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Factors Shaping the Pattern of Seasonal Variations of Microcystins in Lake Xingyun, a Subtropical Plateau Lake in China

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The occurrence of *Microcystis* blooms in eutrophic freshwater bodies has become a worldwide problem. Many strains of *Microcystis* are known to produce cyanobacterial heptatoxins called microcystins (MC). Laboratory studies indicate that production of microcystin by *Microcystis* is affected by various environmental factors such as light, temperature, nutrients, trace elements, salinity, pH and nutrient contents in cells (Gorham 1964; Van der Westhuizen and Eloff 1985; Watanabe and Oishi 1985; Oh et al. 2000; Lee et al. 2000; Long et al. 2001). Environmental variables are likely to influence MC concentration directly by influencing cellular microcystin production and content (Orr and Jones 1998; Long et al. 2001), and indirectly by influencing cyanobacterial species and strain composition (Chorus 2001; Vézie et al. 2002).

In recent years, field surveys on microcystins have been extensively carried out in different parts of the world. However, only a few studies addressed the significance of direct environmental influences on microcystin production by *Microcystis* (Wicks and Thiel 1990; Kotak et al. 1995, 2000; Jacoby et al. 2000). Such studies on cyanobacterial toxins in natural algal blooms are still lacking from subtropical China, especially in the Chinese plateau lakes. The purpose of this paper is to describe seasonal variations of intra- and extracellular microcystins in the subtropical plateau Lake Xingyun, and the effects of physicochemical and biological factors on microcystin dynamics. We also discuss the possible mechanisms underlying these variations.

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Materials and Methods

Lake Xingyun is a shallow freshwater lake, located at 1721 m above sea level in Yungui Plateau, in southwest China (Fig. 1). The surface area is 34 km^2 with a mean depth of 7 m, a maximum depth of 10 m. It supports a large fish industry used by local people, mainly based on silver carp, bighead carp, common carp and crucian carp. The annual yield of edible fish is over 1100 tons since 1990. The natural lake also connects to the south part of Fuxian Lake, which is oligotrophic, and China's second deepest lake, through a narrow Gehe River.

Eight sites (Fig. 1) were sampled in the middle of every month in Lake Xingyun from October 2002 to September 2003. Water temperature was measured by a WMY-01 digital thermometer. pH and conductivity were determined with PHB-4PH meter and DDB-303A, respectively. Dissolved oxygen (DO) was measured by a JPB-607 DO meter. Transparency was measured by a Secchi disk. The mixed water samples were collected from depths of 0 m, 4 m and 6 m for the analysis of total nitrogen (TN), total dissolved nitrogen (TDN), nitrate-nitrogen (NO_3–N), ammonium-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N), total phosphorus (TP), total dissolved phosphorus (TDP), phosphate-phosphorus (PO_4–P), chlorophyll *a* and phytoplankton biomass. All forms of nitrogen and phosphorus concentrations and phytoplankton biomass were determined according to the methods described by Zheng et al. (2004).

Algal cells from natural blooms were collected with a 64-μm-mesh size plankton net. The algal samples were

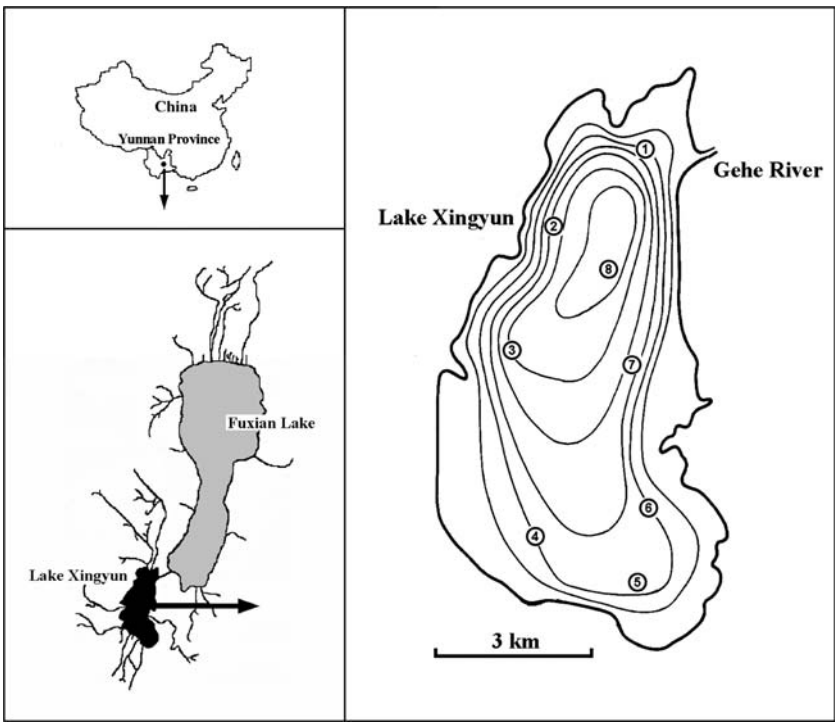

Fig. 1 Geographic location of Lake Xingyun and the sampling sites

immediately frozen at –25°C, and then freeze-dried for toxin analysis (MC content in algal blooms). Lake water (1 liter) was filtered with a filter (Waterman GF/C, UK) to separate toxins dissolved in water (dissolved MC) and toxins in particulate (MC in seston). Both freeze-dried algal cells (0.1 g) and filter papers were extracted with three washes of 30 ml methanol (75%)and the suspensions were centrifuged at 15,000 rpm (30 minutes at 4°C). The supernatant was diluted 1:5 with distilled water, and the filtrates diluted in the methanol were directly concentrated on SPE cartridges (10 ml, C18, 500 mg), which were previously activated with 10 ml methanol (100%) and distilled water. The cartridges were washed with 10 ml methanol (10%) and distilled water. Elution from the cartridges with 10 ml methanol (100%) was evaporated to dryness. The residue was dissolved with 100 µl distilled water and used for the final detection and identification of MCs by LC-MS. MC concentration was measured according to the methods described by Chen and Xie (2005).

Results and Discussion

Changes in the phytoplankton and *Microcystis* biomass were shown in Fig. 2.

Microcystis dominated the phytoplankton community (69–99%) throughout the year. The biomass of *Microcystis* increased dramatically up to 34.4 and 35.8 mg l^{-1} in June and July but remained relatively stable in other months. The chlorophyll *a* concentration (26–330 µg l^{-1}) showed a steady increase from April to July (Fig. 2).

Three MC analogues were identified, with MC-LR and MC-RR being main components. The variations of dissolved MC, MC in seston and algal blooms are shown in Fig. 3. Only dissolved MC-YR was not detected (under the limited detection of 0.002 µg l^{-1}) in October, November and December. The total MC concentration varied greatly during the sample period. MC concentration was at a low level (< 0.34 µg l^{-1}) but increased dramatically (> 1.47 µg l^{-1}) when water temperature was above 20°C, beginning in May. MC in seston composed 67–99% of the total MC.

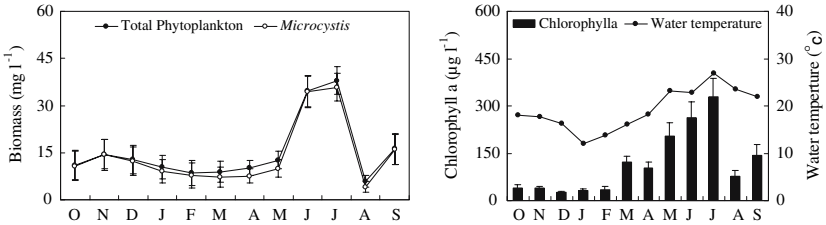

Fig. 2 Changes in biomass of total phytoplankton and *Microcystis*, chlorophyll *a* and water temperature in Lake Xingyun

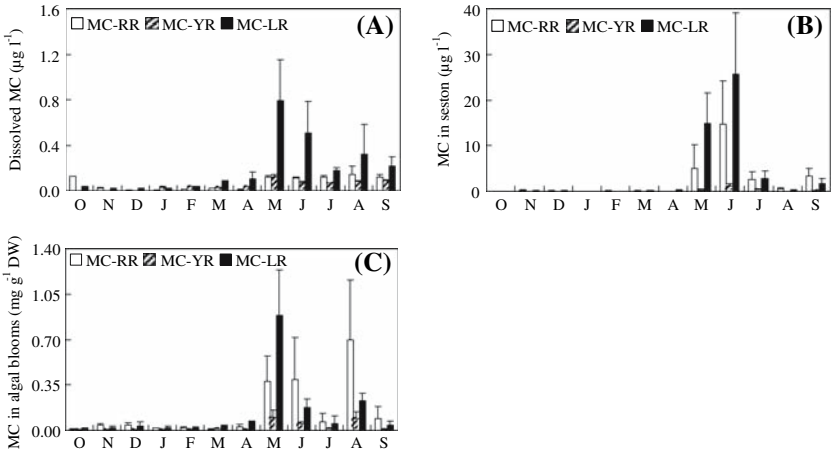

Fig. 3 Seasonal variations of **A** dissolved MC, **B** MC in seston and **C** algal blooms in Lake Xingyun.

The maxima of dissolved MC and MC in seston were 1.03 μg l^{-1} in May and 41.42 μg l^{-1} June, respectively. MC content in algal blooms was relatively high (> 0.61 mg g^{-1} DW) in May, June and August. Significant correlation was found between the total MC and the product of *Microcystis* biomass and MC in algal blooms ($y = 1.9101x - 1.4328$, $r = 0.984$, $P < 0.05$) (Fig. 4).

Table 1 showed the mean, minima and maxima of environmental parameters during the study period. The correlation coefficients between MC concentrations,and *Microcystis* biomass, and different environmental factors are listed in Table 2. The total MC concentration was positively correlated with conductivity, chlorophyll *a*, TN, TDN, NO_3–N, NO_2–N, TP and *Microcystis* biomass; and negatively correlated with transparency and DO. MC in algal blooms was positively correlated with TDN and NO_3–N; and negatively correlated with transparency and DO. The relationship between MC in algal blooms and water temperature is illustrated in Fig. 5. The maximum value of MC in algal blooms occurred at 23.2°C.

Although there were significant correlations between nutrient conditions (TN, TDN, NO_3–N, NO_2–N and TP) and the total MC, only TN and NO_3–N were positively correlated with MC in algal blooms, indicating that MC production by *Microcystis* in Lake Xingyun was regulated by nitrogen (N) concentration. In laboratory studies, Lee et al. (2000) reported that microcystin content of *Microcystis aeruginosa* exhibits a high correlation with total N concentration, regardless of an N-fixed and P-fixed culture. Similarly, the microcystin toxicity of *Microcystis aeruginosa* decreases dramatically when N decreases in the culture media (Watanabe and Oishi 1985; Codd and Poon 1988). Our data agree with laboratory studies, but are in conflict with field studies in natural *Microcystis* blooms. MC concentration in phytoplankton correlated positively with TN, NO_3–N and TP in 50 New Hampshire lakes (Haney and Ikawa 2001), with TP in eutrophic lakes in Alberta, Canada (Kotak et al. 2000), and with soluble reactive phosphorus in Steilacoom Lake (Jacoby et al. 2000); and correlated negatively with orthophosphate in Hartbeespoort Dam, South Africa (Wicks and Thiel 1990).

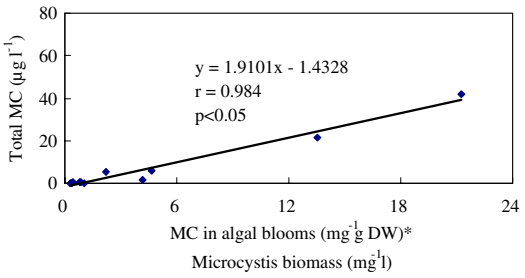

Fig 4 Relationship between total MC (dissolved MC + MC in seston) and the product of MC in algal blooms and *Microcystis* biomass (average value)

Table 1 Environmental parameters in Lake Xingyun from October 2002 to September 2003

Parameter	Unit	Mean	Min	Max
Water temperature	°C	19.2	12.1	26.9
Transparency	cm	99	43	241
DO	mg l^{-1}	9.7	7.3	11.5
pH	-	9.01	7.82	10.19
Conductivity	μs cm^{-1}	334	256	476
Chlorophyll *a*	μg l^{-1}	118	26	330
TN	mg l^{-1}	3.93	1.80	8.16
TDN	mg l^{-1}	1.89	0.76	2.70
NO_3–N	mg l^{-1}	0.52	0.39	0.66
NH_4–N	mg l^{-1}	0.07	0.05	0.10
NO_2–N	μg l^{-1}	7.13	4.19	12.93
TP	mg l^{-1}	0.31	0.12	0.60
TDP	mg l^{-1}	0.18	0.07	0.43
PO_4–P	mg l^{-1}	0.11	0.02	0.32

Table 2 Correlation analysis between MC concentrations and *Microcystis* biomass, and environmental variables ($N = 96$; significant correlations in bold, $P < 0.05$)

Variables	Dissolved MC μg l^{-1}	MC in seston μg l^{-1}	Total MC μg l^{-1}	*Microcystis* biomass mg l^{-1}	MC in algal blooms μg l^{-1}
Transparency	**–0.29**	**–0.38**	**–0.38**	**–0.38**	**–0.27**
DO	**–0.38**	**–0.31**	**–0.31**	**–0.43**	**–0.29**
pH	0.14	-0.01	0.00	0.04	0.16
Conductivity	**0.29**	**0.43**	**0.43**	**0.33**	0.02
Chlorophyll *a*	0.15	**0.47**	**0.36**	**0.59**	0.10
TN	0.15	**0.45**	**0.31**	**0.42**	0.19
TDN	**0.34**	**0.25**	**0.25**	0.16	**0.28**
NO_3–N	**0.49**	**0.50**	**0.48**	**0.26**	**0.38**
NH_4–N	–0.22	–0.18	–0.18	0.16	0.10
NO_2–N	0.00	**0.34**	**0.33**	**0.33**	0.18
TP	0.15	**0.28**	**0.23**	**0.31**	0.05
TDP	0.09	0.09	0.10	0.22	-0.06
PO_4–P	0.08	0.06	0.06	0.20	-0.08
Microcystis	0.27	**0.64**	**0.63**		–0.04

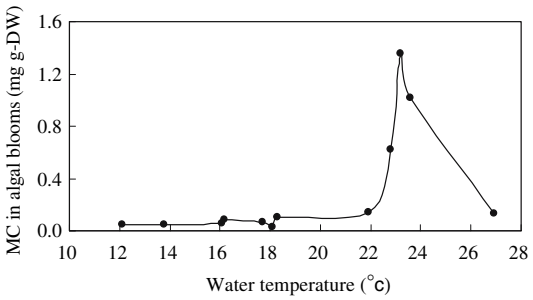

Fig. 5 Relationship between MC content in algal blooms and water temperature (average value)

Contradictory data may be due to different responses of *Microcystis* strains to various environmental factors.

It has been reported that the increase in MC production may be associated with, or related to, high water temperature in Lake Taihu, China (Shen et al. 2003) and Laguna de Bay, the Philippines (Baldia et al. 2003). In our study, there was no significant correlation between water temperature and MC in algal blooms. Relatively high MC concentration in algal blooms (0.13–1.36 mg g^{-1}) was found from May to September, and the maximum value occurred at 23.2°C. The optimal temperature for MC production by *Microcystis aeruginosa* is between 20 and 25°C in previous laboratory studies (Gorham 1964; Van der Westhuizen and Eloff 1985; Watanabe and Oishi 1985). Our results suggest that the optimal water temperature of about 23°C and the abundant nutrients might have jointly contributed to the increased MC production.

No significant correlation was present between MC in algal blooms and *Microscystis* biomass. A similar result was also reported in Steilacoom Lake (Jacoby et al. 2000). According to Sivonen and Jones (1999), the times of maximum MC and maximum biomass are not necessarily coincident. Thus, variation in MC production is independent of the changes in *Microcystis* biomass. The present study is the first to find a significant linear relationship between total MC, and the product of MC in algal blooms and *Microcystis* biomass. These results support the hypothesis proposed by Kotak et al. (2000) that the concentration of MC is probably regulated by both the biomass of toxin-producing phytoplankton in the water column and the concentration of MC produced per unit mass of toxin-producing phytoplankton, and indicate that factors influencing MC production are not necessarily the same with those influencing the biomass of toxin-producing phytoplankton.

In our study, the maximum MC concentration reached as high as 42.12 μg l^{-1} during the warm season and was considerably higher than those of *Microcystis* blooms in other regions of the world (Kotak et al. 1995, 2000; Lahti et al. 1997; Haney and Ikawa 2001). The concentration we measured greatly exceeded the safety limit for drinking (1 μg l^{-1}) and recreation water (20 μg l^{-1}) provided by the WHO. It should be noted, however, that Lake Xingyun produces over 1100 tons of edible fish annually, and is also connected to the south part of Fuxian Lake, which is oligotrophic and the second deepest lake in China. The toxins could have serious ecological impacts on the aquatic food webs of these two lakes. Both monitoring of MC pollution in Lake Xingyun and reduction of the exposure risk of cyanobacterial toxins to both aquatic animals and human beings are urgently needed.

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