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Environmental factors regulating cyanobacteria dominance and microcystin production in a subtropical lake within the Taihu watershed, China*

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Abstract: Understanding the pattern of phytoplankton and their dependence on water quality variables, can help the management of eutrophic lakes. The aim of this study was to determine water quality and environmental factors associated with cyanobacteria dominance and microcystin production in Qingshan Lake, a subtropical lake located in the headwater of the Taihu watershed, China. Water samples collected monthly from 10 study sites in Qingshan Lake were analyzed for the species distributions of freshwater algae and physico-chemical parameters including total nitrogen (TN), ammonia (NH_4^+-N), nitrate (NO_3^--N), total phosphorus (TP), and chlorophyll a (Chl-a) from June, 2008 to May, 2009. Qingshan Lake was found to be eutrophic, based on the calculated trophic state index (TSI). The average TN of 4.33 mg/L during the study period exceeded the Surface Water Quality Standards of China. TP was significantly correlated with relative abundance of cyanobacteria and *Microcystis* biovolume, indicating its important role in regulating cyanobacteria. *Microcystis*, *Anabaena*, and *Oscillatoria* were dominant cyanobacteria in Qingshan Lake from June to November, 2008. Cyanobacteria dominance was regulated by water temperature and TP. Principal component analysis further indicated that microcystin production was most affected by water temperature, TP, and cyanobacteria biomass. Results suggest that the control of TP in summer can mitigate cyanobacteria dominance and microcystin production in Qingshan Lake, and close monitoring should be undertaken in summer.

Key words: Cyanobacteria, Microcystins, Eutrophication, Water quality, Taihu watershed
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1 Introduction

Eutrophication is a threat to freshwater ecosystems worldwide (Lambert and Davy, 2011). Algae blooms caused by eutrophication in freshwater lakes are becoming increasingly a serious problem worldwide (Vasconcelos and Pereira, 2001; Vareli *et al.*, 2009). In China, serious algae blooms have been reported in Taihu and Dianchi Lakes (Liu *et al.*, 2006; Ye *et al.*, 2009). Several studies reported that freshwater cyanobacterial blooms are typically associated with eutrophic waters (Paerl, 1988; Oliver and Ganf, 2000); however, the factors and mechanisms involved in the initiation of cyanobacterial blooms were still unclear. Smith (1983) evaluated algal growing season data from 17 lakes throughout the world, and concluded that cyanobacteria tended to dominate when the total nitrogen to total phosphorus mass ratio (TN/TP) was lower than 29:1. Other factors that potentially influence algal bloom formation, in addition to nutrients, include light limitation, weather, and

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water turbulence. During the summer stratified season, conditions allowing cyanobacteria to dominate over algal assemblages include a stable water column, reduced grazing by large herbivores, low TN/TP ratios, high nutrient levels, and high pH (Paerl, 1988; Havens *et al.*, 2003). Results to date, however, still preclude a precise description of the specific environmental conditions for the formation of algal blooms. Consequently, conducting investigation on seasonal and spatial variations of environmental factors in specific freshwater lakes, and identifying the primary environmental factor associated with algal blooms, can provide effective management measures to reduce bloom development, and contribute to the water quality management at the watershed scale.

Another problem associated with algae blooms is the presence of potentially toxic cyanobacteria in water bodies that hamper the water usage. Several algae species produce toxins that can cause acute liver failure and death (Carmichael *et al.*, 2001), as well as low dose chronic effects in humans such as carcinogenesis and tumor growth promotion (Sivonen and Jones, 1999). These health hazards led the WHO (1998) to establish a provisional guideline for microcystin-LR of 1 μg/L in drinking water. Microcystins can be produced by *Microcystis* spp., *Anabaena* spp., *Oscillatoria* spp. and *Nostoc* spp. (Sivonen and Jones, 1999). Populations of these species are known to have strains that can produce microcystins (Watanabe *et al.*, 1991; Vezie *et al.*, 1998). It is therefore essential to determine the primary environmental factors associated with cyanobacteria dominance and microcystin production.

In the present study, we examined several environmental factors, phytoplankton succession and microcystin concentrations in Qingshan Lake located in the Taihu watershed in southeastern China from June, 2008 to May, 2009. The purposes of this study were to identify which physico-chemical parameters were important for the dominance of cyanobacteria, and to determine the relationships between environmental factors and microcystin concentrations. Since these freshwater lakes generally serve as major drinking water sources, understanding the presence, seasonal distribution, and environmental factors associated with microcystin production can help develop management strategies for protecting water quality, as well as establishing reference standards for safe drinking water in Qingshan Lake.

2 Materials and methods

2.1 Study site

The study site, Qingshan Lake (119°45′42.06″ E –119°48′0.32″ E, 30°13′32.56″ N–30°15′18.64″ N), with a total area of about 10 km^2, is located in the Taihu watershed, about 50 km west of Hangzhou near Lin’an in southeastern China (Fig. 1). The lake serves as a major drinking water source for Lin’an, but pollution from upstream agricultural non-point sources and residential sewage has caused severe eutrophication and algal blooms in the past decade. As shown in Fig. 1, upstream water flows into the lake near Site 1 and Site 1’.

2.2 Field sampling

Surface water samples (top 0–50 cm) collected monthly from 10 sites in Qingshan Lake were analyzed for physico-chemical parameters, algal biomass, algal species composition, and microcystins contents from June, 2008 to May, 2009. Physico-chemical water quality parameters include temperature, dissolved oxygen (DO), turbidity, pH, conductivity, TN, ammonia (NH_4^+-N), nitrate (NO_3^--N),

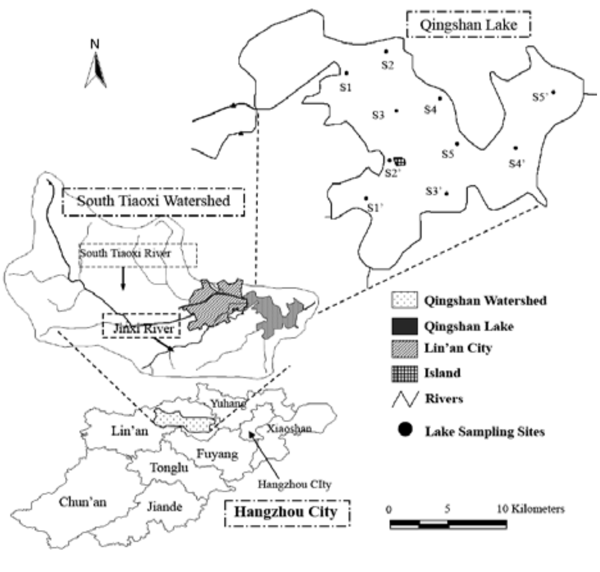

Fig. 1 Study area and sampling sites

and TP. Algal parameters included algal biomass (measured as chlorophyll-a (Chl-a)) and biovolume (estimated from cell number and taxonomic composition of the algae). Temperature, DO, turbidity, conductivity, and pH were measured in situ using a YSI 556 multi-probe system (YSI Environmental, USA). Field collected water samples (5.0 L each site) were transported to the lab and stored in a cold room (4 °C) before further analysis.

2.3 Analytical methods

TN was analyzed by digesting 10 ml sample containing alkaline potassium persulfate ($K_2S_2O_8$) at 120–124 °C for 30 min, following by cooling before 1.0 ml of 10% hydrochloric acid was added. TN was measured via UV spectrophotometry at 220 nm in reference to 275 nm using a Shimadzu UV2450 spectrophotometer (Japan) according to Chinese National Standard methods for water quality analysis (GB 11894-89). Ammonia and nitrate-nitrogen were measured using an HACH DR890 analyzer (USA).

TP was measured by the ammonium molybdate spectrometric method according to Chinese National Standard methods for water quality analysis (GB 11893-89). A water sample of 25 ml was digested at 121 °C for 30 min after adding $K_2S_2O_8$, followed by the addition of 1.0 ml of 10% ascorbic acid. TP levels in the water sample were also measured using a Shimadzu UV2450 spectrophotometer.

2.4 Algal attributes

Water samples were subsampled for Chl-a measurements within 12 h of collection. 250 ml aliquots were filtered through 0.45 μm Whatman AH glass fiber filters, held frozen overnight prior to extraction using ethanol (Jespersen and Christoffersen, 1987). Pigments were measured spectrophotometrically at 665 and 750 nm using a Shimadzu UV2450 spectrophotometer.

1.0 L sample were preserved with 1% Lugol's iodine solution immediately after sampling, and concentrated to 50 ml after settling for 48 h (APHA, 1992). Then the supernatant was removed and the residue was collected. After complete mixing, 0.1 ml of concentrated sample were counted directly through 0.1 ml counting chamber at 400× magnification with YSI 100 microscope (Nikon, Japan). At least 400 cells were identified and counted per sample. Taxonomic identification was performed according to the method by Hu and Wei (2006). Phytoplankton cells were measured to calculate volumes of each taxon from the closest geometric shape (Sun and Liu, 2003). Algal biomass (wet basis) was calculated from biovolume assuming a wet mass density of 1 g/cm^3.

2.5 Analysis of microcystins

Microcystin contents were analyzed by enzyme-linked immunosorbent assay (ELISA) using microcystins plate kits obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The kit consists of a 96-well microtiter plate coated with anti-microcystin-LR antibodies that were immobilized on the walls of the test wells. Assays of standards or samples were performed following the kit instructions. Briefly, 50 μl water sample, standard or negative control, and 50 μl monoclonal antibody solution were introduced into the well. The contents of each well were mixed thoroughly, and the plate was incubated for 90 min at ambient temperature. The wells were washed three times with 0.05% (in volume) Tween-20 in phosphate buffer saline (PBS) with an immuno-wash apparatus (Thermo, USA). 100 μl aliquot of a microcystin-enzyme conjugate solution were then added and incubated for 30 min at ambient temperature. The wells were then washed five times with 0.05% (in volume) Tween-20 in PBS. 100 μl aliquot of substrate were added to each well and incubated for 5–10 min at ambient temperature. The substrate was transformed by the enzyme conjugate into a blue compound. Then 50 μl aliquot of 1 mol/L H_2SO_4 were added to stop the enzyme reaction, and the solutions turned yellow. The absorbance at 450 nm was immediately measured with a microtiter plate reader (Thermo, USA). The detection limit of microcystin was 50 ng/L.

2.6 Statistical analysis

Statistical analysis was conducted using SPSS software, version 16.0 for Windows (Chicago, USA). A paired *t*-test was used to compare the difference of physico-chemical parameters in two sampling sites near the western inlets. Temporal and spatial differences among means of physico-chemical parameters, algal parameters, and microcystin concentrations were determined using ANOVA and least significant difference (LSD) test. The Pearson correlation analysis was used to analyze the correlation among physico-chemical parameters, algal parameters, and microcystins. Furthermore, regression analysis was used to test the relationship between algal biomass and physico-chemical parameters. To determine the main environmental variables affecting the toxin production of cyanobacteria in Qingshan Lake, the Pearson correlation analysis and the principal component analysis (PCA) were performed on data of all 15 environmental variables. The entire dataset (n=120) was used for each test and correlation analysis unless otherwise noted. The criteria for statistical significance was set at p<0.05.

3 Results

3.1 Water quality of Qingshan Lake

Water temperature (T), pH, and TP were identified as the primary factors contributing to cyanobacteria growth, while T and TP were the primary factors contributing to microcystin production. Seasonal variabilities in T, DO, pH, conductivity (Cond.), and turbidity (Turb.) were all significant (ANOVA, p<0.01), while water depth did not show any significant differences among different months (ANOVA, p>0.05) (Table 1). The lake water has an average temperature above 24 °C during the months from May to October, which favors the growth of cyanobacteria and microcystin production.

Significant differences were also observed in nitrate, TN, and TP among different months (ANOVA, p<0.001), but not in ammonia (ANOVA, p>0.05). The average nitrate was 2.56 mg/L, with the highest value of 3.74 mg/L in June, 2008, considerably higher than any other month during the study period (LSD test, p<0.001). The lowest was 2.08 mg/L in August, 2008 (Fig. 2a). Both of TN and TP concentrations were stable in winter (from January to April) (Fig. 2b). TP concentrations in warm months (from June to October) were statistically higher than those in winter (LSD test, p<0.001). The highest TN was 7.95 mg/L in December, 2008, higher than any other month during the study period (LSD test, p<0.001), and the lowest was 2.15 mg/L in October, 2008.

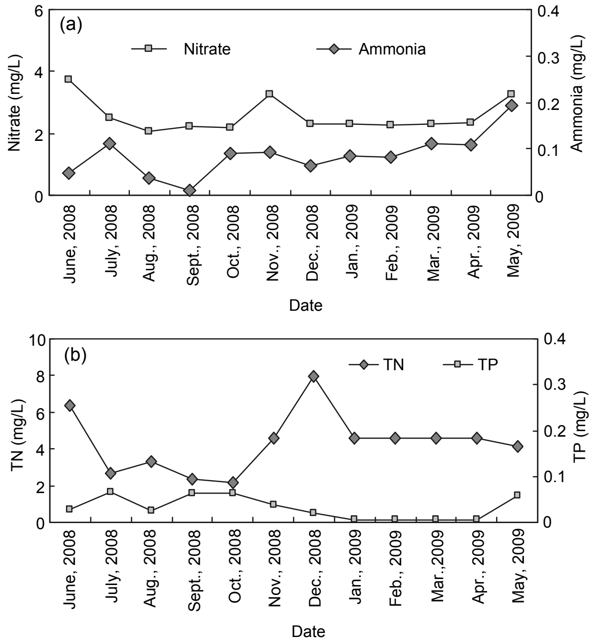

Fig. 2 Average nutrient concentrations of surface water samples in Qingshan Lake, June, 2008-May, 2009 for 10 sampling sites
(a) Nitrate and ammonia; (b) TN and TP

Table 1 Water quality parameters in Qingshan Lake during the period of 2008–2009

Sampling date	T (°C)	DO (mg/L)	pH	Cond. (mS/cm)	Turb. (NTU)	Water depth (m)
June 24, 2008	26.0–27.6	5.70	7.62	0.21–0.24	32	5.8
July 23, 2008	30.2–31.2	5.70	7.05	0.25–0.28	38	5.6
Aug. 25, 2008	28.8–30.1	5.95	5.87	0.26–0.29	48	5.5
Sept. 22, 2008	28.6–29.2	6.82	6.55	0.30–0.32	28	5.3
Oct. 21, 2008	24.0–25.0	6.88	6.47	0.34–0.40	82	5.2
Nov. 25, 2008	14.0–14.8	6.33	6.41	0.30–0.32	87	6.2
Dec. 23, 2008	7.6–8.4	8.37	6.47	0.36–0.60	57	4.1
Jan. 15, 2009	5.0–5.8	8.96	6.12	0.42–0.50	64	5.1
Feb. 15, 2009	10.3–12.4	4.68	6.54	0.43–0.44	57	4.1
Mar. 15, 2009	11.9–14.5	9.76	6.40	0.22–0.25	38	6.7
Apr. 16, 2009	16.7–19.9	9.08	7.11	0.27–0.31	64	5.0
May 14, 2009	18.2–25.0	5.62	7.25	0.34–0.50	140	5.2

With regard to spatial variations, significant differences in pH, water depth, turbidity, TN, and ammonia were identified between the two inlets (Site 1 and Site 1') (Table 2). TN and ammonia in Site 1 were statistically higher than Site 1' (paired t-test, $p<0.05$). In addition, spatial variability was found in pH (ANOVA, $p<0.05$), water depth, turbidity (ANOVA, $p<0.01$), and ammonia (ANOVA, $p<0.001$), but no difference was observed in water temperature, DO, conductivity, nitrate, TN, and TP among the 10 sampling sites (ANOVA, $p>0.05$), indicating that water in Qingshan Lake was well mixed. Average turbidity in Site 1 was 121, which was higher than any other sampling site in Qingshan Lake (LSD test, $p<0.05$), and average water depth in Site 1 was 2.1 m, which was significantly lower than any other site (LSD test, $p<0.001$). The highest water depth was 10.0 m in Site 5', significantly higher than any other site in Qingshan Lake (LSD test, $p<0.001$). Ammonia in Site 1 was statistically higher than any other site during the study period (Fig. 3a) (LSD test, $p<0.05$).

3.2 Trophic state of Qingshan Lake

The analysis of variance identified temporal variations for Chl-a concentrations among different months (ANOVA, $p<0.001$), but no spatial significant difference was observed among the 10 sampling sites (ANOVA, $p>0.05$). During the study period, the average Chl-a concentration in Qingshan Lake was 12.56 mg/m^3, with 25.59 mg/m^3 being the highest in June, 2008 and 1.82 mg/m^3 being the lowest in December, 2008 (Fig. 3b).

Based on the Chl-a level, Carlson's trophic state index (TSI) (Carlson, 1977) was further applied to determine the trophic status of Qingshan Lake. The average TSI of Qingshan Lake was 52, indicating that the Qingshan Lake was eutrophic according to Carlson (1977). TSIs in several months even exceeded 61, indicating that Qingshan Lake reached hypereutrophic state in June (TSI=62) and October (TSI=61).

3.3 Algal species composition and its dependence on nutrient levels

The composition of algal species (measured as percent biovolume) varied from the dominance by cyanobacteria in late summer-fall period to diatoms, euglenoids, and green algae during the winter-spring period (Fig. 4). No significant difference was found among relative abundance of cyanobacteria of 10

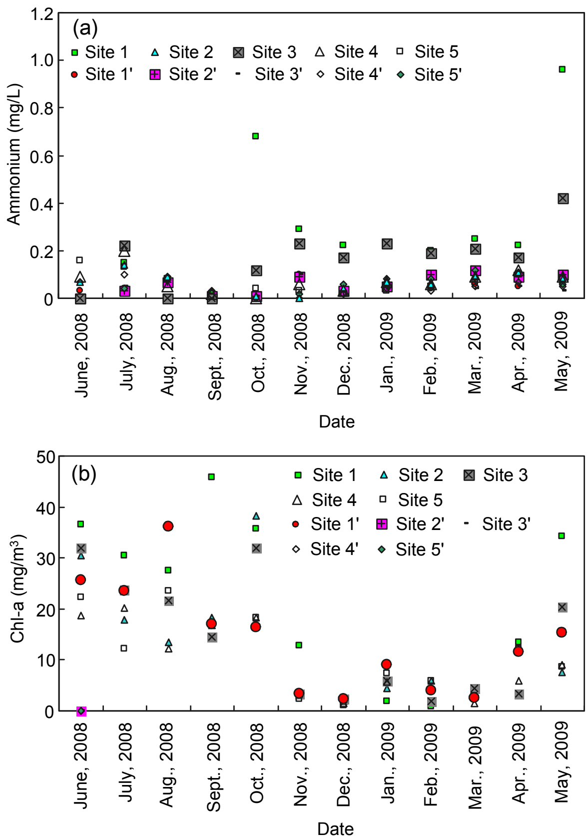

Fig. 3 Spatial variations of ammonia (a) and Chl-a (b) concentrations of surface water samples in Qingshan Lake, June, 2008–May, 2009

Table 2 Mean water quality parameters differences between two inlets (Site 1 and Site 1') during the study period

Parameter	pH	T (°C)	Water depth (m)	DO (mg/L)	Turb. (NTU)	Cond. (mS/cm)	TN (mg/L)	TP (mg/L)	NH_4^+ (mg/L)	NO_3^- (mg/L)
Mean value (Site 1)	6.27	19.67	2.1	6.32	121	0.362	5.34	0.058	0.27	2.69
Mean value (Site 1')	6.89	19.75	3.8	7.36	48	0.324	4.03	0.030	0.04	2.38
p-value (Site 1 vs. Site 1')	0.001	n.s.	0.000	n.s.	0.025	n.s.	0.010	n.s.	0.015	n.s.

The p-values for paired t-tests are presented and p-values greater than 0.05 are denoted as not significant (n.s.)

sampling sites by multiple comparisons (ANOVA, $p>0.05$, data not shown). A succession of cyanobacteria, *Microcystis*, *Anabaena*, and *Oscillatoria*, were observed in Qingshan Lake from June to November, 2008, but no cyanobacteria were observed in the surface water samples from December, 2008 to March, 2009. *Microcystis* was the dominant cyanobacteria species from June to August, 2008, while from September to November, *Oscillatoria* and *Anabaena* accounted for more than 80% of total cyanobacteria biovolume.

The relative abundance of cyanobacteria was positively correlated to TP ($r=0.53$, $p<0.001$), but negatively correlated to TN ($r=-0.43$, $p<0.001$) and TN/TP ratios ($r=0.71$, $p<0.001$) (data not shown). Based on the monthly average of 10 sampling sites, we found a good correlation between relative abundance of cyanobacteria and log-transformed TN/TP ratios (Fig. 5) ($r=0.93$, $p<0.001$, $n=12$) and TP concentrations (Fig. 6) ($r=0.92$, $p<0.001$, $n=12$).

3.4 Microcystin concentrations

There was a significant temporal variation ($p<0.001$, $n=120$) in microcystin concentrations in Qingshan Lake, as shown in Fig. 7. The highest average microcystin concentration was 0.51 μg/L in August, 2008, which was significantly higher than any other month during the study period (LSD test, $p<0.01$). No microcystin was detected from January to March, 2009.

The highest microcystin concentration detected in Qingshan Lake was 1.28 μg/L in Site 3' on Aug. 25, 2008, corresponding to a relative abundance of cyanobacteria of 25%. The second highest microcystin concentration detected at 1.03 μg/L was also in Site 3' on May 14, 2009, with a relative abundance of cyanobacteria under 20%. The maximum microcystin concentration in Qingshan Lake exceeded the value of 1 μg/L recommended by WHO (1998) for drinking water standard, indicating the potential threat to human health.

3.5 Correlation between microcystins and water quality variables

We observed that the period of lower toxin levels in September and October corresponded to an increase in cyanobacteria biovolume. This result implies that the same conditions leading to cyanobacteria

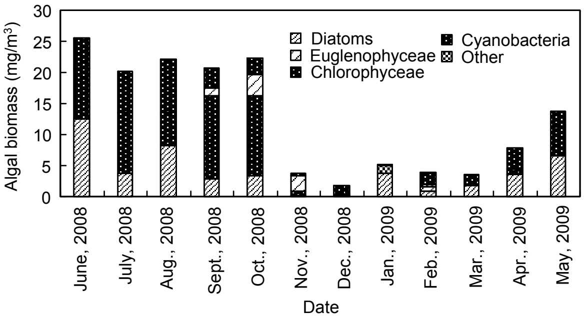

Fig. 4 Seasonal changes in the phytoplankton community composition (Percentage of total biovolume) during June, 2008–May, 2009 in Qingshan Lake

Only four dominant groups that together represented at least 80% at all times are shown, and the remainder being grouped is "Other"

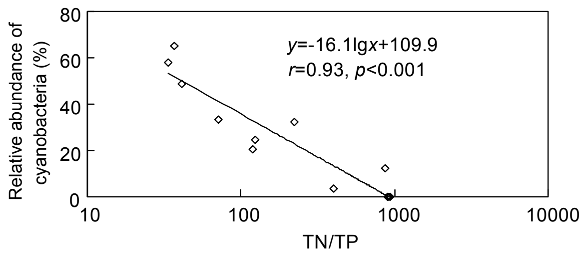

Fig. 5 Relationship between percent of total phytoplankton biovolume due to cyanobacteria with monthly mean TN/TP ratios

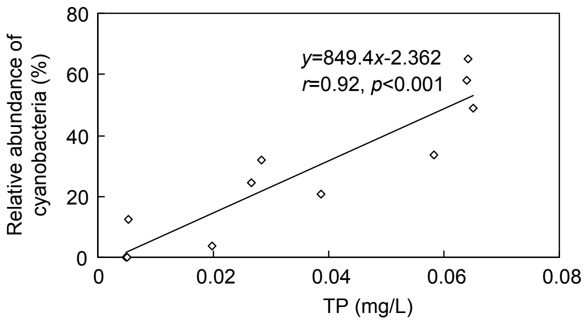

Fig. 6 Relationship between percent of total phytoplankton biovolume due to cyanobacteria and monthly mean TP concentrations

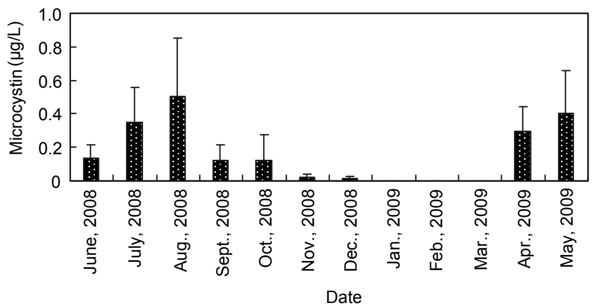

Fig. 7 Average microcystin concentrations of 10 sampling sites in surface water of Qingshan Lake, June, 2008–May, 2009

dominance may not lead to increased toxin concentrations. To identify the potential physico-chemical factors that may influence toxin production in Qingshan Lake, the correlations between the microcystin concentration and physico-chemical water quality parameters were analyzed (Table 3). Microcystin concentrations were positively correlated to water temperature ($p<0.01$), TP ($p<0.05$), Chl-a ($p<0.01$), cyanobacteria biovolume (cyano.) ($p<0.01$), and *Microcystis* biovolume ($p<0.05$), and negatively correlated to TN ($p<0.01$), TN/TP ratios ($p<0.01$), DO ($p<0.01$), and conductivity ($p<0.05$).

Table 3 Correlations between physico-chemical factors, algal parameters, and microcystins (MCs)

Environmental variable	Microcystin	Chl-a	Cyano.	*Microcystis*
TN	-0.292^{**}	-0.342^{**}	-0.349^{**}	-0.261^{**}
TP	0.193^{*}	0.598^{**}	0.596^{**}	0.418^{**}
TN/TP	-0.311^{**}	-0.564^{**}	-0.565^{**}	-0.438^{**}
Microcystin	1	0.353^{**}	0.295^{**}	0.216^{*}
Chl-a	0.353^{**}	1	0.709^{**}	0.512^{**}
Cyano.	0.295^{**}	0.709^{**}	1	0.736^{**}
Microcystis	0.216^{*}	0.512^{**}	0.736^{**}	1
NH_4^+	−0.027	0.116	0.177	−0.007
NO_3^-	0.005	0.167	0.106	0.112
pH	0.154	0.135	0.223^{*}	0.184^{*}
T	0.555^{**}	0.774^{**}	0.672^{**}	0.557^{**}
DO	-0.247^{**}	-0.414^{**}	-0.343^{**}	-0.262^{**}
Turb.	0.011	0.094	0.110	0.019
Depth	0.119	0.020	0.058	0.006
Cond.	-0.229^{*}	-0.310^{**}	-0.232^{*}	-0.233^{*}

* Correlation is significant at $p<0.05$; ** Correlation is significant at $p<0.01$

4 Discussion

4.1 Sources of N and P in Qingshan Lake

The significant difference observed in physico-chemical parameters in the two inlets suggests that the inlet near Site 1 is the main source of nitrogen pollution in Qingshan Lake, because TN and ammonia concentrations in Site 1 were statistically higher than Site 1' across the study period (paired t-test, $p<0.05$), and the average annual flow to the inlet near Site 1 is 1.5 times higher than that near Site 1' (unpublished data). The average TN during the study period in Qingshan Lake was 4.33 mg/L, which is four times higher than the category "III" standard (<1.0 mg/L) of surface water quality of China (GB 3838-2002), nearly twice higher than the concentrations reported in previous studies during the occurrence of *Microcystis* blooms in Taihu Lake (Ke *et al.*, 2007) and impoundants of Huron River (Lehman, 2007).

Forested land runoff upstream may be a significant source of nitrogen in Qingshan Lake in winter. The maximum TN concentration observed was in December, and this time of the year coincides with application of large quantities of organic nitrogen fertilizer (20 fold higher than the other three applications during the year). Therefore, the high TN concentration in Qingshan Lake may be linked to runoff from the bamboo forestry upstream, and nitrogen fertilizer recommendations should be adjusted to improve nitrogen use efficiency and reduce substantial nitrogen losses to water bodies. According to the statistical data from the Management Bureau of Taihu Lake Watershed, Ministry Bureau of Water Resources, nitrogen pollution from agricultural non-point sources accounted for 77% of the TN discharged in the area. Wang *et al.* (2004) also concluded that frequent cyanobacterial blooms in Taihu Lake in summer were correlated to nutrient enrichment, primarily nitrogen and phosphorus, mainly from agricultural non-point source pollution. Residential sewage from Lin'an also contributed to the nitrogen pollution in Qingshan Lake, as ammonia in Site 1 was statistically higher than any other site during the study period (LSD test, $p<0.05$). Dentener *et al.* (2006) reported that nitrogen deposition in southeastern China, where Qingshan Lake is located, has already exceeded the threshold of 1000 mg/(m^2·y), above which changes in natural ecosystems may occur. Chen *et al.* (2008) showed that wet deposition of atmospheric nitrogen could change the nutrient frame of surface water in southeastern China, which can affect the structure of phytoplankton species, subsequently impacting pH and salinity of surface water (Skip *et al.*, 1995; Vitousek *et al.*, 1997). In this study, pH in surface water was positively correlated with cyanobacteria and *Microcystis* (Table 3). Therefore, input of nutrients due to atmospheric wet deposition should also be considered as the source of nitrogen pollution.

The phosphorus pollution in Qingshan Lake was not as serious as nitrogen. The average TP was 0.032 mg/L, which meets the category “III” standard of 0.05 mg/L of China (GB 3838-2002). However, 16.7% of the TP values during the study period were greater than the TP standard. A previous study showed that the internal phosphorus loading in shallow lake induced by resuspension can be 20–30 times greater than the release phosphorus from undisturbed sediment (Sondergaard *et al.*, 1992). The highest value of turbidity was 140 NTU in May, 2009, and a positive correlation was found between TP concentrations and turbidity (p<0.001, r=0.42), which suggests an intense mixing of water column (e.g., boating) and sediment re-suspension could contribute to the increase of TP in Qingshan Lake.

4.2 Algal biomass and species variations in response to water quality

TP and water temperature were found to be the primary factors that stimulate algal biomass development in Qingshan Lake, because Chl-a was positively correlated to TP (r=0.60, p<0.01) and water temperature (r=0.77, p<0.01) (Table 3). A similar positive correlation between TP and Chl-a was also found in Taihu Lake based on five years of monthly data (James *et al.*, 2009). It seems that algal communities exerted a little effect on DO concentrations in Qingshan Lake, as DO was negatively correlated to Chl-a, cyanobacteria biovolume and *Microcystis* biovolume (p<0.01) (Table 3). This result was inconsistent with Xie *et al.* (2003), who found that DO concentrations in the enclosures with occurrence of *Microcystis* blooms were even significantly higher than that at the same depth in Donghu Lake. Chl-a was also negatively correlated to Cond. (r=−0.31, p<0.05), TN (r=−0.34, p<0.01) and TN/TP ratios (r=−0.56, p<0.01). A similar negative relationship between algal biomass and TN/TP ratios was also found in lakes in North America (Kotak *et al.*, 2000; Graham *et al.*, 2004). This may be caused by uptake of nitrogen by algae and release of phosphorus from the sediment in eutrophic and hypereutrophic lakes (Xie *et al.*, 2003).

Algal species composition in Qingshan Lake exhibits a seasonal variation from dominance by cyanobacteria from late summer and early fall to diatoms, euglenoids, and green algae during winter and spring. No cyanobacteria was identified in winter in the water surface, and Wu *et al.* (2008) confirmed that blue-green algae mostly began to overwinter in the sediment in November in Taihu Lake and Chaohu Lake, China. With the increase in water temperature, the sediment surface becomes anaerobic, which will cause *Microcystis* to float upward back to the water and recolonize the water column in spring (Fallon and Thomas, 1981). No cyanobacteria was identified in Qingshan Lake after November, accidently coinciding with the overwinter period confirmed by Wu *et al.* (2008). We are unable to address this question because only surface water samples were measured in the present study.

We observed transitions not only from diatoms to cyanobacteria in summer, but also transitions within cyanobacteria communities from *Microcystis* to *Anabaena* and *Oscillatoria*. Cyanobacteria and *Microcystis* biovolume was positively correlated to T, pH, and TP, negatively correlated to TN and TN/TP, but not significantly correlated to nitrate and ammonia (Table 3). Chen *et al.* (2003) and Ke *et al.* (2008) also indicated that water temperature is the most important discriminant variable for the changes of phytoplankton community in Taihu Lake during the succession period. Moreover, the relative abundance was also positively correlated to TP, suggesting that elevated TP can contribute to the dominance of cyanobacteria in Qingshan Lake.

TP concentrations in July, September, and October in 2008 were around 0.065 mg/L (Fig. 2b), which were significantly higher than other months during the study period (LSD test, p<0.01). Meanwhile, relative abundance of cyanobacteria exceeded 50% in these three months (Fig. 4), which was higher than other months during the year. TN/TP ratios in Qingshan Lake showed a great variation from month to month, ranging from 33–940 during the months when relative abundance of cyanobacteria were more than 50%. TN/TP ratios were lower than 40 (Fig. 5), with TP concentrations higher than 0.06 mg/L (Fig. 6). Therefore, we hypothesized that a combination of high water temperature (>24 °C) and high TP (>0.06 mg/L), together with regulation of TN/TP ratios (less than 40) enhanced the growth of cyanobacteria in Qingshan Lake. TN/TP ratios observed in Qingshan Lake during the algae blooms were similar to those found in Taihu Lake (Ke *et al.*, 2007) and Dianchi Lake (Liu *et al.*, 2006), but much higher than other freshwater lakes (Dai *et al.*, 2008; El Herry *et al.*, 2008).

4.3 Microcystins concentrations in relation to algae species and water quality variables

As mentioned above, higher relative abundance of cyanobacteria may not lead to increased microcystin concentrations in Qingshan Lake. An increase in cyanobacteria biovolume in September and October did not cause higher toxin levels, even though *Anabaena* and *Oscillatoria* accounted for more than 80% of total cyanobacteria biovolume at this time of the year. These findings are similar to Chen *et al.* (2009) in Taihu Lake. Microcystin toxin was positively correlated with Chl-a and *Microcystis* biovolume, but only 12% and 5% of the variances were explained, respectively, by these two parameters. Thus, we suggest that a long-term monitoring program of cyanobacteria and their toxins should be implemented, especially in warm months.

The independence of toxin levels on biovolume has been observed by others (Jacoby *et al.*, 2000; Mankiewicz-Boczek *et al.*, 2006). It is possible that this inconsistency may result from changes within the *Microcystis* bloom because strains differ in their ability to produce toxins. Heterogeneity within *Microcystis* blooms has been observed in previous studies (Watanabe *et al.*, 1991; Kotak *et al.*, 1995). However, we are unable to address this issue because no genotype was performed in the present study. Others have suggested that the conditions optimal for cell growth are not identical to those optimal for toxin production (Lehman, 2007). Lehman *et al.* (2009) also found that the same environmental conditions that predicted dominance of *Microcystis* did not serve to predict toxicity.

The relationship between microcystin variations and environmental factors were further analyzed by PCA. Four significant component (eigenvalue>1) were extracted, explaining approximately 80% of total variance (component 1: 33%, component 2: 22%, component 3: 14%, and component 4: 10%). Component 1 had a positive weighting for *T*, TP and algal parameters (Chl-a, cyanobacteria and *Microcystis* biovolume), but had negative weightings for TN/TP and DO (Fig. 8). Component 2 had positive weightings for turbidity, NH_4^+, and conductivity. Component 3 had positive weightings for pH and nutrients (NO_3^- and TN). Microcystin concentrations had the highest positive correlation with component 1 (Fig. 8).

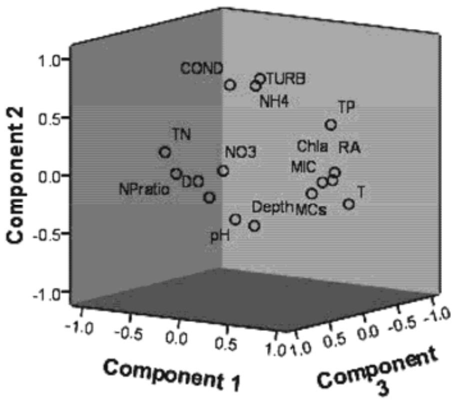

Fig. 8 Component plot of the first three components from a principal component analysis (PCA) performed with 14 environmental variables and microcystin concentrations from all samples during the study period
MIC: *Microcystis* biovolume; RA: relative abundance of cyanobacteria; MCs: microcystins

Understanding the factors capable of predicting algal biomass as well as toxin production is critical for us to design management strategy for algae bloom control. Lehman *et al.* (2009) suggested that the most feasible investigative tool to identify the complex relationship between *Microcystis* and its toxin expression should be laboratory experiments, as we can control confounding variables present in the field. However, conflicting results have been reported on the relationship between microcystin production and nutrient levels from these experimentally based studies. Downing *et al.* (2005) found that *Microcystis* biomass was positively correlated to TN (p<0.05), but no significant correlation was found between microcystin and TN, TP or TN/TP. In contrast, significant positive relations were found between microcystin-LR, microcystin-RR, and TN/TP in a chemostat by Oh *et al.* (2000). Vezie *et al.* (2002) also found microcystin production correlated to TN/TP ratios, and the highest toxin concentration occurred when this ratio was in a range of 207 to 300. Lehman *et al.* (2009) found that microcystin concentration was positively associated with nitrate levels in an urban impoundment, and a similar result was also found in (Jiang *et al.*, 2008), though not in the present study.

5 Conclusions

Qingshan Lake was eutrophic according to the calculated trophic state index, mainly caused by non-point sources upstream. TP was significantly correlated with relative abundance of cyanobacteria and *Microcystis* biovolume. Cyanobacteria dominance was regulated by water temperature and TP in Qingshan Lake. Principal components analysis further showed that microcystin production was most affected by water temperature, TP, and cyanobacteria biomass. Therefore, control of TP in the summer can mitigate cyanobacteria dominance and microcystin production in Qingshan Lake.

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