorophyll *a* and phaeopigments by spectrophotometry. *Achiv fuer Hydrobiologie supplement* 14: 52–59.
- Mueller-Solger, A. B., A. D. Jassby & D. C. Mueller-Navarra, 2002. Nutritional quality for zooplankton (*Daphnia*) in a tidal freshwater system (Sacramento-San Joaquin River Delta, USA). *Limnology and Oceanography* 47: 1468–1476.
- O'Brien, K. R., D. L. Meyer, A. M. Waite, G. N. Ivey & D. P. Hamilton, 2004. Disaggregation of *Microcystis aeruginosa* colonies under turbulent mixing: laboratory experiments in a grid-stirred tank. *Hydrobiologia* 519: 143–152.
- Paerl, H. W., 1988. Nuisance phytoplankton blooms in coastal, estuarine and inland waters. *Limnology and Oceanography* 33: 823–847.
- Park, H. & M. F. Watanabe, 1995. Toxic *Microcystis* in Eutrophic lakes. In M. F. Watanabe, K. Harada, W. W. Carmichael & H. Fujiki (eds), *Toxic Microcystis*. CRC Press, New York: 57–78.
- Reinikainen, M., M. Ketola & M. Walls, 1994. Effects of the concentrations of toxic *Microcystis aeruginosa* and an alternative food on the survival of *Daphnia pulex*. *Limnology and Oceanography* 39: 424–432.
- Reynolds, C. S., G. H. M. Jaworski, H. A. Cmiech & G. F. Leedale, 1981. On the annual cycle of the blue-green alga *Microcystis aeruginosa* Kutz. Emend. Elenkin. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences* 293: 419–477.
- Roberts, R. D. & T. Zohary, 1992. The influence of temperature and light on the upper limit of *Microcystis aeruginosa* production in a hypertrophic reservoir. *Journal of Plankton Research* 14: 235–247.
- Robson, B. J. & D. P. Hamilton, 2003. Summer flow event induces a cyanobacterial bloom in a seasonal Western Australia estuary. *Marine and Freshwater Research* 54: 139–151.
- SAS Institute, Inc., 2004. SAS/STAT User's Guide, Version 8. SAS Institute Inc., SAS Campus Drive, Cary, NC.
- Sellner, K. G., D. C. Brownlee, M. H. Bundy, S. G. Brownlee & K. R. Braun, 1993. Zooplankton grazing in a Potomac River cyanobacteria bloom. *Estuaries* 16: 859–872.
- Sivonen, K. & G. Jones, 1999. Cyanobacterial toxins. In I. Chorus & J. Bartram (eds), *Toxic Cyanobacteria in Water*, World Health Organization, London: 41–112.
- 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.
- Utermohl, H. 1958. Zur Vervollkommung der quantitativen Phytoplankton-methodik. *Mitteilungen Internationale Vereinigung fur Theoretische und Angewandtet Limnologie* 9: 1–38.
- Van der Westhuizen, A. J. & J. N. Eloff, 1985. Effect of temperature and light on the toxicity and growth of the blue-green alga *Microcystis aeruginosa* (UV-006). *Planta* 163: 55–59.
- Watanabe, M. M. 1995. Isolation, cultivation and classification of bloom-forming *Microcystis* in Japan. In M. F. Watanabe, K. Harada, W. W. Carmichael & H. Fujiki (eds), *Toxic Microcystis*. CRC Press, New York: 13–34.
- Watanabe, M. M., K. Kaya & N. Takamura, 1992. Fate of the toxic cyclic heptapeptides, the microcystins, from blooms of *Microcystis* (cyanobacteria) in a hypertrophic lake. *Journal of Phycology* 28: 761–775.
- World Health Organization, 1998. *Guidelines for Safe Recreational-Water Environments: Coastal and Freshwaters*. Draft for consultation. World Health Organization, Geneva.
- Yunes, J. S., P. S. Salomon, A. Matthiensen, K. A. Beattie, S. L. Raggett & G. A. Codd, 1996. Toxic blooms of cyanobacteria in the Patos Lagoon Estuary, Southern Brazil. *Journal of Aquatic Ecosystem Health* 5: 223–229.