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Relationships between microcystins and environmental parameters in 30 subtropical shallow lakes along the Yangtze River, China

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SUMMARY

1. A survey of 30 subtropical shallow lakes in the middle and lower reaches of the Yangtze River area in China was conducted during July–September in 2003–2004 to study how environmental and biological variables were associated with the concentration of the cyanobacterial toxin microcystin (MC).
2. Mean MC concentration in seasonally river-connected lakes (SL) was nearly 33 times that in permanently river-connected lakes (RL), and more than six times that in city lakes (NC) and non-urban lakes (NE) which were not connected to the Yangtze River. The highest MC (8.574 μg L^{-1}) was detected in Dianshan Lake.
3. MC-RR and MC-LR were the primary toxin variants in our data. MC-RR, MC-YR and MC-LR were significantly correlated with Chl *a*, biomass of cyanobacteria, *Microcystis* and *Anabaena*, indicating that microcystins were mainly produced by *Microcystis* and *Anabaena* sp. in these lakes.
4. Nonlinear interval maxima regression indicated that the relationships of Secchi depth, total nitrogen (TN) : total phosphorus (TP) and NH_4^+ with MC were characterised by negative exponential curves. The relationships between MC and TN, TP, NO_3^- + NO_2^- were fitted well with a unimodal curve.
5. Multivariate analyses by principal component and classifying analysis indicated that MC was mainly affected by *Microcystis* among the biological factors, and was closely related with temperature among physicochemical factors.

Keywords: cyanobacteria, LC-MS, microcystin, shallow lakes, Yangtze River

Introduction

Cyanobacteria are the most widespread and problematic freshwater nuisance algal taxa (Paerl *et al.*, 2001). In addition to their unsightly appearance and bad smell, some cyanobacteria produce potent microcystin toxins (MC) that pose a hazard to human health and have been linked to an increased incidence of liver cancer (Fujiki & Suganuma, 1993) or even deaths (Dunn, 1996). Several genera of cyanobacteria, including *Microcystis*, *Anabaena*, *Oscillatoria*, *Nostoc* and *Planktothrix*, produce microcystins (Meriluoto *et al.*, 1989; Namikoshi *et al.*, 1990; Harada *et al.*, 1991; Kotak *et al.*, 1993, 1995; Robillot *et al.*, 2000; Briand *et al.*, 2002).

Two biotic factors affect MC concentration in the field: cellular microcystin production and content (Orr & Jones, 1998; Long, Jones & Orr, 2001), and the species composition of cyanobacteria in lakes (Chorus *et al.*, 2001; Vézie *et al.*, 2002). Environmental conditions also indirectly influence microcystins through their effects on these two factors. Numerous laboratory studies

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and investigations in specific lakes have examined the effects of various environmental factors (e.g. nutrients, light, temperature) on MC production (Van der Westhuizen & Eloff, 1985; Watanabe & Oishi, 1985; Utkilen & Gjølme, 1992; Jacoby *et al*., 2000; Sekadende, Lyimo & Kurmayer, 2005). Regional studies on the interactions between microcystins and physicochemical variables have been conducted in Canada (Kotak *et al*., 2000), America (Graham *et al*., 2004) and Spain (Aboal & Puig, 2005), but such studies have been lacking in Asia, although in lake water generally more eutrophic and toxic cyanobacteria are more common (Jin, 2003). Many of the numerous shallow lakes in the middle and lower reaches of the Yangtze River area in subtropical China, and many of these lakes have cyanobacterial blooms in the warm season because of high nutrient levels and high water temperature. Contamination of lake water by microcystins is becoming a serious concern in this region.

We investigated 30 lakes in the middle and lower reaches of the Yangtze River area during 2003–2004. The main purposes were: (1) to determine concentrations and distributions of different microcystins; (2) to explore relationships between MC concentration and both environmental variables and the dominant cyanobacteria and (3) to propose possible mechanisms underlying MC production in these subtropical lakes.

Methods

Study area

The 30 shallow lakes are located in five provinces (Hubei, Hunan, Jiangxi, Anhui and Jiangsu) in the middle and lower reaches of the Yangtze River (Fig. 1), and most of these lakes are eutrophic or hypereutrophic. Two to ten sites were sampled in each lake based on environmental heterogeneity and surface area of the lakes, and the positions were recorded by a GPS system. Each lake was sampled twice (in summer and winter), and it took 2 years (2003 and 2004) to complete the whole sampling because of a wide geological distribution of these lakes. Only the summer (from July to September) samples were used for microcystin analysis because there were no cyanobacterial blooms in the winter in our study lakes.

The study lakes were divided into four types (Table 1): river-connected lakes (RL) which were connected to the Yangtze River for the whole year; lakes connected to the river only seasonally (SL) and city lakes (NC) and non-urban lakes (NE) which were not connected to the river at all.

Collection and analysis of samples

Water samples at each site were collected with Tygon tubing fitted with a one-way valve. For analysis of total nitrogen (TN), total phosphorus (TP), NO_3^-, NO_2^- and chlorophyll *a* (Chl *a*), a composite sample was taken from the surface (0–1 m), middle and bottom layers at each site. Samples were kept dark in a refrigerator before laboratory analysis. The lake water was filtered through a membrane filter (0.45-μm-pore-diameter) for analysis of NO_3^-, NH_4^+ and NO_2^-. TN was determined by the alkaline potassium persulphate digestion-UV spectrophotometric method (Nydahl, 1978). TP was determined by the ammonium

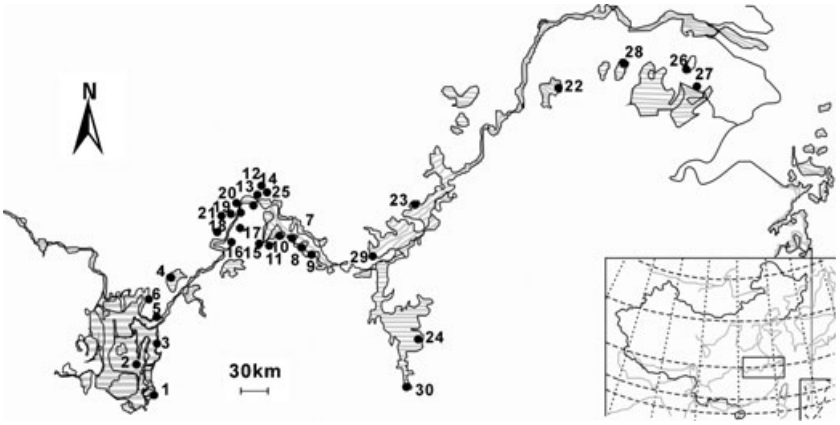

Fig. 1 Geographic location of the lakes surveyed. The number (1–30) represents the code number for each lake (see Table 1).

Table 1 Limnological characteristics and mean MC values for the 30 shallow lakes in the Yangtze River area

Lake name	Lake code	Sampling date	Longitude E	Latitude N	Area (km^2)	Mean depth (m)	Secchi depth (cm)	pH	Temp. (°C)	TN (mg L^{-1})	$NO_3^- + NO_2^-$ (mg L^{-1})	NH_4^+ (mg L^{-1})	TP (mg L^{-1})	MC (μg L^{-1})
RL														
South Dongting	1	20.06.2003	E112°47′	N28°45′	917.1	2.5	106	7.7	26.3	0.65	0.40	0.01	0.02	0.013
West Dongting	2	19.06.2003	E112°10′	N28°50′	520.5	4.8	51	7.9	22.3	0.74	0.61	0.03	0.10	0.048
East Dongting	3	01.08.2004	E113°02′	N29°15′	1478.2	6.1	83	8.4	28.8	0.79	0.86	0.14	0.05	0.048
Poyang	24	03.07.2004	E115°58′	N29°16′	3583.7	2.5	133	6.4	30.8	1.21	0.45	0.19	0.02	0.003
SL														
Honghu	4	01.09.2003	E113°22′	N29°49′	348.4	1.9	58	8.0	30.5	1.69	0.39	0.09	0.08	0.852
Laojianghe	5	04.07.2003	E113°00′	N29°33′	18.4	4.9	52	7.7	28.5	1.07	0.79	0.18	0.08	0.118
Tianezhou	6	03.07.2003	E112°33′	N29°50′	14.5	7.6	108	7.8	28.5	0.70	0.96	0.14	0.02	0.961
Shijiu	22	28.08.2004	E118°53′	N31°30′	210.4	2.3	113	7.8	26.0	0.69	0.20	0.49	0.07	0.599
Yangcheng	26	16.07.2004	E120°49′	N31°25′	119.1	1.6	78	8.3	30.0	0.63	0.43	0.13	0.09	0.897
Dianshan	27	07.08.2004	E120°58′	N31°10′	63.7	2.2	33	8.5	30.5	1.52	1.53	0.76	0.25	1.759
Longgan	29	07.08.2004	E116°10′	N29°57′	316.2	2.1	67	7.8	30.7	0.52	0.40	0.10	0.03	0.035
Junshan	30	28.08.2004	E116°18′	N28°37′	192.5	3.7	221	7.5	30.5	0.62	0.13	0.02	0.05	0.423
NC														
Hongxing	8	26.08.2003	E114°58′	N30°05′	1.0	2.7	55	7.6	29.0	3.39	1.91	0.14	0.25	0.022
Sanliqi	9	26.08.2003	E114°57′	N30°05′	2.7	2.0	50	7.9	30.0	2.57	1.46	0.42	0.18	0.024
Nanhu	17	16.07.2003	E114°20′	N30°30′	8.0	2.4	41	8.5	28.5	3.83	1.59	0.51	0.33	0.553
Houguan	18	13.08.2003	E114°06′	N30°29′	10.1	2.8	107	8.0	25.5	0.99	0.23	0.10	0.06	0.104
Longyang	19	15.08.2003	E114°10′	N30°33′	1.4	1.5	30	7.8	27.0	8.67	1.54	3.19	0.96	0.098
Moshui	20	19.08.2003	E114°13′	N30°32′	2.7	2.1	38	7.9	28.5	5.26	1.53	2.06	1.45	0.022
Sanjiao	21	15.08.2003	E114°09′	N30°31′	2.5	1.5	23	8.1	25.0	8.23	2.76	3.73	0.33	0.051
NE														
Huama	7	11.08.2003	E115°02′	N30°20′	4.7	2.2	53	8.0	30.5	1.20	0.31	0.17	0.06	0.020
Qiaodun	10	26.08.2003	E114°41′	N30°15′	8.0	2.6	138	7.9	33.0	0.59	0.25	0.08	0.04	0.736
Bao'an	11	24.07.2003	E114°43′	N30°12′	3.6	2.6	48	7.9	25.1	0.92	0.29	0.16	0.09	0.115
Qihu	12	28.08.2003	E114°38′	N30°36′	1.0	1.3	103	7.1	30.4	1.31	0.43	0.20	0.08	0.015
Taojiada	13	30.08.2003	E114°36′	N30°37′	1.5	1.7	75	7.5	30.5	0.79	0.33	0.38	0.07	0.008
Zhangdu	14	28.08.2003	E114°41′	N30°39′	41.1	1.7	162	7.7	30.5	1.00	0.39	0.08	0.05	0.001
Niushan	15	21.07.2003	E114°30′	N30°20′	38.2	3.9	382	7.5	28.5	0.66	0.24	0.15	0.04	0.178
Qingling	16	20.08.2003	E114°14′	N30°26′	2.1	1.3	30	8.1	25.5	2.51	0.46	0.43	0.23	0.118
Wuchang	23	20.06.2004	E116°40′	N30°16′	100.5	2.1	109	7.9	26.2	0.48	0.19	0.14	0.06	0.062
Daoshui	25	30.08.2003	E114°37′	N30°38′	n.d	2.9	96	n.d	28.5	0.85	0.52	0.07	0.07	0.055
Gehu	28	21.07.2004	E119°49′	N31°40′	146.5	1.3	35	n.d	32.1	1.22	0.59	0.41	0.25	0.084

Area, lake area; Temp., water temperature; n.d, no data.

molybdate method after potassium persulphate digestion (Prepas & Rigler, 1982). Ammonium (NH_4^+-N) was measured by the Nessler method, nitrite by the α-naphthylamine method and nitrate by the UV spectrophotometry method (Eaton *et al*., 1995). Chl *a* was measured spectrophotometrically (Zhang & Huang, 1991). Mean water temperature was measured *in situ* using a thermometer and Secchi depth was recorded at one central site.

Phytoplankton in the composite sample were preserved in Lugol's solution. Phytoplankton species were identified according to Prescott (1978) and enumerated with a microscope equipped with a calibrated micrometer (Zhang & Huang, 1991).

For cyanobacterial toxin analysis, 1 L of the composite lake water was filtered through a glass fibre filter (Whatman GF/C, Maidstone, U.K.) using a vacuum pump. The filters were frozen immediately, and after return to the laboratory were extracted thrice in 75% methanol. The supernatant was diluted 1 : 5 with distilled water and MCs were directly concentrated on solid phase extraction cartridges (10 mL, C18, 500 mg), which were previously activated with 10 mL of methanol (100%) followed by 10 mL distilled water. The cartridges were then washed with 10 mL of 10% methanol followed by 10 mL distilled water. Microcystins were eluted from the cartridges with 10 mL 100% methanol and then evaporated to dryness. The residue was dissolved in 100 μL distilled water and used for the final detection and identification of MCs by LC-MS.

Microcystin concentration was measured using a Finnigan LC-MS system (Thermo Electron Corporation, San Jose, U.S.A.) consisting of a thermo surveyor auto sampler, a surveyor MS pump, a surveyor PDA system, and a Finnigan LCQ-Advantage MAX ion trap mass spectrometer equipped with an atmospheric pressure ionisation fitted with an electro spray ionisation source. The sample was separated on a Hypersil GOLD 5 μm column (2.1 mm i.d. × 150 mm) with a linear gradient run of acetonitrile (30–100%) and acidified water. Both water and acetonitrile were acidified with 0.05% formic acid. Data acquisition was in the positive ionisation centroid mode with full mass mode at a mass range between 400 and 1400. MS tuning and optimisation were achieved by infusing microcystin-RR and monitoring the $[M + 2H]^{2+}$ ion at m/z 520 (Chen & Xie, 2005). Microcystins in cyanobacterial extracts were identified against standards of MC-RR, MC-LR and MC-YR.

Statistical analyses

To examine the correlations between MC concentrations and cyanobacterial biomass and limnological variables, we used data from all sites in the 30 lakes. STATISTIC for Windows statistical software (version 6.0) was used for all analyses. To characterise the relationships between MC and environmental variables, we used interval maxima regression (IMR) and principal component and classifying analysis (PCCA).

Bivariate plots of the relationships between particulate MC and environmental variables showed nonlinear trends. Based on the descriptions by Graham *et al.* (2004), each variable was divided into equal increments, resulting in 10–16 intervals. The maximum MC values were obtained from each interval and used in nonlinear regression analysis. Curve fitting employing a least-square method was used to fit equations and to estimate coefficients. IMR relationships were considered to be significant at $\alpha = 0.05$ (Graham *et al*., 2004).

Principal component and classifying analysis was used to perform multivariate analyses. PCCA removes the redundancy from the original data set so that only the first few principal-components scores are required to describe most of the information contained in the original data set. PCCA transforms a number of (possibly) correlated variables into a smaller number of uncorrelated principal-components. The first principal-component accounts for the most variability in the data and each accessorial component axis accounts for as much of the remaining as possible. Original environmental and biological data were used to fit active variables and microcystin data for supplemental variables.

Results

Some basic limnological characteristics of the 30 lakes are shown in Table 1. Most lakes (80%) were very shallow (<3 m). Based on TP level, most of the sampling sites (80%) were of eutrophic and hypereutrophic status (Wu *et al*., 2006). According to the TN : TP ratio criteria of Forsberg & Ryding (1980), 33% sites were potentially P-limited (TN : TP > 17),

36% were co-limited (17 > TN : TP > 10) by N and P, and 31% were N-limited (TN : TP < 10).

The mean nutrient concentrations were much higher in the city lakes (NC) than in other lakes; for example, there was a difference of over four times for TN and over five times for TP. The mean nutrient concentrations were lowest (TP 0.05 mg L^{-1} and TN 0.85 mg L^{-1}) in the river-connected lakes (RL).

Examination of the community structure of cyanobacteria in these lakes revealed five genera that have the potential to produce MC: *Anabaena*, *Aphanocapsa*, *Microcystis*, *Nostoc* and *Oscillatoria* (Paerl *et al*., 2001). Cyanobacterial biomass was dominated by MC-producing genera (67% sites), with *Microcystis* ranking the first (37%), followed by *Anabaena* (14%), *Oscillatoria* (13%) and *Aphanocapsa* (3%), whereas *Nostoc* was rare.

Microcystin was detected at 176 sites (97% of sites), although MC concentrations were low in some sites (Table 1). The maximum mean MC value (1.759 μg L^{-1}) was in Dianshan Lake and the minimum in Zhangdu Lake (0.001 μg L^{-1}).

Mean total MC concentrations were 0.579 μg L^{-1} (MC-RR 0.390 μg L^{-1}, MC-YR 0.049 μg L^{-1} and MC-LR 0.347 μg L^{-1}) in seasonally river-connected lakes (SL), nearly 33 times (MC-RR 34 times, MC-YR 52 times and MC-LR 30 times) those in the river-connected lake (RL), and more than six times those in city lakes (NC) or non-urban lakes (NE) (Fig. 2).

There were strong correlations between MC-RR, MC-YR, MC-LR and total MC, and MC-LR had the strongest relationship with total MC ($r = 0.98$, $P < 0.001$). MC-RR, MC-YR and MC-LR all showed significant correlations with Chl *a*, and with biomass of cyanobacteria, *Microcystis* and *Anabaena* (Table 2), suggesting that these two cyanobacterial taxa are the principal producers of microcystin. NO_3^- showed significant correlation with MC-RR and MC-LR, whereas NH_4^+ showed significant correlation with MC-YR and MC-LR. On the other hand, biomass of *Microcystis* and *Anabaena* showed significant correlations with Chl *a*, biomass of cyanobacteria, COD and TP.

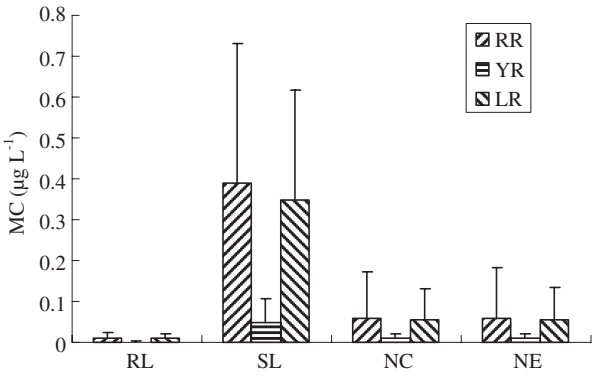

Fig. 2 Distributions of MC-RR, MC-YR and MC-LR (means with positive standard deviations) in the middle and lower reaches of the Yangtze River. RL, river-connected lakes; SL, seasonal river-connected lakes; NC, non-connected city lakes; NE, non-connected non-urban lakes.

The MC (-RR, -YR and -LR) maxima in relation to TP were characterised by a unimodal curve (RR, $r = 0.728$, $P < 0.01$; YR, $r = 0.811$, $P < 0.05$; LR, $r = 0.920$, $P < 0.01$; Table 3, Fig. 3). Along the TP gradient, the greatest MC values occurred between 0.05 and 0.25 mg L^{-1} (Fig. 3). The MC (RR, YR and LR)–TN : TP maxima were fitted with a negative exponential (RR, $r = 0.991$, $P < 0.01$; YR, $r = 0.984$, $P < 0.01$; LR, $r = 0.770$, $P < 0.01$), and the maximum MC (-RR, -YR and -LR) values occurred when TN : TP < 20 (Fig. 4).

Interval maxima regression analysis was also performed using *Microcystis* biomass and cyanobacterial biomass (Figs 3 & 4, Table 3). *Microcystis* biomass–TN, *Microcystis* biomass–TP, *Microcystis* biomass–(NO_3^- + NO_2^-), *Microcystis* biomass–NH_4^+ and *Microcystis* biomass–TN : TP relationships were strikingly similar to *Microcystis* biomass–MC (RR, YR and LR) relationships. This indicates that the three MC variants showed similar change with chemical factors and cyanobacterial biomass (Figs 3 & 4, Table 3).

Also, other bivariate plots between MC (-RR, -YR and -LR), *Microcystis* biomass, cyanobacterial biomass and environmental variables had maxima described by either negative exponential relationships or unimodal curves (Table 3). For example, the Secchi depth and NH_4^+ relationship was characterised by negative exponential curves. The relationships between MC and TN, NO_3^- + NO_2^- could be fitted with a unimodal curve. Peak values of MC and *Microcystis* biomass occurred when Secchi depth <50 cm, NH_4^+ <0.5 mg L^{-1} and within a TN range of 0.25–1.0 mg L^{-1} (Table 3).

With the PCCA, more than 60% of the environmental and biological variation in the data was explained by Component Axis 1 (36.79%) and Component Axis 2 (23.38%). Component Axis 1 had high positive weighting for Secchi and water depth, but

Table 2 Correlations between MC (-RR, -YR and -LR) concentrations (μg L^{-1}), *Microcystis* and *Anabaena* biomass (mg L^{-1}) and limnological variables based on log-transformed combined data

	RR			*YR*			*LR*			*MC*			*Microcystis*			*Anabaena*		
Variable	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*
Chla	0.24	**	153	0.40	***	138	0.26	**	158	0.26	***	172	0.31	**	103	0.35	**	75
Cyanobacteria	0.34	***	135	0.42	***	125	0.38	***	136	0.41	***	150	0.74	***	106	0.64	***	75
Microcystis	0.29	**	92	0.43	***	87	0.36	***	95	0.31	**	100						
Anabaena	0.41	**	64	0.45	***	62	0.42	***	68	0.40	***	71	0.41	**	53			
Synechocystis	0.29	*	54	0.28	*	52	0.24	ns	54	0.19	ns	59	0.41	**	51	0.60	**	20
Oscillatoria	–0.13	ns	42	–0.08	ns	35	0.10	ns	42	–0.01	ns	46	0.11	ns	37	0.51	*	23
Aphanocapsa	0.27	ns	32	0.39	*	29	0.29	ns	33	0.32	ns	35	0.26	ns	28	0.52	*	17
Water depth	–0.02	ns	157	–0.07	ns	142	0.01	ns	162	0.01	ns	176	–0.03	ns	106	–0.47	***	75
Secchi depth	0.02	ns	157	–0.10	ns	142	–0.06	ns	162	–0.03	ns	176	–0.05	ns	106	–0.44	***	75
Water temperature	0.06	ns	157	0.19	*	142	0.09	ns	162	0.10	ns	176	–0.06	ns	106	0.05	ns	75
COD	0.15	ns	157	0.29	***	142	0.20	**	162	0.17	*	176	0.26	**	106	0.32	**	75
NO_3^-	–0.32	***	157	–0.08	ns	142	–0.22	**	162	–0.29	***	176	0.12	ns	106	0.08	ns	75
NH_4^+	0.06	ns	157	0.20	*	142	0.18	*	162	0.15	*	176	0.07	ns	106	0.03	ns	75
NO_2^-	–0.17	*	157	–0.03	ns	142	–0.06	ns	162	–0.09	ns	176	–0.07	ns	106	–0.19	ns	75
TP	0.02	ns	157	0.24	**	142	0.09	ns	162	0.04	ns	176	0.29	**	106	0.39	***	75
TN	0.01	ns	157	0.11	ns	142	0.08	ns	162	0.02	ns	176	0.09	ns	106	0.14	ns	75

*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, ns, not significant ($P > 0.05$).

Table 3 Relationships between *Microcystis* or cyanobacterial biomass and several important growth factors

	RR		YR		LR		*Microcystis*		Cyanobacteria		
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	High value in
TP (UC)	182	0.73*	182	0.81**	182	0.92**	144	0.90**	177	0.81**	0.05–0.25 mg L^{-1}
TN (UC)	182	0.78**	182	0.68**	182	0.85**	144	0.79**	177	0.60	0.25–1.0 mg L^{-1}
$NO_3^- + NO_2^-$ (UC)	182	0.98**	182	0.95**	182	0.80**	144	0.94**	177	0.95**	0.2–0.7 mg L^{-1}
Secchi (NE)	182	0.99**	182	0.99**	182	0.85**	143	0.80**	176	0.58	<50 cm
NH_4^+ (NE)	181	0.99**	181	0.96**	181	0.99**	143	0.88**	176	0.90**	<0.5 mg L^{-1}
TN : TP (NE)	182	0.99**	182	0.98**	182	0.77**	144	0.65*	177	0.96**	<20

*$P < 0.05$, **$P < 0.01$.
UC, mean unimodal curves relationships; NE, mean negative exponential relationships; TN, total nitrogen; TP, total phosphorus.

negative weighting for water temperature and for some chemical and biological variables (such as N, P, *Microcystis*, *Anabaena*) (Fig. 5). Component Axis 2 had high positive weighting for most biological variables and water temperature, but negative weighting for other environmental variables (Fig. 5). MC-RR and MC-LR had high negative correlations with factor 1 and MC-YR had positive correlations with factor 2.

Discussion

In recent years, more than 70 structural variants of microcystins have been isolated and characterised from cyanobacterial blooms and cultures (Sivonen & Jones, 1999). The present study indicates that MC-LR and MC-RR were the primary microcystins in the lakes of the Yangtze River area. Pronounced prevalence of these variants is often reported to be characteristic of *Microcystis*-dominated blooms, although the variants do not always concur and their relative shares are variable (Shen *et al.*, 2003). In the present study, *Microcystis* and *Anabaena* were the main cyanobacteria in the Yangtze River, which is in accordance with the results of regional studies in American and Finnish lakes (Sivonen *et al.*, 1990; Yoo *et al.*, 1995).

In the present study, there was a distinct difference in MC concentration among the different lake types.

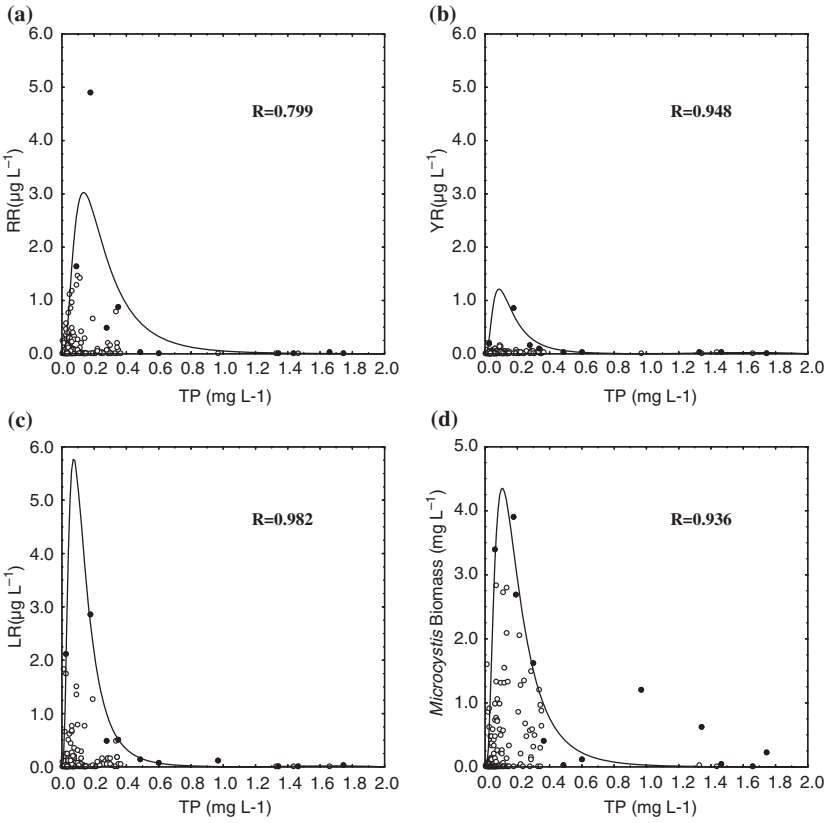

Fig. 3 Relationship to TP for microcystin (MC-RR, MC-YR and MC-LR) concentration and *Microcystis* biomass. Curves were estimated using interval maxima regression (IMR). Black points indicate the data used for IMR analysis; *r* values are for this fitted line only. (a) MC-RR-TP relationship ($n = 182$, IMR $P < 0.01$), (b) MC-YR-TP relationship ($n = 182$; IMR $P < 0.01$), (c) MC-LR-TP relationship ($n = 182$; IMR $P < 0.01$), (d) *Microcystis* biomass-TP relationship ($n = 144$; IMR $P < 0.01$).

The seasonally connected lakes had the highest MC value (0.786 μg L^{-1}), while the river connected lakes had the lowest mean value (0.024 μg L^{-1}). The seasonally connected lakes had water exchange with the Yangtze River one or two times per year, and were eutrophic (TP > 0.035 mg L^{-1}), usually with thick cyanobacterial blooms in warm seasons in some of these lakes. However, the city lakes had a similar MC level to the non-urban lakes, although there was a great difference in nutrient level between these two types of lakes.

In the present study, significant correlations between MC concentration and biomass of *Microcystis* and *Anabaena* spp. ($P < 0.01$) suggest that these (and especially *Microcystis*) were the major MC-producing cyanobacteria in the lakes of the Yangtze River. Similarly, in three hypereutrohic Canadian lakes, *Microcystis* was present in 97% of samples with detectable MC-LR, and was significantly correlated with MC (Kotak *et al.*, 1995). In 13 lakes in the same area, significant correlation was present between MC and *Microcystis* (Kotak *et al.*, 2000). Similar results were also observed in Finnish and Czech lakes (Sivonen *et al.*, 1990; Petr *et al.*, 2006).

As MC concentrations in field samples of phytoplankton are affected both directly by MC-producing cyanobacteria and indirectly by environmental factors, and these factors vary temporally and spatially, it is not surprising that the relationships between MC and environmental factors are not always consistent. For example, MC in phytoplankton was strongly correlated with TN of the lake water in America (Graham *et al.*, 2004), but not in Canada (Kotak *et al.*, 2000), Germany (Chorus *et al.*, 2001) and in the present study.

Interval maxima regression enabled us to define the upper limit of MC along an environmental gradient, which represented the potential maximum if all conditions for MC production and MC producing cyanobacteria were optimal (Graham *et al.*, 2004). The unimodal curves showed that environmental gradients were optimal for MC only in some ranges, while the negative exponential curves showed that the environmental gradient was optimal when the values were very low. MC (-LR, -YR and -RR) values often fell below the potential maxima because other abiotic and biotic factors were sub-optimal (Kaiser, Speckman & Jones, 1994).

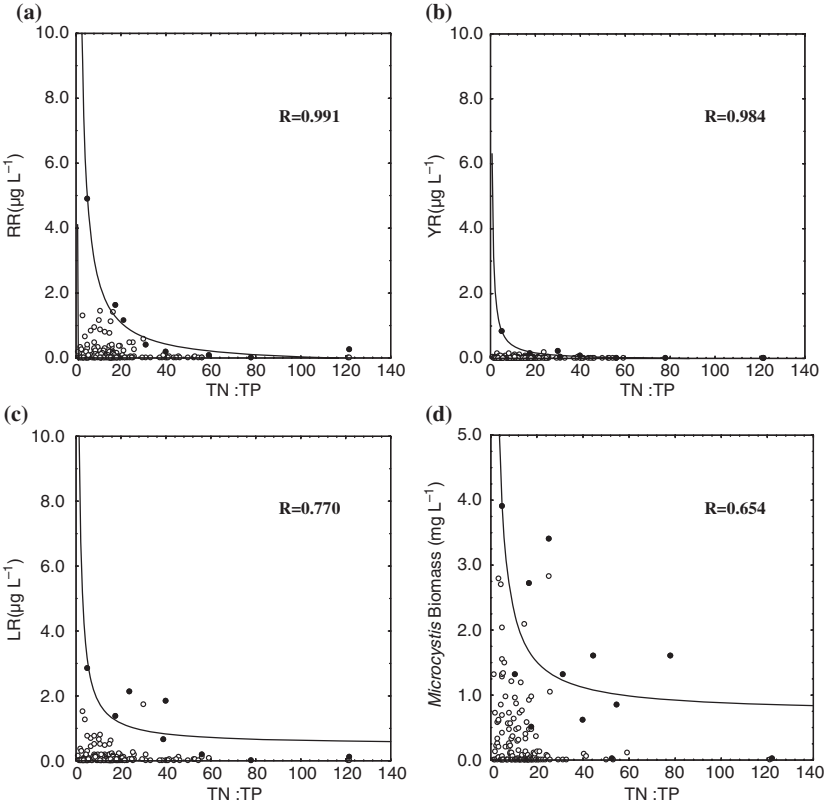

Fig. 4 Relationship to TN : TP ratio for microcystin (MC-RR, MC-YR and MC-LR) concentration and *Microcystis* biomass (MB). Curves were estimated using interval maxima regression (IMR). Black points indicate the data used for IMR analysis; *r* values are for this fitted line only. (a) MC-RR-TN : TP relationship ($n = 182$, IMR $P < 0.01$), (b) MC-YR-TN : TP relationship ($n = 182$; IMR $P < 0.01$), (c) MC-LR-TN : TP relationship ($n = 182$; IMR $P < 0.01$), (d) MB-TN : TP relationship ($n = 144$; IMR $P < 0.05$).

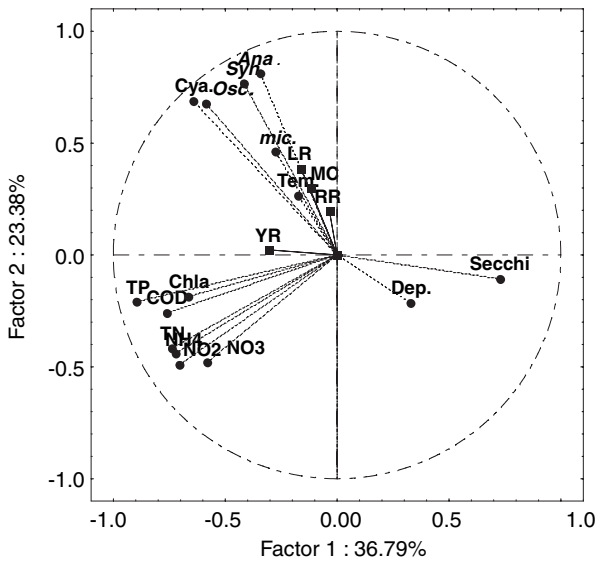

Fig. 5 Scatterplot of first two components from a PCCA performed with 15 physiochemical variables and MC variables from all samples. (●, the active variables; ■, the supplementary variables; Cya, Cyanophyta; *Mic*, *Microcystis*; *Osc*, *Oscillatoria*; *Syn*, *Synechocystis*; *Ana*, *Anabaena*; Dep, water depth).

In the present study, the MC maxima were characterised by unimodal curves with respect to TN, TP and $NO_3^- + NO_2^-$, while the MC maxima along the Secchi depth, TN : TP and NH_4^+ gradients were characterised by negative exponential relationships; and along the $NO_3^- + NO_2^-$ gradient, maximal MC values occurred between 0.3 and 1.0 mg L^{-1}. The lower MC maxima at TN < 0.25 mg L^{-1}, TP < 0.05 mg L^{-1} and $NO_3^- + NO_2^-$ < 0.3 mg L^{-1} might be due to nutrient limitation of cyanobacterial growth, while lower maxima at TN > 1.0 mg L^{-1}, TP > 0.25 mg L^{-1} and $NO_3^- + NO_2^-$ > 1.0 mg L^{-1} might imply that either excessive nutrients or biological factors influenced the shape of the curve. In lakes of central Alberta, where $NO_3^- + NO_2^-$ concentration was relatively low (almost <0.2 mg L^{-1}) and easily assimilated by cyanobacteria in summer, maximum MC-LR concentration occurred when $NO_3^- + NO_2^-$ < 10 μg L^{-1} (Kotak *et al.*, 2000). In contrast, in the present study $NO_3^- + NO_2^-$ was sufficient or even in excess. The negative exponential relationship between the three MC analogues and NH_4^+ in our

study lakes indicates that NH_4^+ was depleted earlier than NO_3^- by cyanobacteria, and it is known that when NH_4^+ is available, cyanobacteria do not assimilate alternative N sources (Turpin, 1991; Blomqvist, Pettersson & Hyenstrand, 1994; Ochoa de Alda *et al*., 1996).

In the present study, MCs had negative exponential relationships with TN : TP, which is similar to results from America (<50) and Canada (<5) (Graham *et al*., 2004; Kotak *et al*., 2000). This may be because nitrogen was consumed by algae and microbes while phosphorus was released from the sediment in eutrophic and hypereutrophic lakes (Xie *et al*., 2003; Wu *et al*., 2006). In the present study, maximal MC values increased with increasing TN and TP with a peak between 0.25 and 1.0 mg L^{-1} of TN and a peak between 0.05 and 0.25 mg L^{-1} of TP. These values are lower than those reported by Graham *et al*. (2004) from lakes in Midwestern America (1.5–4.0 mg L^{-1} TN and 0.1–0.6 mg L^{-1} TP), probably due to different sampling methods. The composite surface or integrated epilimnetic samples were chosen in midwestern American lakes, while composite samples were taken from surface, middle and bottom layers in our study. As some cyanobacteria can adjust their buoyancy according to irradiance and thus assemble in surface waters (Kirk & Oliver, 1995), higher TN and TP concentrations may appear in the surface than in the whole water body.

There have been few multivariate analyses examining the relationship between MCs and biological and physiochemical factors at a regional scale. The present study used PCCA to explore relationships between MC and various biological and physiochemical factors. Cyanobacterial species and microcystin variables had negative weightings on Component Axis 1 and positive weightings on Component Axis 2. Among biological variables, MC, MC-RR and MC-LR showed closer positive relationships with the biomass of *Microcystis* than with that of *Anabaena*, *Synechocystis*, *Oscillatoria* or total cyanobacteria, suggesting that *Microcystis* was the single most important MC producer. Among the environmental factors, temperature was an important variable closely related with MC, suggesting that high temperature in summer could promote MC production. In the present study, MC was negatively correlated with Secchi and water depth, suggesting that deeper and clear waters may not favour MC production by cyanobacteria (cf. Jacoby *et al*., 2000; Graham *et al*., 2004).

Natural systems are characterised by a vast array of biotic and abiotic gradients coupled with multiple species interactions (Reynolds, 1998). In the Yangtze River lakes variation of MC concentration along environmental gradients proved similarly complex. Further investigation of the influence of environmental factors on MC production are necessary to assist effective lake management and minimisation of risks to human health risks from exposure to MC, especially in the seasonally river-connected lakes in the Yangtze River area.

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