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Seasonal Dynamics of Microcystins with Associated Biotic and Abiotic Parameters in Two Bays of Lake Taihu, the Third Largest Freshwater Lake in China

Y. Q. Liu · P. Xie · D. W. Zhang · Z. R. Wen

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The occurrence of heavy cyanobacterial blooms in eutrophic freshwater has been a worldwide problem (Carmichael 1992). Cyanobacteria are a nuisance in inland water bodies mainly because of bloom formation and toxin production (Chorus and Bartram 1999). Microcystins (MCs), which are secondary metabolites and are produced by some cyanobacteria (Carmichael 1992), are health hazard to livestock, wildlife and even humans (Carmichael 2001), because these cyclic heptapeptide are hepatotoxic by virtue of their accumulation in the liver via multispecific bile acid transporters (Eriksson et al. 1990) and inhibition of serine/threonine protein phosphatases 1 and 2A (Mackintosh et al. 1990).

Because of public health concerns, many scientists have tried to elucidate the factors controlling the toxicity of cyanobacteria. External nutrient concentrations are suggested as an important factor for different toxicity of water blooms (Rapala and Sivonen 1998). There have been extensive laboratory studies to evaluate effects of environmental factors on MC production, such as the incidence of light on MC biosynthesis (Hesse and Kohl 2001), the response of MC-producer strains at different temperatures (Sivonen and Jones 1999; María et al. 2003), and the production of MC under different nutrient limitation, mainly P and N restriction (Oh et al. 2000; Hesse and Kohl 2001; Long et al. 2001; Vézie et al. 2002; María et al. 2003). However, some of these results are contradictory. It is likely that concentrations of phosphorus and/or nitrogen act indirectly via an influence on growth rates. It is reported that MC concentrations are correlated with total and dissolved phosphorus (Kotak et al. 1995; Lahti et al. 1997), total nitrogen (Lahti et al. 1997), and NO_3-nitrogen (Vézie et al. 1997). On the other hand, negative correlations between MC concentration and NO_3-nitrogen (Kotak et al. 1995; Lahti et al. 1997) have been also reported.

The main purposes of this study were to describe seasonal dynamics of *M. aeruginosa* and to analyze the relationship between MC production and major biological and physical–chemical parameter in Lake Taihu. This study was conducted in two major bays with different nutrient levels. Such information is essential for us to develop a monitoring program of water quality in Lake Taihu.

Materials and Methods

Lake Taihu (30°56′–31°34′N, 119°54′–120°36′E) is situated on the ancient Yangtze River delta with a catchment of 36,500 km^2, and surrounded by most heavily industrialized cities with almost the highest population density in China. It is a large, shallow and unstratified lake with an area of 2,338 km^2 and mean depth of about 2 m. In recent years, with the rapid development of economy and intensive use of water resources, water pollution has become increasingly serious and the water quality of Lake Taihu is deteriorating rapidly (Pu et al. 1998a). Because of the considerable eutrophication, heavy cyanobacterial blooms often occur in warmer seasons over wider areas, sometimes

Y. Q. Liu · P. Xie (✉) · D. W. Zhang · Z. R. Wen
Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, People's Republic of China
e-mail: xieping@ihb.ac.in

Y. Q. Liu
e-mail: yaqliu@126.com

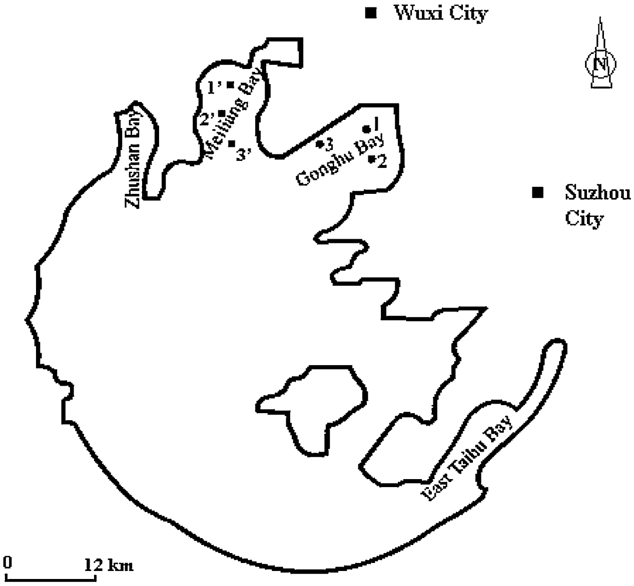


Fig. 1 Sketch map of Lake Taihu showing the sampling sites in Meiliang Bay and Gonghu Bay of Lake Taihu

covering an area of approximately 1,000 km^2 (Pu et al. 1998b). Meiliang Bay, one of the hypertrophic parts in Lake Taihu (Chen et al. 2001), services as the main water supply for Wuxi, an industrial city with a population of 1 million located approximately 2 km northeast of Lake Taihu. Gonghu Bay, a mesotrophic bay in the northeast part of the Lake Taihu, with 10–95% of its area covered with abundant submerged plants, flourishing from early April to late October every year (Fan et al. 2005). Three sampling sites were chosen in either bay (Fig. 1).

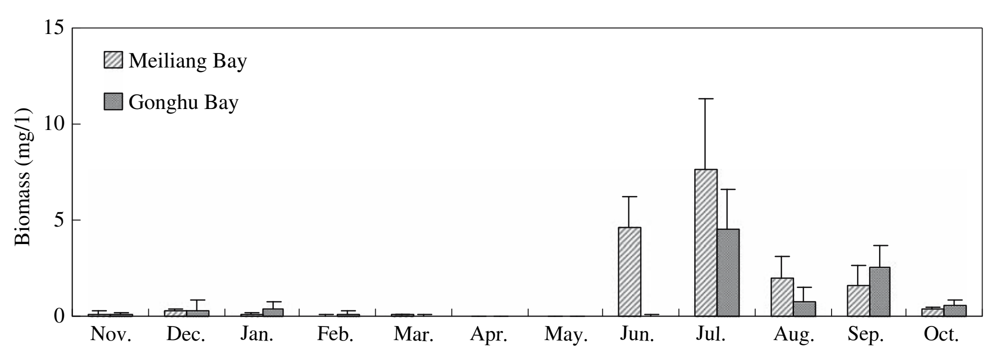
Integrated water was collected from the surface and near the bottom layers using a 5-L modified Patalas's bottle sampler. Water temperature, Secchi depth (SD) and water depth were measured in situ. Subsamples for phytoplankton were preserved with 1% acidified Lugol's iodine solution and concentrated to 30 ml after sedimentation for 48 h. After mixing, 0.1 ml concentrated samples were counted directly under 600× magnification. Colonial *Microcystis* spp. cell were separated using a high-speed blender (Ultra-Turrax) and counted. Taxonomic identification was processed according to Hu et al. (1979) and biomass was estimated from approximate geometric volumes of each taxon, assuming that 1 mm^3 equals 10^{-6} μg fresh weight (Shei et al. 1993).

In the laboratory, total phosphorus (TP) concentration was measured by colorimetry after digestion of the unfiltered samples with $K_2S_2O_8$ + NaOH to orthophosphate (Ebina et al. 1983). After digestion simultaneously with TP, total nitrogen (TN) was measured as nitrate and absorbance was measured at 220 nm. Ammonium (NH_4–N) was determined by the Nessler method and Nitrate (NO_3–N) was analyzed using the automated Korolev/Cadmium reduction method. Chlorophyll a was determined

Table 1 Annual mean values of major environmental parameters in Gonghu Bay and Meiliang Bay of Lake Taihu

	NH_4–N (mg/L)	NO_2–N (mg/L)	NO_3–N (mg/L)	TN (mg/L)	TDN (mg/L)	PO_4–P (μg/L)	TP (mg/L)	TDP (mg/L)	Chl-a (°C)	WT (°C)	SD (cm)	WD (m)
Gonghu bay												
Lower	0.07	0.01	0.24	0.4	0.17	0.41	0.01	0.01	5.05	3.7	34	1.68
Upper	1.42	0.07	2.56	4.61	3.99	3.38	0.25	0.05	47.56	32.0	95	2.45
Mean	0.54	0.02	1.26	2.50	1.87	1.20	0.09	0.03	19.63	18.1	58	1.97
Meiliang Bay												
Lower	0.21	0	0.47	1.4	0.74	0.87	0.03	0.02	3.20	3.1	12	1.97
Upper	3.87	0.08	2.86	7.26	6.43	7.01	0.42	0.09	89.50	32.2	58	2.73
Mean	1.61	0.01	1.79	4.01	3.40	2.83	0.19	0.05	33.35	18.9	30	2.27
Sig. (2-tailed)	0.000	0.094	0.000	0.000	0.001	0.000	0.000	0.000	0.070	0.214	0.001	0.000

Fig. 2 Seasonal changes in biomass of *Microcystis* in Gonghu Bay and Meiliang Bay of Lake Taihu (data are mean±SD)



by a spectrophotometer (Lorenzen 1967) after filtration on the glass–fiber filter (GF/C, Whatman, UK) and 24 h extraction in 90% acetone (Chemical sources and purity: Tianjin Bodi Chemicals Co., Ltd., China; ≥99.5%)

MCs were measured as intra- and extracellular MCs according to the methods of Zheng et al. (2004). Both intra- and extracellular MCs were determined by a reverse-phase highperformance liquid chromatography (HPLC) equipped with an ODS column (Cosmosil 5C18-AR, 4.6 × 150 mm, Nacalai, Japan) and a SPD-20A UV–vis spectrophotometer set at 238 nm. MC concentrations were determined by comparing the peak areas of the test samples with those of the standards available (MC-LR, MC-RR and MC-YR, Wako Pure Chemical Industries-Japan, purity ≥95.0%). Average recoveries of water samples were 78% for MC-RR, 81% for MC-LR. The limit of detection for the MCs was 0.02 μg ml^{-1}.

Results and Discussion

The annual mean values of the major environmental parameters are showed in Table 1. All nutrient levels were higher in Meiliang Bay than in Gonghu Bay ($p < 0.05$, n = 36), except NO_2–N. The mean concentration of Chl-*a* was much higher in Meiliang Bay than in Gonghu Bay and according to the Chl-*a* criterion by OECD (1982), Meiliang Bay was classified as eutrophic and Gonghu Bay as mesotrophic.

During the survey period, we identified 71 phytoplankton genera belonging to eight phyla, among which Cyanaphyta and Chlorophyta comprised >90% of total numeric abundance. The dominant genera were Chlorophyta in May, June and July and in the rest time. *M. aeruginosa* was the absolute dominant species among Cyanaphyta and there was a significant difference in *Microcystis* biomass between the two bays ($p < 0.05$, n = 36) (Fig. 2). *Microcystis* biomass was negatively correlated with concentrations of various N forms (NH_4-N, NO_3-N, TN, TDN), but not with those of various P forms (PO_4-P, TP, TDP) (Table 2), indicating that *M. aeruginosa* bloom appeared to be more closely associated with N concentrations than P concentrations. However, previous studies indicate that *M. aeruginosa* was closely associated with TP (Downing et al. 2001). Perhaps P loading in Lake Taihu was at a saturation level to the growth of *M. aeruginosa*.

In the present study, the intracellular MC content, ranging from undetectable to 5.80 μg/l, was relatively higher in summer (from July to September) when heavy cyanobacterial blooms occurred. The mean concentration of intracellular MCs was much higher in Gonghu Bay than in Meiliang bay ($p < 0.01$) (Fig. 3), which indicating that there was no positive relation between MC production and

Table 2 Relationship between MCs concentration and phytoplankton biomass and limnological variables

	Microcystis	Phytoplankton	Chl-a	NH_4–N	NO_3–N	TN	TDN	PO_4–P	TP	TDP	WT	SD	WD
Gonghu Bay													
Microcystis	1	0.510**	0.476	−0.309	−0.495**	−0.408*	−0.367*	−0.127	−0.034	−0.096	0.465	−0.538	0.722*
Phytoplankton	0.510**	1	0.742**	−0.091	0.060	0.144	0.164	0.248	0.442**	0.209	0.600	−0.620	0.499
Intra-MC	0.843**	0.535**	0.630*	−0.308	−0.422*	−0.303	−0.344	0.025	0.160	0.049	0.655	−0.504	0.807*
Extra-MC	−0.037	−0.135	−0.424	−0.208	−0.127	−0.172	−0.129	−0.131	−0.133	−0.113	−0.248	0.406	−0.808*
Meiliang Bay													
Microcystis	1	0.615**	0.291	−0.437**	−0.482**	−0.365*	−0.349*	−0.129	−0.097	−0.025	0.630	−0.389	0.083
Phytoplankton	0.615**	1	0.829**	−0.158	−0.106	0.034	−0.067	−0.232	0.467**	−0.153	0.584	−0.472	−0.206
Intra-MC	0.462**	0.179	0.183	−0.408*	−0.541**	−0.371*	−0.319	0.110	−0.124	0.035	0.602	−0.505	0.660*
Extra-MC	0.008	0.035	−0.049	0.204	0.017	−0.310	−0.214	−0.217	−0.030	−0.182	−0.398	0.703*	0.052

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

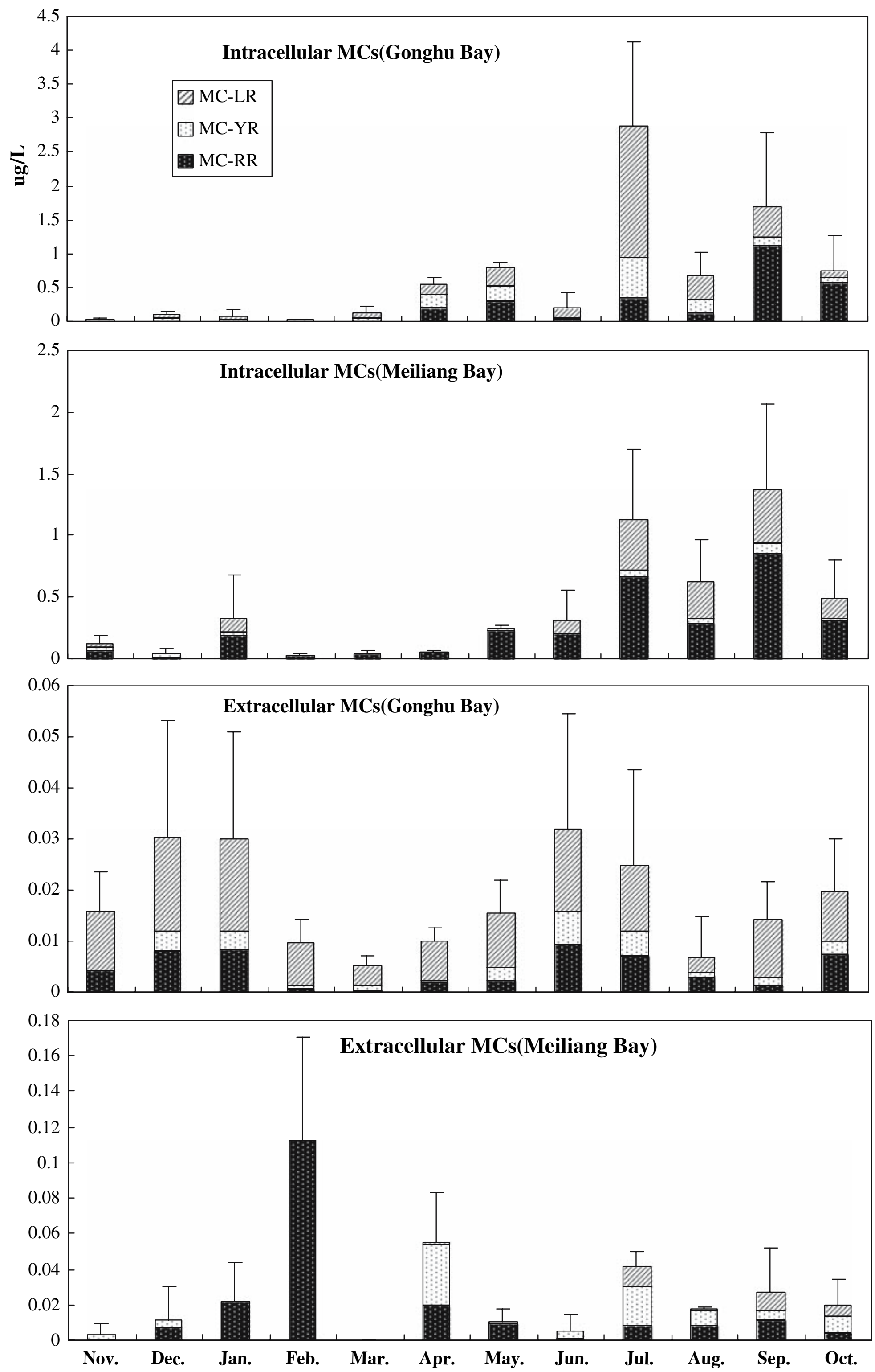


Fig. 3 Seasonal changes in the intra- and extra-cellular MC concentrations in the water column of Gonghu Bay and Meiliang Bay (data are mean±SD)

nutrient levels. Similar results were also found in Lake Ringsjön, southern Sweden (Gertrud et al. 1999). On the other hand, some studies indicate positive relation between trophic status and MC production (Vézie et al. 2002). In the present study, MC production was dependent on the growth of *Microcysts* because of the positive linear correlation between intracellular MC content and *Microcystis* biomass in both bays. The reason for the lower MC concentration in Meiliang Bay whereas *Microcystis* biomass was higher might be due to a lower ratio of toxic to nontoxic strains. Ohtake et al. (1989) suggest that MC production is closely associated with the ratio of toxic to nontoxic strains.

Intracellular MC concentrations showed a positive correlation with *Microcytis* biomass, and negative correlations with NH_4-N and NO_3–N concentrations ($p < 0.05$, n = 36) (Table 2). The negative linear correlations between MC and N concentrations indicate that N is a regulating factor for MC production in Lake Taihu. This disagrees with the view that high nitrogen level enhances MC production of *Microcystis* (Vézie et al. 2002; Downing et al. 2005). Previous studies about this were quite contradictory: in some studies, MC concentration was positively correlated with TN (Lahti et al. 1997) and NO_3–N (Vézie et al. 1997), but in other cases, it was negatively correlated with NO_3–N (Lahti et al. 1997). The negative linear correlation between the concentrations of intracellular MCs and NH_4–N in the present study coincides with the view that ammonium is toxic to *M. aeruginosa* (María, 2003, 2005). Zheng et al. (2004) also report that negative correlation was present between NH_4–N and intracellular MCs in a small eutrophic lake. In the present study, Perhaps N loading affected MC production indirectly through influencing *Microcystis* biomass, because of the significant correlation among N concentrations, *Microcystis* biomass and intracellular MC content.

In the present study, the toxins of the samples collected from Lake Taihu were identified as MC-LR, MC-RR and MC-YR, but the ratio of MC-LR to MC-RR was much higher in Gonghu Bay than that in Meiliang Bay ($p < 0.01$). It is reported that MC-RR/MC-LR ratio tends to be high in higher TP water but to be low in lower TP water (Yang et al. 2006). Previous study indicate invariable patterns of MCs and persisting patterns of MCs are probably due to a stable strain composition of field populations, while changes in MC composition of monospecific populations might be due to changes in the strain composition of this population (Fastner et al. 2001). In the present study, perhaps TDN and TDP favored certain *Microcystis* strains to produce MC-LR more than MC-RR because of the positive correlations between the ratio of MC-LR to MC-RR and the concentrations of TDN, TDP in Gonghu Bay (Table 2).

The World Health Organization (WHO 1998) has determined a provisional guideline value for MC of 1 μg/L MC-LR in drinking water. According to Gupta et al. (2003), the intraperitoneal (i.p.) medium lethal dose (LD50) in mice for MC-RR and -YR is as about 5- and 2.5-fold as that for MC-LR, respectively, then in the present study, 66.7% of the water samples were above the safety limit of 1 μg/l MC-LR required for drinking water during the warm seasons from July to September. As a drinking water source, MC pollution in this lake was very serious. The removal of particles, including cyanobacteria cells, from water resources is recommended to effectively reduce the risk of MCs, and the long-term impacts of MCs on aquatic ecosystem and public health cannot be overlooked.

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