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ORIGINAL ARTICLE

Temporal dynamics of cyanobacterial community structure in Dianshan Lake of Shanghai, China

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Abstract In recent years, Dianshan Lake, located in Shanghai, China, has experienced increasing eutrophication and frequent algal blooms mainly caused by cyanobacteria. In this study, the temporal dynamics of the cyanobacterial community structure in Dianshan Lake were investigated using molecular techniques in order to identify important abiotic factors influencing such dynamics. Whole-cell PCR analysis showed that cyanobacteria were more abundant in spring and summer than in autumn and winter. This result was further supported by denaturing gradient gel electrophoresis profile analysis. *Microcystis* was observed to be the dominant species of cyanobacteria in Dianshan Lake. Approximately 50 % of cyanobacteria species were influenced by abiotic parameters, such as temperature, orthophosphate, and total phosphorus. The significant correlation between the temporal dynamics of the cyanobacterial community structure and abiotic factors suggests that temperature and phosphorus are essential parameters influencing the dynamic changes of algal blooms in Dianshan Lake.

Keywords Temporal dynamics · Cyanobacteria community structure · Algal bloom · Dianshan Lake

Introduction

Dianshan Lake is located downstream of Taihu Lake basin (31° 04' –31° 12' N, 120° 53' –121° 01' E) west of Shanghai, China. The lake covers an area of 62 km^2, with average depth of 2.1 m and maximum depth of 3.6 m. It is one of the five largest natural freshwater lakes in China and a major water supply source for the Shanghai drinking water system (Lin et al. 2009; Wu et al. 2011). However, with the rapid economic development of Shanghai and adjacent provinces, the water quality in Dianshan Lake has diminished in recent years with increasing eutrophication and frequent algal blooms (Zheng and Wang 2009).

Eutrophication in lakes usually stimulates microbial growth, and an algal bloom occurs if such growth has not been restricted, which will not only destroy the stability of the water ecological system as well as lower water transparency and dissolved oxygen content, but also presents enormous threats to the safety of public drinking water due to toxins produced by some cyanophyta (Liang et al. 2013). Therefore, the cyanobacterial bloom is one of the most serious problems that can occur in a eutrophic freshwater system in terms of drinking water safety and quality (Sheng et al. 2012).

Because most microorganisms in a natural environment cannot be cultured, analyzing the microorganisms via the use of genetic information and special functional genes of different microbes is important (Briones and Raskin 2003; Katariina and Matti 2011). Molecular biology technologies, such as analysis of gene libraries based on cloning methods, polymerase chain reaction (PCR), and denaturing gradient gel electrophoresis (DGGE) can be applied to analyze quickly the diversity of a planktonic algal community and to reveal the regulation on succession, phylogenetic, and population dynamics of the algal community (Foulds et al. 2002; Hisbergues et al. 2003; Malik et al. 2008). However, the dynamics of the cyanobacterial community structure in Dianshan Lake have not been well studied using modern molecular biology technology, which limits the study of relationships between the cyanobacteria and abiotic parameters.

In order to understand better the dynamics of the cyanobacterial community in Dianshan Lake, the temporal variation of the cyanobacterial community was investigated

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using PCR-based molecular methods in this study. The relationships between the structure of the cyanobacterial community and abiotic parameters were analyzed statistically, and the results are expected to provide fundamental information useful for the control of eutrophication and algal blooms in Dianshan Lake.

Materials and methods

Sampling site layout

The water flow into Dianshan Lake is from the northwest to the southeast with entrances at Dazhuku (P8) and Jishui port (P9) and outlets at P2 (Natatorium) and P3 (Xizha) into the Huangpu River (Fig. 1). The hydraulic retention time is approximately 29 days. The water flow rate into Dianshan Lake is only 0.03 m/s and decreases from near the shore to the center of the lake.

Water samples were collected once per month from May 2009 to February 2010 from Dianshan Lake. Thirteen sampling sites were selected (P1 to P13 in Fig. 1): Dianfeng, Natatorium, and Xizha in the east; Zhaotian Lake and Qiandun Port in the north; Dazhuku and Jishui Port in the middle; and Baishiji and Cage fishery in the south. P6, P8, P9, and P12 are inflow monitoring points located on the border of Jiangsu Province. P1 and P3 are the major outlets of Dianshan Lake located in Shanghai city.

According to the HouWen division method of the four seasons, which is commonly used in China (Zhang et al. 2008), March to May is spring, June to September is summer, October to November is autumn, and December to February is winter in Shanghai.

Water sampling and analysis

Water samples were collected in parallel using an organic glass hydrophore HQM-1 (2.5 L) from the subsurface, 0.5 m beneath the water surface. Samples were collected in 1-L sampling bottles and stored at low temperature. Temperature, dissolved oxygen (DO), and pH were measured onsite with portable equipment (Shanghai Leici Co., China). Other water physical and chemical parameters, such as biological oxygen demand (BOD_5), ammonia (NH_3-N), total nitrogen (TN), orthophosphate (MPO_4), and total phosphate (TP) were determined according to standard methods (APHA 1998).

Whole-cell PCR assay of cyanobacteria

For whole-cell PCR assay of cyanobacteria in water, microbial samples were collected by centrifugation (10,000 rpm, 10 min) and re-suspended in 30 μL double distilled water for in situ whole-cell detection.

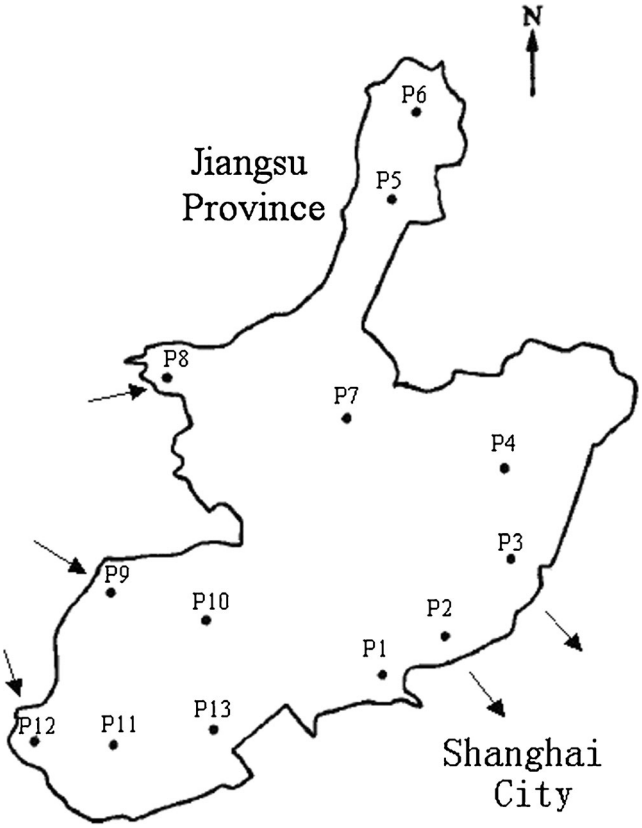

Fig. 1 Sampling sites in Dianshan Lake. The direction of the arrows represents the water flow. P1 Dianfeng, P2 Natatorium, P3 Xizha, P4 The east area, P5 Zhaotian Lake, P6 Qiandun Port, P7 The north area, P8 Dazhuku, P9 Jishui Port, P10 The middle area, P11 The south area, P12 Baishiji, and P13 Cage fishery

To detect the presence of cyanobacteria in the water samples, conserved 16S rRNA sequences of cyanobacteria in samples were amplified using primers for 27 F1 (5'-AGAG TTTGATCCTGGCTCAG-3') and 409R (5'-TTAC AA(C/T)CCAA(G/A)(G/A)(G/A)CCTTCCTCCC-3') (Zhang et al. 2005). The length of the PCR products was 400 bp. The detection limit of PCR could reach 10^2 cells/mL.

Each 25-μL PCR mixture contained 4 μL 10× PCR buffer (containing Mg^{2+}) (final Mg^{2+} concentration was 3.2 mmol/L), 0.8 μL (10 mmol/L) dNTPs, 10 pmol primer 27 F1, 25 pmol primer 409R, 2 U Taq enzyme, 2 μL BSA (5 mg/μL), 10 μL of a water sample, and the remainder was double distilled water. The following PCR program was performed on a PCR thermocycler (MJ research PTC 200, USA): predenaturing temperature was at 95 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 57 °C for 1 min, and 65 °C for 2 min. The final extension step was set at 65 °C for 30 min. After amplification, the PCR products were detected by 1.0 % (W/V) high resolution agarose gel electrophoresis. After electrophoresis, agarose gel was dyed in the dark for 10 min with nucleic acid dye (SDNA) before being visualized under ultraviolet light, and digital images were recorded.

DNA extraction from cyanobacteria

To extract total DNA in water samples, 200 mL of each water sample was filtered through a 0.22-μm cellulose acetate filter membrane, and total DNA was extracted using DNA extraction kits produced by Shanghai Biocolor Bioscience & Technology Company Ltd. (China). Detailed extraction procedures were performed following the manufacturer's instructions. Extracted DNA was stored at –20 °C for subsequent PCR-DGGE analysis.

PCR-DGGE analysis

To elucidate the temporal variation of diversity and structural changes in the cyanobacterial community, PCR products of specific 16S rRNA gene fragments of cyanobacteria were analyzed with DGGE. The 16S rRNA genes of cyanobacteria in samples were amplified using primers for CYA359F (5'-GGGGAATYTTCCGCAATGGG-3') and CYA781R (Ye et al. 2011). The reverse primer for CYA781R was an equimolar mixture of CYA781R (a) (GACTACTGGGGTATCT AATCCCATT) and CYA781R (b) (GACTACAGGGGTAT CTAATCCCTTT). The length of the PCR products was 422 bp. The 5' end of the CYA359F sequence was coupled to a GC clamp, which was CYA359F-GC (CGCCCGCCGC GCCCCGCGCCGGTCCCGCCGCCCCCGCCC).

Each 50-μL PCR mixture contained 5 μL 10× PCR buffer, 1 μL (10 mmol/L) dNTPs, 3 μL $MgCl_2$, 0.5 μL primer (10 mmol/L) CYA781R (a), 0.5 μL primer (10 mmol/L) CYA781R (b), 1 μL primer (10 mmol/L) CYA359F, 2 U Taq enzyme, and 2 μL template DNA, and the remainder was double distilled water. The amplification program was as follows: predenaturing temperature was set at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 50 s, and 72 °C for 1 min. The final extension step was set at 72 °C for 10 min.

The PCR product of each sample was used for DGGE electrophoresis analysis. DGGE was performed using the Bio-Rad Dcode System (Bio-Rad, Hercules, CA, USA). The PCR products were loaded onto 8 % (w/v) polyacrylamide gels in 1 TAE (20 mM Tris, 10 mM acetate, 0.5 mM EDTA, pH 8.0). The polyacrylamide gel had a linear denaturant gradient ranging from 20 % to 60 % [100 % denaturant contained 7 mo1/L urea and 40 % (v/v) deionized formamide]. Electrophoresis was performed for 3.5 h at 200 V at a constant 60 °C. Then the gel was stained in GelRed (NaCl 0.1 mol/L) and digitized using a gel documentation system (Shanghai Tannon Instrument Co., China).

After imaging, the dominant bands and the characteristic bands on DGGE gels were cut and transferred into a centrifuge tube for recycling. Samples were sterilized with 30 μL of 70 % cold ethanol, followed by trituration of the gel, 25 μL sterile double distilled water was added, and samples were dissolved at 4 °C for 24 h. After centrifugation, a 1 or 3 μL template was taken according to the original fragment PCR reaction system (without GC clamp) and procedures for re-amplification, and the products of amplification, were sent to Shanghai Sangon Co., Ltd. for sequencing.

All the sequences were queried against the GenBank database (http://www.ncbi.nlm.nih.gov) by BLAST algorithm (Altschul et al. 1990). Molecular Evolutionary Genetics Analysis (MEGA) 4.0 software was used to establish the phylogenetic tree (Kumar et al. 2002). Using the p-distance algorithm and neighbor-joining method, the bootstrap method was used to construct the evolutionary tree according to the evolutionary distance matrix and a replicate value of 1,000. The sequences determined in this study are available in GenBank under JF431074~JF431085.

Statistical correlation analysis

Data for the variations in physicochemical parameters over time in Dianshan Lake are given as mean ± standard deviation (SD). All statistical analyses of the data were performed using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA), and $P< 0.05$ was considered statistically significant.

To verify the relationships between the cyanobacterial community structure and abiotic parameters, the Canoco 4.5 software package was used for canonical correspondence analysis (CCA) of DGGE profiles for cyanobacteria and water quality parameters (Braak and Verdonschot 1995). The arrows represent different water quality parameters, and the direction of the arrows represents the correlation between each variable and the canonical axes. The length of the arrows represents the relative contribution of water quality parameters to the axes. Cyanobacterial community data were transformed to the files named species.dta and environment.dta using WcanoImp. Canoco 4.5 was applied for Windows computing, and the double sequence diagram was drawn in Canodraw.

Results

Temporal variation of the water physicochemical factors

The changes in average values of water parameters over time in Dianshan Lake are shown in Table 1. From May 2009 to February 2010, the water temperature was in the range of 5.12 °C to 29.07 °C, and the annual average water temperature was 19.06 °C. The annual pH value in Dianshan Lake changed little, remaining between 7.18 and 7.55. The concentration of DO was higher in winter than in summer and was in the range of 6.23 mg/L to 13.06 mg/L. The average concentration of BOD_5 was maintained at 6.14 mg/L, and the organic pollution also was maintained at a stable level.

The average concentration of TN was 3.84 mg/L, and TN in winter (5.63 mg/L) was higher than that in summer (3.07 mg/L) or autumn (3.06 mg/L). The concentration of NH_3-N ranged from 0.16 mg/L to 0.24 mg/L, and it was

Table 1 Variations in physico-chemical factors in Dianshan Lake

Index time	Temperature (°C)	pH	DO (mg/L)	BOD_5 (mg/L)	NH_3-N (mg/L)	TN (mg/L)	MPO_4 (mg/L)	TP (mg/L)
2009.05	22.41	7.33	6.89	5.93	0.20	3.39	0.077	0.20
2009.06	28.91	7.29	6.61	5.74	0.24	2.73	0.136	0.24
2009.07	29.07	7.27	7.19	6.21	0.24	3.01	0.150	0.24
2009.08	27.49	7.35	6.23	6.13	0.21	3.15	0.132	0.21
2009.09	26.37	7.18	7.70	5.74	0.18	1.77	0.085	0.18
2009.10	24.00	7.42	7.55	6.31	0.16	2.74	0.038	0.16
2009.11	10.97	7.51	8.71	6.26	0.19	4.65	0.106	0.19
2009.12	5.12	7.55	11.54	6.31	0.18	4.98	0.094	0.18
2010.01	6.50	7.42	13.06	6.29	0.23	5.95	0.062	0.23
2010.02	9.78	7.42	10.03	6.50	0.19	5.98	0.076	0.19
Average± SD	19.06±9.78	7.37± 0.11	8.55± 2.28	6.14± 0.26	0.20± 0.03	3.84± 1.46	0.10± 0.04	0.20± 0.03

SD: standard deviation.

higher in summer and lower in autumn. The concentration of MPO_4 was in the range of 0.038 mg/L to 0.150 mg/L, and the overall trend was the same as that for NH_3-N. The concentration of TP in Dianshan Lake showed small fluctuations between 0.16 mg/L and 0.24 mg/L and was highest in summer.

Statistical analysis revealed that among the N- and P-related factors in Dianshan Lake, NH_3-N had a significantly positive correlation with TN ($P<0.01$), and these parameters both had a positive correlation with DO ($P<0.05$) and a significant negative correlation with temperature ($P<0.01$). By contrast, MPO_4 and TP showed no correlation with the other factors ($P>0.05$).

Whole-cell PCR assay of cyanobacteria

The results of the whole-cell PCR amplification test (Table 2) showed that the cyanobacteria began to appear at different sampling sites in April, with the positive rate of cyanobacteria in this month at 46 %. From May to June, the positive rate of cyanobacteria increased. From July to September in summer, the positive rate of cyanobacteria reached 100 % among the sampling sites. After October, the rate of cyanobacteria presentation in samples decreased significantly and was reduced to only 23 % in autumn and winter, which mainly occurred at the P6, P7, and P8 sites located in the north area of the lake. These results show that the quantities of cyanobacteria in spring and summer were larger than those in autumn and winter in Dianshan Lake, and that P8, one of the influent sites, had the highest positive rate.

DGGE profile analysis of the cyanobacterial community

As cyanobacteria grow well in summer and have a potential risk for bloom (O'Neila et al. 2012), the DGGE profile of July was selected and bands were sequenced (Fig. 2). Figure 2

Table 2 Whole-cell PCR test of cyanobacteria in Dianshan Lake

Sampling sites	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Positive rate
P1	−	−	−	+	+	+	−	−	+	44 %
P2	−	+	−	+	+	+	−	−	+	56 %
P3	−	+	+	+	+	+	−	−	+	67 %
P4	+	+	−	+	+	+	−	−	−	56 %
P5	−	+	+	+	+	+	−	−	−	56 %
P6	−	+	+	+	+	+	+	−	−	67 %
P7	−	+	−	+	+	+	+	+	+	78 %
P8	+	+	+	−	+	+	+	+	+	89 %
P9	+	+	+	+	+	+	−	−	−	67 %
P10	−	+	−	+	+	+	−	−	−	44 %
P11	+	−	+	+	+	+	−	−	−	56 %
P12	+	+	+	+	+	+	−	+	−	78 %
P13	+	−	+	+	+	+	−	−	−	56 %
Positive rate	46 %	77 %	62 %	92 %	100 %	100 %	23 %	23 %	38 %	

Note: "+" denotes positive; "–" denotes negative.

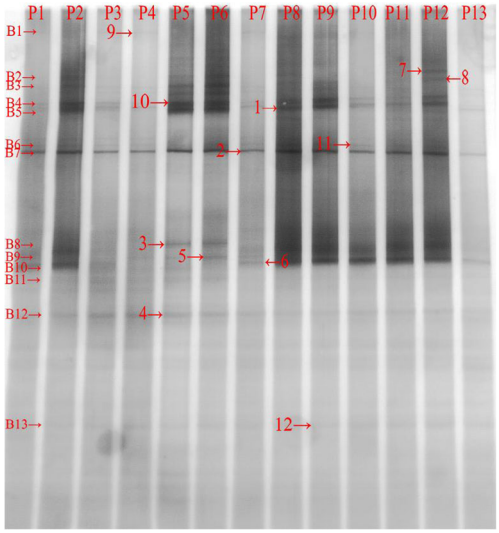

Fig. 2 DGGE profiles of cyanobacteria 16S rRNA gene fragments in Dianshan Lake. B1 ~ B13 represent the shared bands in DGGE profiles, and marked bands 1 ~ 12 were excised and sequenced. P1 ~ P13 represent different sampling sites

shows the DGGE profile of the cyanobacterial community of all 13 sampling sites in Dianshan Lake for samples collected in July. Differences in the number and brightness of bands were observed among the different sites. However, the shared bands, such as 2, 4, and 11, appeared for different sites and were prevalent in summer. From the Blast results of Genbank, bands 2, 4, and 11 are known to represent *Microcystis* (Table 3), and their higher brightness in the lane shows that *Microcystis* was the predominant species of cyanobacteria in Dianshan Lake. For the sites of P6, P9, and P12, the numbers of bands were significantly higher than that for other sites, suggesting that the population density of cyanobacteria was higher at inflow sites.

The sequence results of bands 1 to 12 (Fig. 2) are shown in Table 3, and 50 % of the bands represented the *Microcystis* genus, which is the dominant species of cyanobacteria in Dianshan Lake in summer. The rest of the bands belonged to the *Synechococcus* genus and uncultured cyanobacterium. Most bands had considerable similarity with known sequences (99 % similarity), which were detected previously in the Lake of China and other European countries.

The sequences obtained in this experiment, named DSHCYAN 1 to 12, were used to establish a phylogenetic tree by MEGA 4.0 software. Figure 3 shows that most cyanobacteria in Dianshan Lake were similar to *Microcystis*, whereas some cyanobacteria were similar to *Synechococcus* sp. and uncultured cyanobacterium.

The number of bands on DGGE profiles represents the population abundance of cyanobacteria, and the statistical results are shown in Fig. 4. Over the four seasons, the abundance of the cyanobacterial population was highest in summer and lowest in autumn. The general characteristics were consistent with the whole-cell PCR detection rate.

CCA of the cyanobacterial community and water quality parameters

The results of the CCA-based analysis of DGGE data and water quality parameters demonstrate mutual relationships between the communities and water quality parameters, including temperature, pH, DO, BOD_5, NH_3-N, TN, MPO_4, and TP (Fig. 5).

The relationships between cyanobacterial community composition and water quality parameters, and those between water samples from different sites collected during the four seasons and water quality parameters are shown in Fig. 5a and b, respectively. The pH and DO along Axis 1 were positively correlated with correlation coefficients of 0.8861 and 0.5172, respectively. MPO_4 and TP along Axis 1 were negatively correlated with correlation coefficients of -0.7077 and -0.4743, respectively. The correlation coefficients for TN, NH_3-N, and BOD_5 along Axis 1 were smaller at 0.3722, 0.0641, and 0.1162, respectively. The correlation coefficients between the water quality parameters along Axis 2 were lower than the absolute value of 0.3 and, therefore, the correlations were not significant.

In Fig. 5a, the vertical distance from the sorting position of 46.2 % of the bands, including B4 and B9 to B13 vs temperature, MPO_4, and TP was much shorter than the vertical distance of the other bands, and these dominating bands indicate that cyanobacterial species were influenced significantly by these three water parameters. In addition, 23.1 % of bands, such as B2, B3, and B8, were influenced by pH and DO; 7.7 % of bands, such as B6, were influenced by TN; 7.7 % of bands, such as B7, were influenced by NH_3-N; and 15.3 % of bands, such as B1 and B5, had no significant correlation with water quality parameters. In Fig. 5b, when the points were closer on the ordination diagram, they had more similar features to adapt to the environment. Samples taken in July during summer and in May during spring were basically found on the left portion of the ordination, whereas samples collected in autumn and winter were basically found on the right side of the ordination. These results show that temperature, MPO_4, and TP had significant effects on the cyanobacterial community structure during spring and summer, whereas TN, pH, and DO strongly influenced and were significantly correlated with the cyanobacterial community structure during autumn and winter.

Discussion

The concentration of TN and TP in Dianshan Lake showed different trends across the seasons. For example, TN in winter

Table 3 Sequence similarities to closest relatives in Genbank based on DNA recovered from DGGE gels

Band			Closest relative			
No.	Accession No.	Similarity (%)	Organism	Accession No.	Phylogenetic affiliation	Source
1	JF431074	99 %	*Microcystis aeruginosa* LMECYA 7	EU078485	Cyanobacteria; *Microcystis*	*Microcystis aeruginosa* LMECYA 7
2	JF431075	98 %	*Microcystis flos-aquae*	AF139329	Cyanobacteria; *Microcystis*	*Microcystis flos-aquae* UWOCC-C3
3	JF431076	99 %	Uncultured cyanobacterium DGGE gel band LT-CYA-22	GQ848178	Cyanobacteria; environmental samples	China: Lake Taihu
4	JF431077	99 %	*Microcystis aeruginosa* CALU 972	DQ786007	Cyanobacteria; *Microcystis*	Russia: Lake Kroshnoozero
5	JF431078	99 %	*Microcystis* novacekii MCYS-CH01	EU541973	Cyanobacteria; *Microcystis*	Algeria: *Microcystis novacekii* MCYS-CH01
6	JF431079	99 %	*Microcystis* sp. CYN06	EF634465	Cyanobacteria; *Microcystis*	New Zealand: Lake Hakanoa
7	JF431080	99 %	*Microcystis aeruginosa* NIES-298	FJ461749	Cyanobacteria; Chroococcales; *Microcystis*	*Microcystis aeruginosa* NIES-298
8	JF431081	98 %	*Synechococcus* sp. 0tu30s01	AM259220	Cyanobacteria; *Synechococcus*	Finland: Lake Tuusulanjarvi
9	JF431082	99 %	*Microcystis* sp. CHAB731	FJ595695	Cyanobacteria; *Microcystis*	China: Lake Ulungur, Xinjiang
10	JF431083	97 %	Uncultured cyanobacterium clone TH_c287 16S rRNA gene	EU373159	Cyanobacteria; environmental samples	China: Lake Taihu
11	JF431084	99 %	*Microcystis viridis* NIES-1058	DQ648029	Cyanobacteria; *Microcystis*	*Microcystis viridis* NIES-1058
12	JF431085	99 %	Uncultured cyanobacterium clone TH_f17 16S rRNA gene	EU980172	Cyanobacteria; environmental samples	China: Lake Taihu

was significantly higher than that in summer and autumn ($p < 0.05$), and it reached a maximum in January. One reason is the dry season when the volume of the lake decreased and the nutrient concentration of upstream Taihu Lake increased. Another reason is that the low temperature in winter is not conducive to the growth of cyanobacteria, which decreases the uptake of the nitrogen from the water (O'Neila et al. 2012; Böhme 1998). For TP, the temporal variation amplitude was small. The concentration of TP was higher in summer than in autumn and winter when it was relatively stable. The temporal difference was not significant ($P > 0.05$). In summer, the phytoplankton bloomed and the associated microbial metabolism absorbed and converted soluble phosphorus. Therefore, the concentration of TP and orthophosphate began to decrease after August. After October, because the phytoplankton grew slowly and utilized limited nutrients, the concentration of TP stabilized. However, compared with the National Surface Water Standard (GB3838-2002), the TN concentration was beyond the level V (the lowest level of the surface standard) of 2 mg/L in most samples, whereas TP in half of the samples surpassed the level V of 0.2 mg/L, showing that Dianshan Lake is polluted with nitrogen and phosphorus, which may support the growth and reproduction of cyanobacteria by leading to eutrophication.

A whole-cell PCR assay was performed to detect directly the presence of cyanobacteria in natural water samples. This method is simple and fast without requiring DNA extraction, and thus, provides an effective and rapid method for large scale monitoring and early detection of cyanobacteria in surface freshwater (Barón-Sola et al. 2012). The PCR results verified the pattern proposed by the correlational research. The cyanobacterial community began to appear in May during late spring and reproduced to generate a large population from July to September during summer, which extended to October in early autumn and finally declined gradually in December during winter (Domingues et al. 2005). At the P6, P7, and P8 sites, which represent the north area of the lake, a certain concentration of cyanobacteria were detected after October, indicating that the north area of the west lake was influenced by effluent of Taihu Lake with slight pollution (Yang and Liu 2010). Even in December, cyanobacteria could still be detected in these areas, suggesting that the overall density of cyanobacteria in this area was higher than that in other areas. Although the whole-cell PCR test could not provide information regarding cyanobacteria species, the detection rate still rapidly shows the distribution and scope of cyanobacteria in Dianshan Lake.

PCR products of DNA fragments of the same length and different sequences can be separated in DGGE. In DGGE profiles, more bands in a lane implies a higher abundance of the cyanobacteria, and the brightness of a band represents the relative quantity of a certain species (Hansel et al. 2008). The DGGE profiles for the cyanobacterial community from April 2009 to March 2010 in Dianshan Lake showed that the abundance of the cyanobacterial community was higher in spring and summer than in autumn and winter. The occurrence

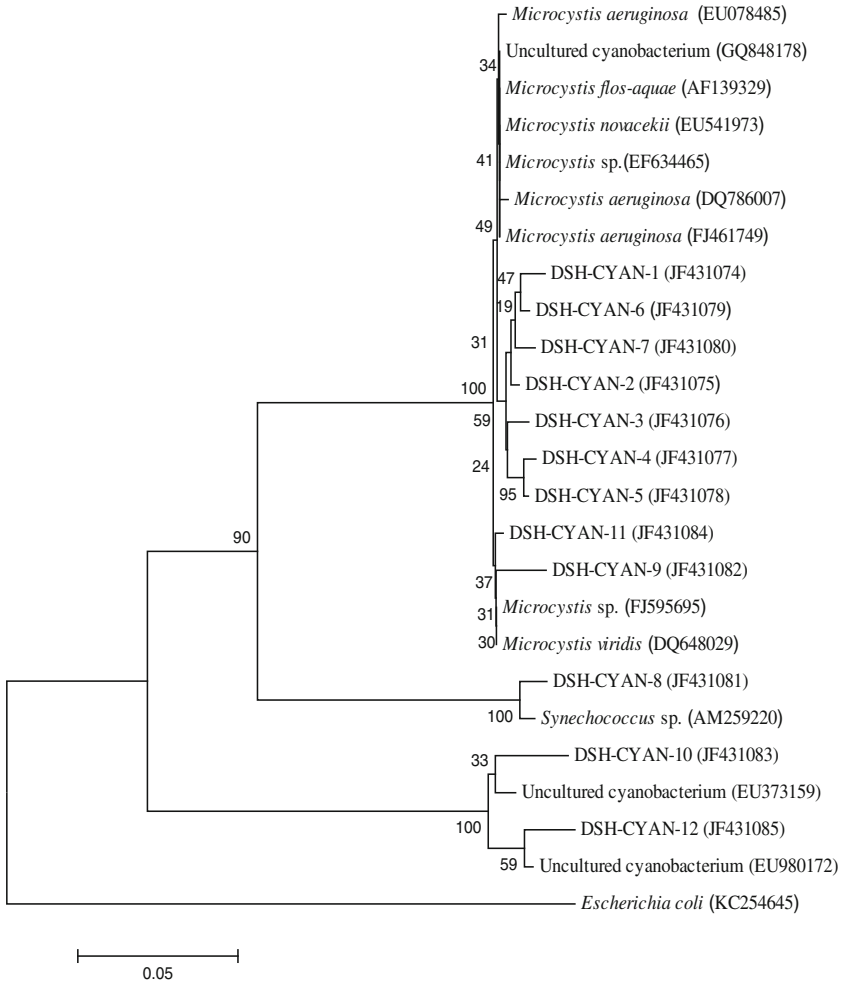

Fig. 3 Phylogenetic tree of 16S rRNA gene sequences of cyanobacteria in Dianshan Lake

of cyanobacteria could be attributed to high temperature, slightly alkaline conditions, and nutrient-rich freshwater, which favored cyanobacterial growth (George et al. 2012). The average water temperature in Dianshan Lake in summer was 27.96 °C, which falls within the optimum temperature range of 25 °C to 35 °C for the growth of most cyanobacteria. However, the water temperature in winter was low, and most cyanobacteria entered a decline phase in winter. The cyanobacterial community differed among the sampling sites (e.g. P6, P9, and P12), and the abundance of cyanobacteria

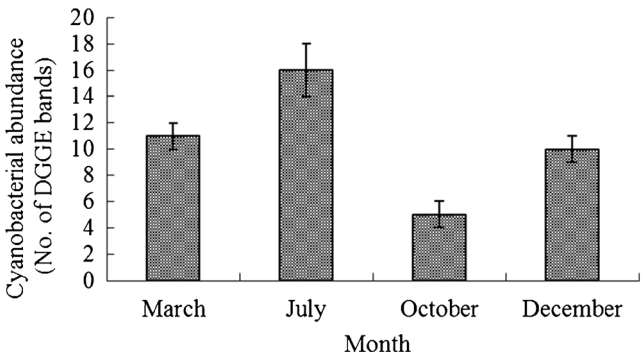

Fig. 4 Cyanobacterial abundance in Dianshan Lake during different seasons

was greater due to pollution from upper Taihu Lake effluent, which resulted in massive growth of cyanobacteria. The dominant species in Dianshan Lake was *Microcystis*, including *Microcystis aeruginosa* and *Microcystis flos-aquae*. This species not only grew well during spring and summer, but also during autumn and winter. The overwhelming dominance of *Microcystis* in various lakes around the world has been reported previously (Tan et al. 2009; Missona et al. 2012). At the same time, *Synechococcus* presents certain advantages. These species are in marine and fresh water environments and make important contributions to the primary production of the entire water ecosystem (Jochem 1988; Chiang et al. 2002). Some cyanobacteria species are very similar to those in Taihu Lake, suggesting that these cyanobacteria are common species in the Taihu basin, because Taihu Lake is in the upper reaches of Dianshan Lake.

The results of CCA showed that the presence of most identified species was correlated with water quality parameters. The reason might due to the common characteristics of the species (Bonilla et al. 2005). From the results shown in Fig. 5a, it was obvious that nearly 50 % of the cyanobacterial species, including *Microcystis aeruginosa* and other *Microcystis* sp., were influenced by temperature, MPO_4, and

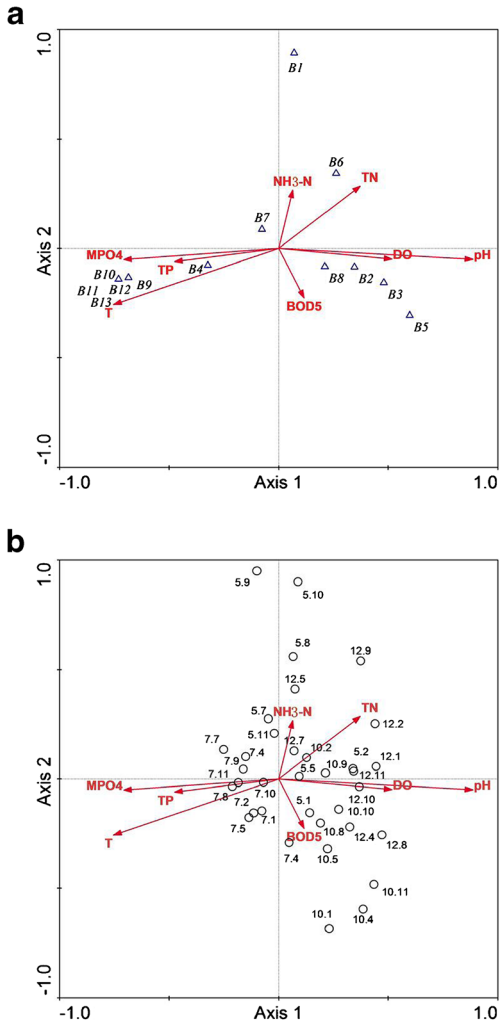

Fig. 5 CCA analysis of cyanobacterial community and water quality indexes in Dianshan Lake **a**. Δ B1~B13 represent the shared bands of May, Jul, Oct, and Dec in DGGE profiles, and the numbers represent the different dominant bands in DGGE profiles. **b**.○ 5.1~12.11 represent the water samples collected in May during spring, in July during summer, in October during autumn, and in December during winter, respectively. The number after the decimal point represents the sampling site. Sites 1, 2, and 4 represent the east area of the lake; 5, 7, and 8 represent the north area of the lake; 9 represents the west area of the lake; 10 represents the middle area of the lake; and 11 represents the south area of the lake

TP, suggesting that temperature and phosphorus were the main factors influencing the growth of these cyanobacteria species and had a major impact on the distribution of the cyanobacterial community. In addition, 23.1 % of cyanobacteria species including *Microcystis flos-aquae* and *Synechococcus* sp. among others were influenced by pH and DO, indicating significant roles of these two parameters in cyanobacterial distribution. From the results shown in Fig. 5b, it can be seen that the cyanobacterial community was highly correlated with temperature and phosphorus, demonstrating that temperature, MPO_4, and TP greatly influenced the cyanobacterial community during spring and summer. The reason may be that an appropriate temperature and high phosphorus concentration promote cyanobacteria growth and thus lead to large-scale water eutrophication. Therefore, it is essential to control the emission of phosphorus in order to prevent eutrophication. TN, pH, and DO had a significant impact on the cyanobacterial community during autumn and winter, which suggests that the cyanobacterial community is sensitive to TN, pH, and DO during these seasons.

Conclusions

TN and TP concentration at most sampling sites in Dianshan Lake were beyond level V of the National Surface Water Standard (GB3838-2002) during this investigation, showing the Dianshan Lake faces a risk of eutrophication.

The whole-cell PCR assay results indicated that cyanobacteria were more abundant during spring and summer than during autumn and winter in Dianshan Lake. This result was further supported by DGGE profile analysis. *Microcystis* was the dominant species of the cyanobacterial community in Dianshan Lake.

Nearly 50 % of cyanobacteria species were affected by temperature, MPO_4, and TP. MPO_4 and TP had significant effects on the cyanobacterial community structure during spring and summer. The cyanobacterial community was highly associated with temperature and phosphorus concentration in Dianshan Lake during spring and summer, and therefore, the control of phosphorus concentration is essential for preventing algal blooms in Dianshan Lake.

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