Seasonal variation of *Microcystis* in Lake Taihu and its relationships with environmental factors

TAN Xiao¹,², KONG Fanxiang¹,*, ZENG Qingfei¹, CAO Huansheng³, QIAN Shanqin¹,², ZHANG Min¹

¹. State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China. E-mail: tanxiao1030@gmail.com
². Graduate School of Chinese Academy of Sciences, Beijing 100049, China
³. Department of Biological Sciences, Fordham University, New York 10458, USA

Received 06 August 2008; revised 29 October 2008; accepted 06 November 2008

Abstract

In order to monitor the changes of *Microcystis* along with temporal and spatial variations, seasonal variation of *Microcystis* in Lake Taihu was investigated by 16S-23S rRNA internal transcribed spacer denaturing gradient gel electrophoresis (16S-23S rRNA-ITS DGGE) and microscopic evaluation. Samples were collected quarterly at four sites (River Mouth, Meiliang Bay, Cross Area, and Lake Center) from August 2006 to April 2007. Results showed that *Microcystis* dominated total phytoplankton abundance at the four sites in all seasons except winter. The average annual abundance of *Microcystis* was relatively high at River Mouth and Meiliang Bay, reaching $81.22 \times 10^6$ and $61.32 \times 10^6$ cells/L, respectively. For temporal variations, Shannon-Wiener diversity index ($H'$) according to DGGE profile revealed the richness of *Microcystis* in summer ($H' = 1.375 \pm 0.034$) and winter ($H' = 1.650 \pm 0.032$) was lower than that in spring ($H' = 2.078 \pm 0.031$) and autumn ($H' = 2.365 \pm 0.032$) ($P < 0.05$). While for spatial variations, the richness of *Microcystis* at River Mouth ($H' = 2.015 \pm 0.074$) was higher than at other sites during four seasons ($P < 0.01$). Very few differences of *Microcystis* diversity in the same season were observed among the other three sites ($P > 0.05$). Canonical correspondence analysis (CCA) was performed to elucidate the relationships between *Microcystis* operational taxonomic units (OTUs) composition and the environmental factors. Results of CCA revealed that temperature was strongly positively correlated with the first axis ($r = 0.963$), while TSS was negatively correlated with the second axis ($r = -0.716$). Phylogenetic tree based on the sequencing results of target bands on DGGE gel indicated that samples collected in summer and winter constituted two separated clusters.

Key words: Lake Taihu; blooms; *Microcystis*; rRNA-ITS; DGGE; canonical correspondence analysis
DOI: 10.1016/S1001-0742(08)62359-1

Introduction

Lake Taihu is located between 30°05′N and 32°08′N and between 119°08′E and 122°55′E, which is one of the large eutrophic freshwater lakes in China (with an area of 2338 km², the annual average water depth of 1.9 m and maximum of 2.6 m) (Hu et al., 2006). Major cyanobacteria blooms composed of *Microcystis* have appeared for decades in lake (Chen et al., 2003). Meiliang Bay lies in the northern part of Lake Taihu, where several rivers (including Zhihugang River) flow into Meiliang Bay carrying wastewater from cities of Wuxi and Changzhou. Therefore, Meiliang Bay has become one of the most eutrophic regions in Lake Taihu (Chen et al., 2003; Xing and Kong, 2007).

In these eutrophic regions, different species of *Microcystis* dominated phytoplankton community from May to October each year (Chen et al., 2003). It was hard to distinguish these morphologically similar species within genus *Microcystis* in field samples by microscopic analysis alone. Previous studies has reported that the molecular diversity of cyanobacteria in Antarctica, which was much greater than the diversity currently known based on traditional microscopic analysis (Taton et al., 2003).

Denaturing gradient gel electrophoresis (DGGE) has become a powerful tool for monitoring genetic diversity in various environmental samples (van Hannen et al., 1998; Xing and Kong, 2007; Zwart et al., 2005). DGGE is capable of resolving minute DNA fragments differences in the same genus such as *Synechococcus* and *Aphanizomenon* (Becker et al., 2002; Laamanen et al., 2002). In recent years, DGGE was applied to 16S-23S rRNA internal transcribed spacer (16S-23S rRNA-ITS) analyses for *Microcystis* investigation in field samples (Janse et al., 2003; Kardinaal et al., 2007). Additionally, some toxic and nontoxic strains of *Microcystis* have been distinguished according to 16S-23S rRNA-ITS (Otsuka et al., 1999).

A seasonal succession of different *Microcystis* genotypes can be a key factor in determining microcystin...
concentrations in Microcystis-dominated lakes (Kardinaal et al., 2007). Furthermore, it is necessary to obtain intensive information about the composition of different Microcystis genotypes among the blooming seasons and non-blooming seasons. This information is useful to explore the forming and developing mechanisms of Microcystis blooms in Lake Taihu. Thus, four sampling sites were selected to investigate minute differences within Microcystis in each season using 16S-23S rRNA-ITS DGGE and microscopic analysis, so as to monitor Microcystis change along with temporal and spatial variations. In addition, the relationships between Microcystis variation and environmental factors were explored.

1 Materials and methods
1.1 Sampling and environmental factors
A 2-m long sampler was used to collect integrated water samples at four sites (River Mouth, Meiliang Bay, Cross Area, and Lake Center) (Fig. 1). Samples were collected seasonally (summer: August 2006; autumn: November 2006; winter: January 2007; spring: April 2007). One liter of each water sample was fixed with Lugol’s iodine and sedimented for 48 h prior to phytoplankton microscopic analysis. Meanwhile, 200 mL of each water sample was sieved through GF/C membranes (Whatman, UK). Filters were stored at –20°C until DNA extraction.

The physico-chemical parameters, including temperature, concentrations of dissolved oxygen (DO), concentrations of chlorophyll a (Chl-a), and pH were recorded real-time by a multi-parameter water quality sonde (YSI 6600, USA). Other parameters, such as total nitrogen (TN), total phosphorus (TP), dissolved inorganic phosphorus (DIP), and total suspended solids (TSS) were analyzed according to the standard methods (Huang, 2000).

1.2 DNA extraction and PCR amplification
The DNA extraction was performed according to the method by Zhou et al. (1996) with modification. Filters were mixed with 800 µL of DNA extraction buffer (100 mmol/L Tris-HCl (pH 8.0), 100 mmol/L sodium EDTA, 100 mmol/L sodium phosphate, 1.5 mol/L NaCl, 1% (W/V) hexadecyltrimethyl-ammonium bromide) and 20 µL of proteinase K (20 mg/mL) in a 2-mL centrifuge tube. Tubes were incubated at 37°C for 30 min. After that, 480 µL of 20% (W/V) SDS (sodium dodecyl sulfate) was added, and then, tubes were laid in 65°C water bath for 2 h with gentle shakes every 15 min. Supernatants were collected after centrifugation at 6000 r/min for 10 min. Subsequently, they were transferred into fresh centrifuge tubes and mixed with an equal volume of phenol/chloroform/isooamyl alcohol (25:24:1, V/V/V). Aqueous phase was obtained by centrifugation at 8000 r/min for 10 min and extracted again with chloroform/isooamyl alcohol (24:1, V/V) to remove phenol. Thereafter, aqueous phase was recovered by centrifugation of 8000 r/min for 10 min. Total nucleic acids were precipitated from aqueous phase with 0.6 volumes of precooled isopropanol. The crude DNA was obtained by washing in cold 70% ethanol and air dried prior to resuspension in 50 µL TE buffer (pH 8.0).

16S-23S rRNA-ITS of cyanobacteria in samples were amplified by using primers CSIF (5′-GYCA CGCCCGAAGTCTTAC-3′) and ULR (5′-CCTGGTGCCTAGGTATC-3′), a 40-bp GC clamp (5′-GGCAAAAGTCRTTAC-3′) and ULR (5′-CCTGGTGCCTAGGTATC-3′) was added to the 5′ end of CSIF for DGGE analysis (Janse et al., 2003). A 50-µL of PCR mixture contained Tris-HCl (pH 8.8) 10 mmol/L, KCl 50 mmol/L, MgCl₂ 1.5 mmol/L, NP-40 0.08% (W/V), template DNA 100 ng, dNTP 0.2 mmol/L, primers each 0.2 µmol/L, BSA 0.8 µg/µL, Taq DNApolymerase (Takara, Japan) 2 U. PCR program was performed on a PCR thermocycler (MJ research PTC-200, USA) as the following manner. Initial denaturing temperature was at 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 2 min. The final extension step was at 72°C for 15 min. The PCR products were detected by 1.5% (W/V) high resolution agarose gel electrophoresis. Bands of 550 bp fragments yielded by Microcystis (Janse et al., 2003) were excised by an axenic scalpel and purified through Agarose-Gel DNA extraction mini kit-spin column (Tiangen, China) prior to DGGE.

1.3 DGGE procedure
DGGE was carried out on the DGGE-2001 system (C.B.S., USA). A 500-ng of each target PCR product yielded by Microcystis was loaded into 6% (W/V) polyacrylamide gel (acylamide/ N,N’-methylene bisacrylamide, 37.5/1, W/W). The gel contained a linear denaturant gradient ranging from 25% to 55% (100% denaturant contained 7 mol/L urea and 40% (V/V) de-ionized formamide). Electrophoresis was performed at 60°C in 1× TAE buffer (pH 8.0) for 16 h. After that, gel was dyed in 0.01% (W/V)
SYBR Green I (Molecular Probes, USA) for 30 min, and then photographed by gel explorer (Omega 10, USA).

1.4 Data analysis

Quantity One software (version 4.6) was used for DGGE profile analysis. Lanes and bands were detected automatically; the densitometric curve of each lane was calculated. Bands with a relative intensity of less than 0.3% were discarded. These analyses procedures were performed three times.

Shannon-Wiener diversity index ($H'$) (Shannon and Weaver, 1949) of each lane was calculated by the following Eq. (1): $H' = -\sum_{i=1}^{n} p_i \ln p_i$

where, $p_i$ (the importance probability of a band in a lane) equals $n_i/N$, $n_i$ is the peak height of the band, $N$ is the sum of all peak heights in lane (Gafan et al., 2005; Xing and Kong, 2007).

Each band (a relative intensity greater than 0.3%) on DGGE was considered to be one operational taxonomic unit (OTU). A matrix was constructed according to the location of each OTU and its relative intensity. Canonical correspondence analysis (CCA) was performed by using CANOCO software (version 4.5) to elucidate the relationships between Microcystis OTUs composition and environmental factors. Furthermore, environmental factors were identified by forward selection with 499 unrestricted Monte Carlo permutations to test the intensity of their influences (ter Braak and Šmilauer, 2002).

1.5 Sequencing of bands on DGGE gel and phylogenetic analysis

Some bands on DGGE gel were excised and mixed with 50 µL TE (pH 8.0). They were laid at 4°C overnight before 16S-23S rRNA ITS fragments were amplified again using 1 µL of the eluent as template together with original primers. Then, bands were confirmed by rerunning DGGE. Subsequently, target bands were excised again and amplified with primers without GC clamp. These amplified products were linked with pGEM-T easy vector (Promega, USA) and transformed into E. coli DH5α competent cells (Takara, Japan). Finally, successfully inserted plasmids were purified and sequenced using an automated sequencer (ABI 3730, USA). Programs of CLUSTAL X (version 1.8) and MEGA (version 4.0) were applied to analyze phylogenetic relationships of the sequencing results. Phylogenetic tree was constructed by the neighbor-joining method. Support for tree branch points was estimated by the interior branch test approach through 500 replicates.

2 Results and discussion

2.1 Microcystis dynamics

In this study, Microcystis was dominated at all four sites in summer, autumn, and spring. According to the microscopic analysis, variations in Microcystis community composition did not show evident trends during four seasons at these sites. Differences of dominant species (Microcystis aeruginosa, Microcystis wesenbergii, and Microcystis flos-aquae) in different samples were not significant (Fig. 2, $P > 0.05$). Microcystis abundance decreased obviously in samples collected in winter, where considerable amount of Bacillariophyta or Chlorophyta were found. Average annual abundances of Microcystis at River Mouth ($81.22 \pm 2.43 \times 10^6$ cells/L) and Meiliang Bay ($61.32 \pm 1.92 \times 10^6$ cells/L) were higher than those at the other two sites ($P < 0.05$) (Fig. 3).

The overwhelming dominance of Microcystis in various lakes of the world has been explained by water temperature, underwater climate (turbidity or suspended solids or Secchi-depth), nutrients, buoyancy regulation, and so on (Brookes and Ganf, 2001; Dokulil and Teubner, 2000; Ibelings et al., 1991; Okino, 1973; Reynolds et al., 1987; Roberts and Zohary, 1987; Smith, 1983). Among these factors, temperature plays an important role (Chen et al., 2001; Del Giorgio et al., 1991; Komárková et al., 2003). In this study, the water temperature from April to November...
at sampling sites was in the range of 18.2–30.4°C. It is commonly observed that Microcystis was in favor of the warm temperature and established dominance during this period. These results are in agreement with previous studies in Lake Taihu (Chen et al., 2001, 2003).

Average cell densities of Microcystis in four seasons at River Mouth and Meiliang Bay were higher than those at other two sites, which perhaps was attributable to two reasons. First, eutrophication was more serious at River Mouth and Meiliang Bay. Higher concentration of nutrient salts yielded more Microcystis (Chen et al., 2003). Second, wind speed and direction affected the distribution of phytoplankton abundance (Cao et al., 2006). Microcystis aggregated and formed blooms on the water surface, which was more prone to be pushed by wind and wave than other phytoplankton. Sometimes serious Microcystis blooms in Meiliang Bay can be regarded as an accumulation from the open lake to Meiliang Bay (Chen et al., 2003).

2.2 Annual diversity variation of Microcystis

The 16S-23S rRNA-ITS DGGE profile displayed the bands yielded by different genotypes of Microcystis (Fig. 4). $H'$ was applied to analyze Microcystis diversity according to DGGE profile (Fig. 5). For temporal variations, the richness of Microcystis in summer ($H' = 1.375 \pm 0.034$) and winter ($H' = 1.650 \pm 0.032$) were lower than that in spring ($H' = 2.078 \pm 0.031$) and autumn ($H' = 2.365 \pm 0.032$) ($P < 0.05$). While for spatial variations, the richness of Microcystis at River Mouth ($H' = 2.015 \pm 0.074$) was higher than other sites during four seasons ($P < 0.01$). Very few differences in Microcystis diversity were observed among the other three sites in the same season ($P > 0.05$).

16S-23S rRNA ITS was less subject to selection pressure and accumulated more mutation sites than functional genes, it was a suitable marker for discriminating cyanobacteria strains at the intra- or inter-specific level (Casamayor et al., 2002; Otsuka et al., 1999). In this study, CSIF and ULR were selected as primers to amplify ITSc (the complete 16S-23S rRNA-ITS fragment). Compared with other cyanobacteria, unique band (550 bp) of Microcystis was amplified using this pair of primers (Janse et al., 2003, 2005). Thus, it was easy to excise target bands from PCR products and exclude disturbances from other cyanobacteria (Neilan, 2002). All these above were very suitable for Microcystis genus analysis. Moreover, changes in the relative abundance of each band can be revealed by quantitative analysis of DGGE profile (Casamayor et al., 2002). It was convenient to assess the diversity and relative abundance of each genotype of Microcystis in samples.

For the temporal variations, $H'$ derived from quantitative analysis of DGGE profile demonstrated that the richness of Microcystis was fairly low in summer and winter. Therefore, in summer blooms overwhelmingly consisting of only several genotypes of Microcystis, and some genotypes of Microcystis decreased rapidly from spring to summer. In winter, low abundances of Microcystis also comprised only a few genotypes of Microcystis. These phenomena were in agreement with a general ecological principle that more-extreme environments are expected to be inhabited by less diverse community (Frontier, 1985). In the spatial variations, $H'$ of samples collected at River Mouth was higher than that at other sites in four seasons. It may be caused by the inflow of Microcystis from Zhihugang River, which could enhance the richness of Microcystis at River Mouth to a certain degree. A previous study reported that moderate wind (3.3–5.0 m/s) could cause a strong mixing of the water in Lake Taihu during almost two-thirds of the year (Zhang et al., 2003). Intensive wind agitation may reduce differences in Microcystis diversity among the other three open sites in same season.

In the present study, $H'$ was applied to reflect the diversity of Microcystis genotypes. The number and relative intensity of bands both influenced $H'$, thus similar band patterns revealed different diversities. Moreover, in a mixture system, various DNAs present at different concentrations and the target templates varied in the PCR

![Fig. 4 Microcystis 16S-23S rRNA-ITS DGGE profile. Samples 1–4 were collected in summer, 5–8 in autumn, 9–12 in winter, 13–16 in spring. Samples collected in the same season were arranged in the order of River Mouth, Meiliang Bay, Cross Area, and Lake Center from the left to the right. Numbers in the bands indicated the excised fragments for sequencing.](image1)

![Fig. 5 Diversity of Microcystis in samples based on Shannon-Wiener diversity index ($H'$) derived from DGGE profile analysis.](image2)
amplified efficiency. The less abundant sequences would not be amplified sufficiently to visualize as bands on DGGE gel. Therefore, quantitative analysis of DGGE profile must be interpreted only as an indication and not an absolute measure (Xing and Kong, 2007).

2.3 Relationships between Microcystis variation and environmental factors

Mean and ranges of the physicochemical parameters at the four sites during four seasons are displayed in Table 1. Results of CCA are given in Table 2, and are illustrated in Fig. 6. The Mont Carrol permutation test demonstrated that temperature contributed significantly to Microcystis OTUs composition (Fig. 6). The eigenvalues of the first and second axis were 0.609 and 0.252, respectively, showing both axes were influential. These two axes explained 67.6% of the observed variation in Microcystis OTUs composition. The first axis showed a high positive canonical correlation with temperature ($r = 0.963$), while the second axis showed a negative canonical correlation with TSS ($r = -0.716$).

Based on the results of CCA, it was obvious that temperature was a deterministic factor for Microcystis OTUs composition in Lake Taihu. Thus, not only the abundance but also genotypes composition of Microcystis was influenced by temperature. Warmer temperature favors blooms and leads to higher Chl-α concentration (Chen et al., 2003; Tan et al., 2006). Extremely growing Microcystis decayed and resulted in lower DO. These two factors also showed markedly canonical correlations with the first axis (Chl-α, $r = 0.912$; DO, $r = -0.895$). As to the second axis, TSS showed a negative correlation with Microcystis OTUs composition. High TSS usually resulted from intensive wind agitation, and meanwhile rapid attenuated underwater light occurred due to more suspended particles. The both were disadvantage to Microcystis (Zhang et al.,

| Table 1 | Mean and ranges of physicochemical parameters at the four sites during four seasons |
|-------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Temperature (°C) | 30.1 (29.5–30.4) | 22.1 (20.7–22.5) | 4.4 (4.1–4.6) | 18.5 (18.2–18.7) |
| TN (mg/L) | 5.86 (5.55–6.18) | 4.72 (4.47–4.95) | 2.19 (2.01–2.27) | 3.36 (3.23–3.49) |
| TP (mg/L) | 0.53 (0.46–0.57) | 0.45 (0.42–0.49) | 0.31 (0.27–0.34) | 0.36 (0.31–0.39) |
| DIP (mg/L) | 0.034 (0.028–0.039) | 0.026 (0.025–0.032) | 0.041 (0.037–0.043) | 0.026 (0.024–0.029) |
| Chl-α (µg/L) | 18.02 (15.13–23.47) | 13.42 (11.98–14.87) | 2.16 (1.94–2.45) | 4.5 (4.12–4.86) |
| TSS (mg/L) | 75.93 (63.4–88.7) | 63.89 (58.4–66.5) | 44.79 (38.67–49.21) | 51.89 (47.44–63.26) |
| DO (mg/L) | 4.97 (4.41–5.23) | 6.29 (6.05–6.46) | 10.09 (9.85–10.64) | 9.17 (8.76–9.43) |
| pH | 8.11 (7.96–8.19) | 7.96 (7.89–8.03) | 7.59 (7.48–7.72) | 7.68 (7.55–7.79) |

TN: total nitrogen; TP: total phosphorus; DIP: dissolved inorganic phosphorus; Chl-α: concentration of chlorophyll a; TSS: total suspended solids; DO: dissolved oxygen.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Summary results of the canonical correspondence analysis (CCA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axis 1</td>
<td>0.609</td>
</tr>
<tr>
<td>Axis 2</td>
<td>0.252</td>
</tr>
<tr>
<td>OTU-environment correlations</td>
<td>0.963</td>
</tr>
<tr>
<td>Cumulative percentage variance of OTU data</td>
<td>46.9</td>
</tr>
<tr>
<td>Cumulative percentage variance OTU-environment relation</td>
<td>71.8</td>
</tr>
</tbody>
</table>

OTUs composition in Lake Taihu. Thus, not only the abundance but also genotypes composition of Microcystis was influenced by temperature. Warmer temperature favors blooms and leads to higher Chl-α concentration (Chen et al., 2003; Tan et al., 2006). Extremely growing Microcystis decayed and resulted in lower DO. These two factors also showed markedly canonical correlations with the first axis (Chl-α, $r = 0.912$; DO, $r = -0.895$). As to the second axis, TSS showed a negative correlation with Microcystis OTUs composition. High TSS usually resulted from intensive wind agitation, and meanwhile rapid attenuated underwater light occurred due to more suspended particles. The both were disadvantage to Microcystis (Zhang et al.,

![Fig. 6](image-url) Canonical correspondence analysis (CCA) ordination diagram of Microcystis OTUs composition in relation to the eight selected environmental variables.
In this study, concentrations of nutrient salts (TN, TP, and DIP) did not show very high correlations with the first two axes (TN, $r = 0.339$; TP, $r = 0.697$; DIP, $r = 0.724$). Therefore, influences of nutrient salts concentrations on Microcystis OTUs composition were not very strong at studied sites.

2.4 Phylogenetic relationship analysis of the sequencing results

Fifty-one sequences were obtained in this study. They were deposited at GenBank and were assigned accession numbers EU513213 to EU513263. These sequences were all products amplified from Microcystis 16S-23S rRNA-ITS, which was confirmed by BLAST search. Phylogenetic tree of the fifty-one sequences showed that samples collected in summer and winter consisted of two separated clusters (Fig. 7), which was supported by the interior branch test. While, samples derived from spring and autumn were mixed together, perhaps both of them made up of the transition group from the overwintering group to the prevalent blooming group.

Previous multivariate analysis had revealed strong relationships of temperature with phytoplankton variation (Chen et al., 2001; Del Giorgio et al., 1991; Komárková et al., 2003). In this study, results of CCA displayed temperature played a key role in affecting the Microcystis OTUs composition in Lake Taihu. Moreover, phylogenetic analysis showed that the prevalent blooming Microcystis and the overwintering Microcystis were two different groups, which can be distinguished by means of 16S-23S rRNA ITS analysis. Physiology characters of these two

---

**Fig. 7** Neighbor-joining tree of the fifty-one sequences amplified by Microcystis 16S-23S rRNA-ITS. Interior branch test values higher than 50% were indicated near nodes. The accession numbers were given in the parentheses. Scale bar = 0.005 substitutions per site. Numbers indicated the excised bands for sequencing.
3 Conclusions

Microscopic analysis and 16S-23S rRNA-ITS DGGE were both used to study seasonal variation of Microcystis at four sites in Lake Taihu. Additionally, the relationships between Microcystis variation and environmental factors were explored. According to the results of microscopic analysis, Microcystis dominated total phytoplankton abundance at four sites studied in all of seasons except winter. The average annual abundance of Microcystis was relatively high at River Mouth and Meiliang Bay. To a certain extent, quantitative analysis of DGGE profile revealed the spatio-temporal changes of Microcystis genotypes composition. For spatial variations, the richness of Microcystis at River Mouth was higher than any other site during four seasons. For temporal variations, the richness of Microcystis in summer and winter was lower than that in spring and autumn. Temperature was strongly positively correlated with the Microcystis OTUs composition, while TSS showed a negative correlation. Samples collected in summer and winter made up two separated clusters in the phylogenetic tree derived from the sequencing results of target bands on DGGE gel. However, according to the morphological analysis results, variations in Microcystis community composition did not show evident trends during four seasons at these sites. Thus, morphological and molecular approaches were supplemented each other in the present study.

Acknowledgments

This work was supported by the State Key Fundamental Research and Development Program (No. 2008CB418000), the National Natural Science Foundation of China (No. 40671068), and the Special Program for Lake Taihu Water Pollution Governing of Jiangsu Province (No. BK2007748).

References


Kardinaal W E A, Janse I, Kamst-van Agterveld M, Meima...
seasonal variation of Microcystis in Lake Taihu and its relationships with environmental factors


