WATER RESEARCH 45 (2011) 4169–4182

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Phytoplankton community succession shaping bacterioplankton community composition in Lake Taihu, China

Yuan Niu [a], Hong Shen [a], Jun Chen [a], Ping Xie [a,*], Xi Yang [a], Min Tao [a], Zhimei Ma [a], Min Qi [b]

[a] Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China
[b] College of Fishery, Huazhong Agricultural University, Wuhan, Hubei, 430070, PR China

ARTICLE INFO

Article history:
Received 6 December 2010
Received in revised form
15 March 2011
Accepted 22 May 2011
Available online 31 May 2011

Keywords:
Bacterioplankton community composition (BCC)
Phytoplankton community succession
Denaturing gradient gel electrophoresis (DGGE)
Canonical correspondence analysis (CCA)
Flavobacteriaceae
ML-5-51.2
Lake Taihu

ABSTRACT

PCR-denaturing gradient gel electrophoresis (DGGE) and canonical correspondence analysis (CCA) were used to explore the relationship between succession of phytoplankton community and temporal variation of bacterioplankton community composition (BCC) in the eutrophic Lake Taihu. Serious *Microcystis* bloom was observed in July–December 2008 and Bacillariophyta and Cryptophyta dominated in January–June 2009. BCC was characterized by DGGE of 16S rRNA gene with subsequent sequencing. The DGGE banding patterns revealed a remarkable seasonality which was closely related to phytoplankton community succession. Variation trend of Shannon–Wiener diversity index in bacterioplankton community was similar to that of phytoplankton community. CCA revealed that temperature and phytoplankton played key roles in structuring BCC. Sequencing of DGGE bands suggested that the majority of the sequences were affiliated with common phylogenetic groups in freshwater: *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes* and *Actinobacteria*. The cluster STA2-30 (affiliated with *Actinobacteria*) was found almost across the sampling time at the two study sites. We observed that the family *Flavobacteriaceae* (affiliated with *Bacteroidetes*) tightly coupled to diatom bloom and the cluster ML-5-51.2 (affiliated with *Actinobacteria*) dominated the bacterioplankton communities during *Microcystis* bloom. These results were quite similar at the two sampling sites, indicating that BCC changes were not random but with fixed pattern. Our study showed insights into relationships between phytoplankton and bacterioplankton communities at species level, facilitating a better understanding of microbial loop and ecosystem functioning in the lake.

1. Introduction

In natural freshwater systems, bacterioplankton plays a key role in the breakdown of organic matter and nutrients cycling (Cole et al., 1988; Caron, 1994). Despite recent advances in the characterization of freshwater bacterioplankton diversity, our knowledge about the factors regulating the bacterioplankton community composition (BCC) is far from holistic understanding (Lindström et al., 2005; Hahn, 2006). Several environmental factors have been suggested to be related to the

* *Corresponding author*. Tel./fax: +86 27 68780622.
E-mail address: xieping@ihb.ac.cn (P. Xie).
0043-1354/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2011.05.022

temporal variation in BCC in experimental and natural systems, which include water temperature (Crump and Hobbie, 2005; Kan et al., 2007; Shade et al., 2007), phytoplankton succession (Höfle et al., 1999; Pinhassi et al., 2004; Rooney-Varga et al., 2005) and predation (van Hannen et al., 1999b; Langenheder and Jürgens, 2001; Muylaert et al., 2002). A previous study has shown that in eutrophic shallow lakes, seasonality of bacterial community structure is dependent on the dominant substrate source as well as on the food web structure (Muylaert et al., 2002). Rooney-Varga et al. (2005) also suggest that changes in phytoplankton community composition may influence the composition of bacterial communities that function as part of the microbial loop. Differences in the quality of organic matter produced by different phytoplankton communities probably result in changes in the composition of bacterioplankton community utilizing this organic matter (van Hannen et al., 1999a). Despite the fact that bacterioplankton and phytoplankton are thought to be closely linked in aquatic ecosystem, our knowledge about how these communities interact with each other at the species composition level is still limited especially in shallow eutrophic lakes.

Lake Taihu, the third largest freshwater lake in China, is a typical shallow eutrophic subtropical lake located in east China (surface area: 2338 km^2, mean depth: 1.9 m). In general, like most eutrophic lakes (Kalff and Knoechel, 1978), diatoms dominate in spring phytoplankton, while heavy cyanobacterial blooms are characteristic for late-summer on its northern part (including Zhushan Bay, Meiliang Bay and Gonghu Bay) each year. Microbial communities are key players in ecological processes related to water quality, so the detailed knowledge of their diversity and function in the system is an essential prerequisite for the sustainable management of freshwater resources (Hahn, 2006). Although a few studies have focused on bacterioplankton community diversity and its regulating factors including submersed macrophytes, phytoplankton community, nutrients, etc. in Lake Taihu (Wu et al., 2007a, 2007b; Xing and Kong, 2007; Tian et al., 2009), our understanding of the relationships between phytoplankton and specific bacterioplankton taxa, and how bacterioplankton are affected by environmental change are far from complete. We hypothesized that phytoplankton community succession might be crucial for bacterioplankton community composition and the specific bacterioplankton taxa could change in accordance with the succession of predominant phytoplankton genus. To this end, links between phytoplankton community succession and BCC variation at the species composition level are badly needed. In addition, this was the first time to explore the BCC in Gonghu Bay, which provides the bulk of drinking water of Wuxi city for millions of residents.

Since the first publication by Muyzer et al. (1993) the PCR-denaturing gradient gel electrophoresis (DGGE) fingerprinting has been widely used in environmental microbiology, and has been recognized to give an acceptable view on differences and similarities of the dominant populations of microbial communities (Muyzer, 1999; Muyzer and Smalla, 1998; Lindström, 2000, 2001; Dorigo et al., 2005). In the present study, the DGGE and canonical correspondence analysis (CCA) methods were used to explore whether there was a close relationship between phytoplankton community succession and temporal variation in BCC in Lake Taihu. Our results clearly demonstrated the above hypothesis, namely, we observed that the family Flavobacteriaceae (affiliated with *Bacteroidetes*) might be in close relation to diatom bloom, while the cluster ML-5-51.2 (affiliated with *Actinobacteria*, Wu et al., 2007b) was in association with *Microcystis* blooms. These may facilitate to understand the relationships between phytoplankton and bacterioplankton communities in eutrophic lake.

2. Materials and methods

2.1. Site and sampling

GonghuBay, where a terrible drinking water malodor incident occurred in the early summer of 2007, is located in the northeastern part of Lake Taihu and has a surface area of approximately 120 km^2. The two sampling sites (Site A 31°25′11.51"N, 120°15′35.04"E; Site B 31°26′49.16"N, 120°22′11.10"E) are located near the Gonghu Waterworks and Xidong Waterworks, respectively (Fig. 1). Lake Taihu has become increasingly eutrophic over the past three decades (Qin et al., 2007) and the ratio of area in hypertrophic state had risen to 77% (Wu et al., 2007b). According to the results of our field survey at 30 sites distributed throughout the lake from 2008–2009 (unpublished data), the succession of predominant phytoplankton and zooplankton communities presented similar patterns at most sites. So the two sampling sites could represent the majority of cases in the entire lake.

Water samples were collected monthly from July 2008 to June 2009, from surface waters (0–0.5 m) with a 5 l Schindler sampler. Water temperature, pH and dissolved oxygen (DO) were measured on location by an YSI 6600 Multi-Parameter Water Quality Sonde. Bacterioplankton samples (200–300 ml of water) for BCC analysis were collected on 0.2 μm-pore-size filters after pre-filtration through 5.0 μm-pore-size filters (diameter 45 mm; Whatmann, UK). Filters were stored at −80 °C until analysis. Water samples (50 ml) were fixed with glutaraldehyde to a final concentration of 2% for determination of total bacterial abundance. 1 l water samples for identification and counts of phytoplankton were preserved with 1% Lugol's iodine solution. Zooplankton were collected by straining 10 l water samples through a 64 μm plankton net and fixed with formalin.

2.2. Chemical analysis

Total nitrogen (TN), ammonium (NH_4-N), nitrate (NO_3-N), total phosphorus (TP), ortho-phosphorus (PO_4-P) and chlorophyll *a* (chl *a*) were analyzed according to standard methods (Jin and Tu, 1990).

2.3. Identification and counting of phytoplankton and zooplankton

Phytoplankton samples were concentrated to 50 ml after sedimentation for 48 h. Then 0.1 ml concentrated samples were counted on a microscope under 400× magnification after mixing. Colonial *Microcystis* spp. cells were separated using an ultrasonic device and then counted. Phytoplankton species

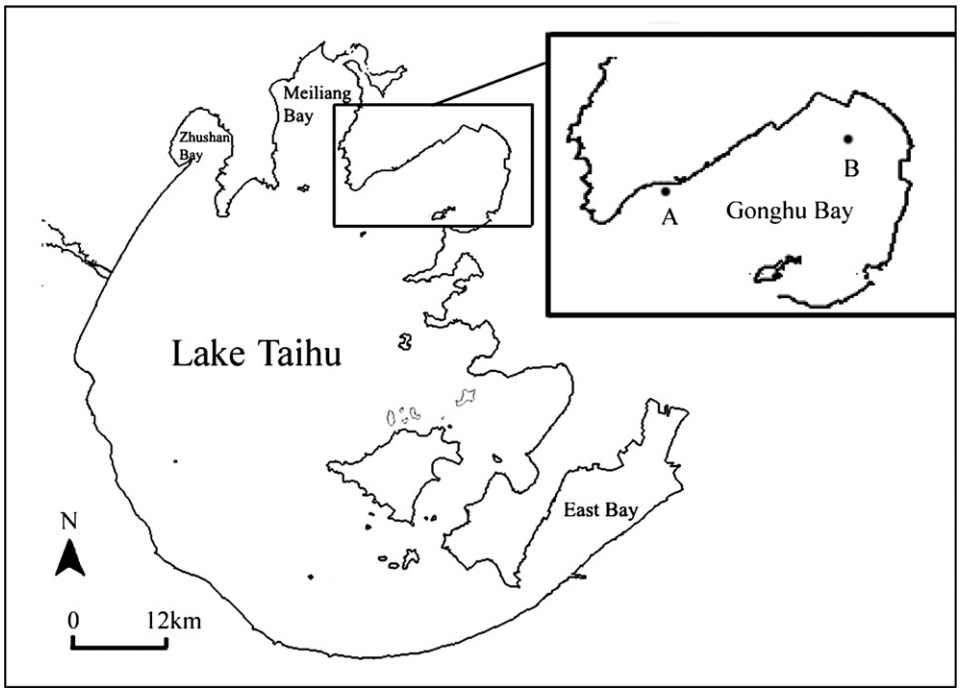

Fig. 1 – Locations of the two sampling sites (A and B) in Gonghu Bay of Lake Taihu.

were identified according to Hu and Wei (2006). Biomass was estimated from cell numbers and cell size measurements, assuming that 1 mm^3 of algal volume equals 1 mg of fresh weight biomass. Zooplankton were identified according to Chiang and Du (1979) and Sheng (1979) and counted using a microscope at a magnification of 100×.

To estimate changes in phytoplankton community, we used the Shannon–Wiener Index (H'_p, bits)

$$H'_p = -\sum_{i=1}^{s} \frac{n_i}{n} \ln \frac{n_i}{n}$$

where n_i is biomass of the ith genus, n is the total biomass of all genera, and s is the total number of genera.

2.4. Total bacterial abundance

2 ml pre-fixed water samples were stained with 4′,6′-diamidino-2-phenolindole (DAPI, Sigma, final concentration 1 μg ml^{-1}) for 15 min (Poter and Feig, 1980) and filtered onto 0.2 μm pore size black polycarbonate filters (Whatman). Total bacterial cells were enumerated under an epifluorescence microscope (Zeiss Axioshop 20). Atleast 1000 cells and a minimum 10 fields of view were counted for each sample.

2.5. DNA extraction and PCR amplification

The filters containing microbes were cut into small pieces with a sterile scalpel and then bacterial genomic DNA was extracted using a Bacterial DNA kit (Omega) following the protocol, followed by purification on QIAamp DNA Kit (Qiagen,Valencia, CA, USA). The purified DNA was used as template to amplify the 16S rRNA gene fragment with the Bacteria-specific primers 357F (5′-CCTACGGGAGGCAGCAG-3′) with a 40-bp GC clamp attached to its 5′ end and the universal primer 518R (5′-ATTACCGCGGCTGCTGG-3′) (Muyzer et al., 1993). PCR mixtures of 50 μl contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 2.5 U of Taq DNA polymerase (Takara) and 50 ng of template DNA. 5 min initial denaturation at 94 °C was followed by a thermal cycling program as follows: 1 min denaturation at 94 °C; 1 min annealing at an initial 65 °C, decreasing 1 °C every cycle to a final of 55 °C; 1 min extension at 72 °C. 30 cycles were run followed by a final 10 min extension at 72 °C. A negative control, in which the template was replaced by an equivalent volume of sterile deionized water, was included. PCR products were confirmed by 1.5% agarose gel electrophoresis.

2.6. Denaturating gradient gel electrophoresis (DGGE)

A total of 800 ng of PCR product for each sample was loaded on a 8% (w/v) polyacrylamide gel (37.5:1 acrylamide: bisacrylamide) with a denaturing gradient that ranged from 40 to 60%, where 100% denaturant is defined as 7M urea and 40% deionized formamide. DGGE was performed with a Dcode system (Bio-Rad Laboratories, USA) using 1 × TAE running buffer (20 mM Tris, 10 mM acetic acid, 0.5 mM EDTA, pH 8.0) at 60 °C for 7 h at 150V. The gel was stained in 1:10000 diluted GelRed (Biotium, USA) nucleic acid staining solution for 30 min and photographed using a Bio Image System (Gene Com.) under UV light. DGGE were performed separately for Sites A and B.

2.7. Sequencing of DGGE bands

All visible DGGE bands were excised with a sterile razor blade and eluted overnight at 4 °C in 40 μl MilliQ water. 2 μl of the supernatant was used as template for reamplification with the

same primer set as described above (without a GC clamp). The amplicons were electrophoresed again on a DGGE gel to check the position of the original band and then purified with Gel Recovery Purification Kit (AxyPrep™) and ligated into pMD18-T plasmid vector (Takara, Japan) following the manufacturer's instruction. The ligated DNA was transformed into *Escherichia coli* DH5α-competent cells. The recombinant clones were selected and then submitted for sequencing using an M13 primers and an automated ABI sequencer at the Genomics Company (Wuhan, China).

2.8. Cluster analysis of DGGE profiles and Statistics

Cluster analyses of DGGE profiles were performed with the NTSYS program version 2.10e (Exeter software, Setauket, NY, USA). A binary matrix was constructed by scoring presence or absence of DGGE bands. Pairwise similarities between gel banding patterns were quantified using the Dice coefficient as: $S_D = (2N_{AB})/(N_A + N_B)$, where N_{AB} is the number of bands common to the samples A and B, and N_A and N_B are the number of bands in samples A and B, respectively. The similarity coefficients were then used to construct a dendrogram using unweighted pair group method with arithmetic average (UPGMA) through the sequential, hierarchical, agglomerative, and nested clustering (SHAN) routine of the NTSYS program.

Gel images were analyzed using Gel-Pro Analyzer (version 4.5). A densitometric curve was calculated for each lane and the relative intensities of all bands were obtained. The Shannon–Wiener Index (H'_b, bits) was calculated to estimate changes in bacterioplankton community composition.

$$H'_b = -\sum_{i=1}^{n} P_i \ln P_i$$

where *Pi* is the relative intensity of each band and *n* is the total number of bands in each lane.

To reveal relationships between BCC and environmental variables (including the physical and chemical parameters, abundances of crustacean zooplankton and biomasses of different taxonomic groups of phytoplankton), canonical correspondence analysis (CCA) was carried out with the software CANOCO (version 4.5, Microcomputer Power, Ithaca, New York, USA) as the length of the first DCA axis run on species data was >4 (ter Braak, 1987; ter Braak and Verdonschot, 1995). By scoring presence (1) and absence (0) of particular DGGE bands, the DGGE data were used to compile a binary matrix by scoring presence (1) and absence (0) of particular DGGE bands. The environmental variables significantly related to BCC were tested by forward selection and Monte Carlo permutation test. CCA was run separately for data sets of Sites A and B.

Statistical analysis of the physical–chemical parameters and abundances of crustacean zooplankton and total bacterial was conducted by SPSS software, version 13.0 for Windows (Chicago, USA). Significance was determined at an alpha level of 0.05 ($p < 0.05$).

2.9. Phylogenetic analysis

The obtained 16S rRNA gene sequences were aligned to known sequences using BLAST. Sequences were aligned with the CLUSTAL W program (Thompson et al., 1994) and the phylogenetic analyses were performed with the MEGA4.0 (Tamura et al., 2007) software package using neighbor-joining methods (Saitou and Nei, 1987). The sequences with similarities greater than 97% were grouped in one operational taxonomic unit (OTU).

2.10. Nucleotide sequences accession numbers

The 16S rRNA gene sequences have been deposited to GenBank under the Accession No. HQ259136 to HQ259213.

3. Results

3.1. Environmental parameters

The nutrient concentrations (TN, NH_4-N, NO_3-N, TP, TN:TP mass ratios and PO_4–P), physical parameters (water temperature, pH, dissolved oxygen, water depth and secchi depth), chlorophyll *a* (chl *a*) and abundances of crustacean zooplankton of each site are shown in Table 1. The highest concentrations of TN and TP at Site A both occurred in October, whereas the maximum TN and TP at Site B were detected in May and September, respectively (Fig. 2b and c). There were no significant differences ($p > 0.05$) in nutrient concentrations (TN, NH_4-N, NO_3-N, TP, TN:TP mass ratio and PO_4–P) and pH between the two sites. The level of chlorophyll

Table 1 – Environmental parameters (mean and range) of the two sampling sites located in Gonghu Bay of Lake Taihu from July 2008 to June 2009.

Variable	Site A		Site B	
	Mean	Range	Mean	Range
Total nitrogen (TN, mg L^{-1})	2.12	0.21–5.48	1.50	0.36–2.72
Total phosphorus (TP, mg L^{-1})	0.138	0.033–0.620	0.088	0.039–0.156
TN:TP	21	6–52	18	7–37
NH_4–N (mg L^{-1})	0.23	0.12–0.58	0.31	0.07–0.95
NO_3–N (mg L^{-1})	0.51	0.04–1.34	0.50	0.03–1.92
PO_4–P (mg L^{-1})	0.008	0.001–0.018	0.010	0.004–0.022
Dissolved oxygen (mg L^{-1})	10.2	6.4–19.0	9.8	7.2–14.0
Water temperature (°C)	18.7	3.1–31.7	18.1	2.5–30.3
Water depth (m)	1.6	1.3–1.8	2.0	1.9–2.4
Secchi depth (cm)	36	9–70	54	25–103
chlorophyll *a* (μg L^{-1})	168.8	12.8–940.0	17.4	2.3–46.2
pH	8.4	7.8–9.6	8.5	7.9–8.8
Cladoceran abundance (ind. L^{-1})	241	0.1–1054	67	1–313
Copepods abundance (ind. L^{-1})	44	0.7–339	37	0.2–180
Total bacterial abundance (10^6 cells ml^{-1})	5.93	3.10–12.00	5.08	2.10–7.80

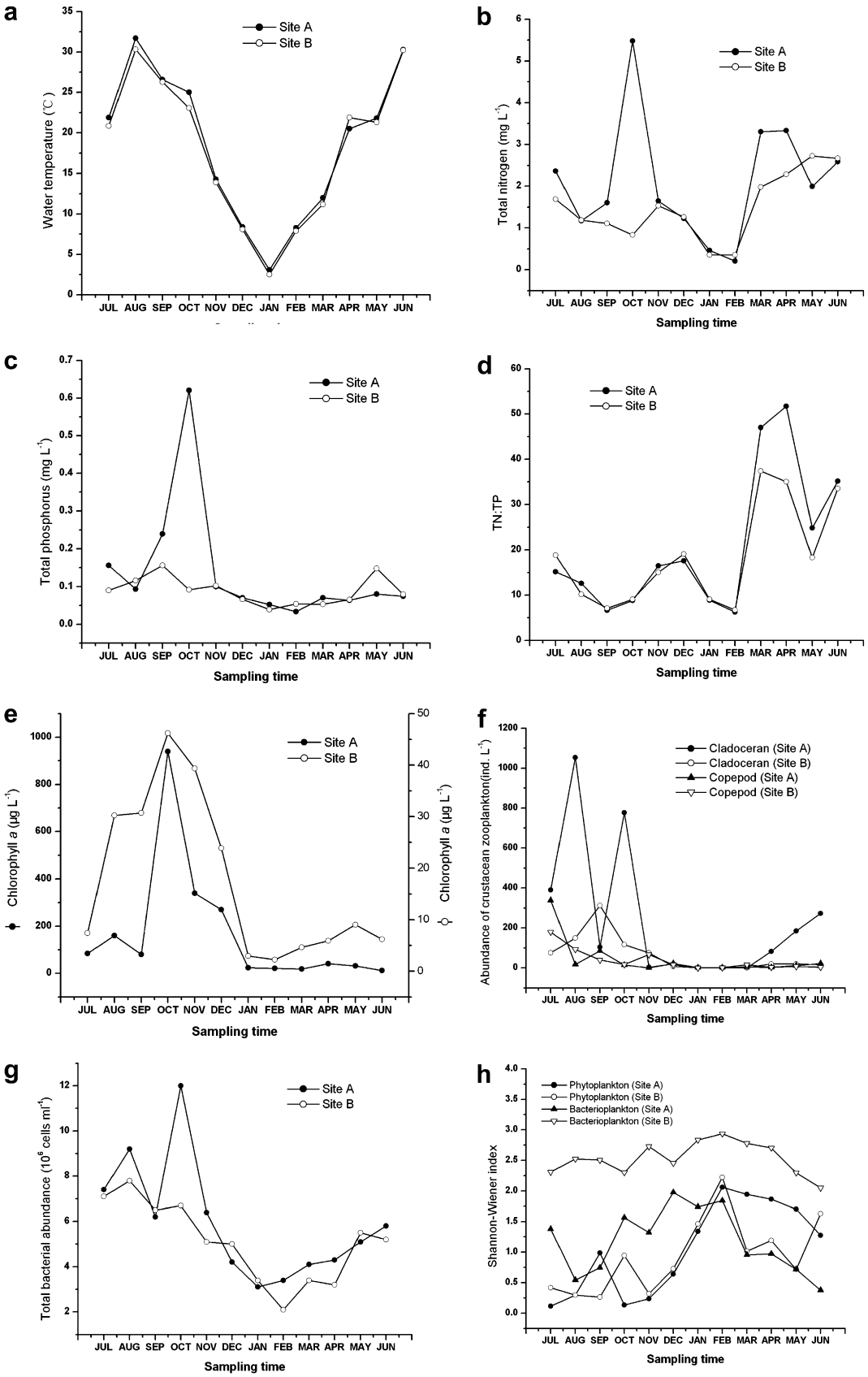

Fig. 2 – Seasonal variations in (a) water temperature, (b, c, d) total nitrogen (TN), total phosphorus (TP) and TN:TP ratio, (e) chlorophyll *a* (chl *a*), (f) abundance of crustacean zooplankton, (g) total bacterial abundance and (h) Shannon–-Wiener diversity index of phytoplankton and bacterioplankton at the two sampling sites from July 2008 to June 2009.

a during the cyanobacterial bloom was far in excess of the yearly average and the highest concentrations occurred in October at both sites. The chlorophyll *a* concentrations were significantly higher ($p < 0.05$) at Site A (with more serious cyanobacterial bloom) than at Site B. Additionally, Site B had higher water depth and secchi depth than Site A ($p < 0.05$).

The seasonal variations in abundance of crustacean zooplankton are shown in Fig. 2f. There were no significant differences ($p > 0.05$) between the two sites in abundance of both cladoceran and copepod. The peaks of cladocerans at Sites A and B appeared in August and September, respectively. *Ceriodaphnia cornuta* (only in summer and autumn) and *Bosmina coregoni* were the dominant species at both sites. The peaks of copepod appeared in July and *Limnoithona sinensis* as well as *Sinocalanus dorrii* were the dominant species at both sites.

3.2. *Total bacterial abundance*

The seasonal variations in total bacterial abundance are shown in Fig. 2g. There was no significant difference ($p > 0.05$) between the two sites. The peaks of total bacterial abundance at Sites A and B appeared in October and August, respectively. There was significant correlation between the total bacterial abundance and biomass of Cyanophyta ($r = 0.757$, $p = 0.000$), indicating that high total bacterial abundance was always companied with the occurrence of cyanobacterial bloom.

3.3. *Succession of phytoplankton community*

Variations in composition of phytoplankton community during the sampling period (from July 2008 to June 2009) are shown in Fig. 3. We can clearly see that Cyanophyta and Bacillariophyta were the two major dominant phyla at both sites in Gonghu Bay (Fig. 3a). Generally, Cyanophyta was the absolutely dominant phylum in July–December 2008 (cyanobacterial-bloom-period), whereas Bacillariophyta dominated in January–June 2009 (diatom-dominated period). In addition, Cryptophyta dominated in March and April at Site B. *Microcystis* was the dominant genus in the phylum of Cyanophyta (>99% at both sites) and in the phylum of Bacillariophyta

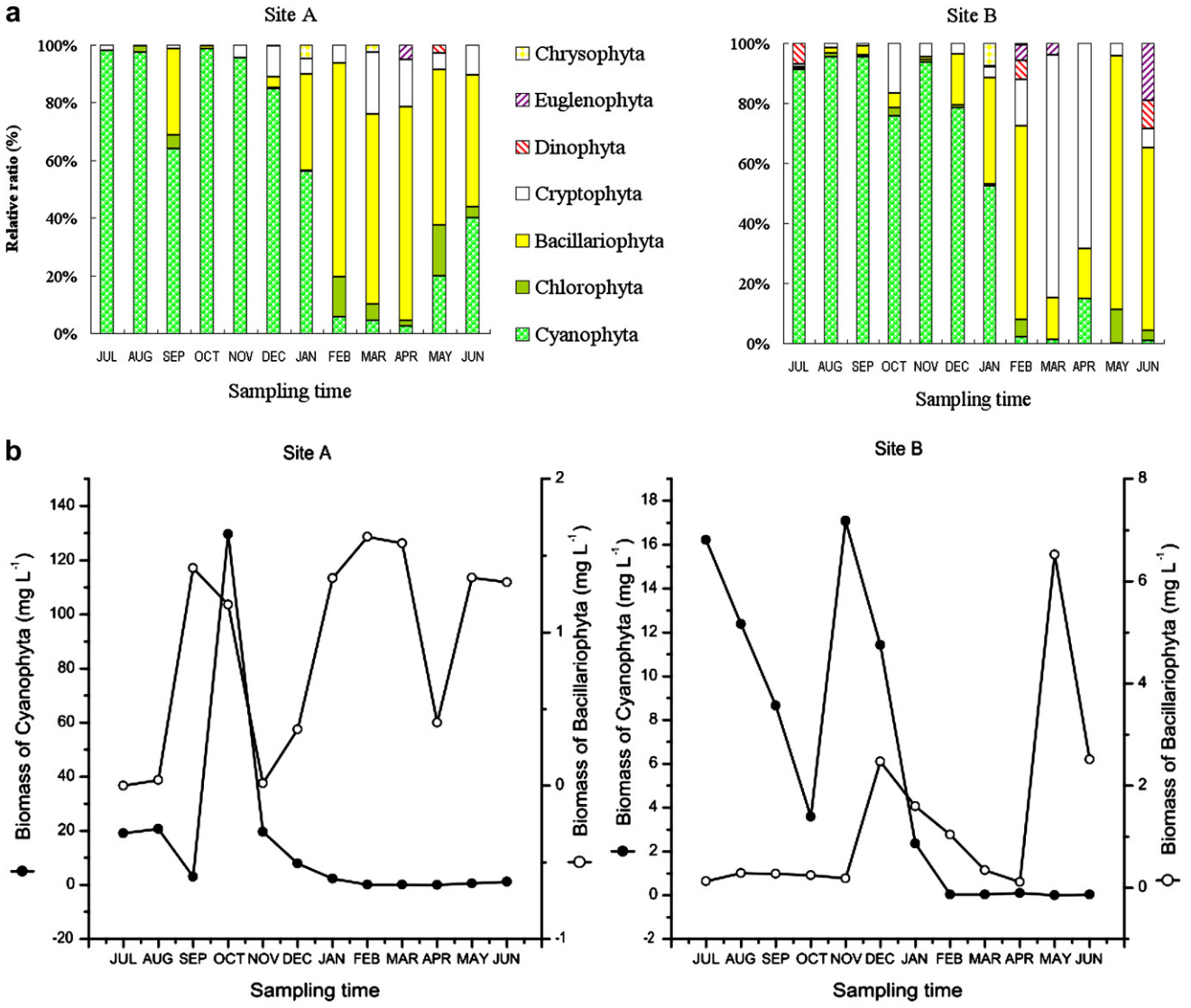

Fig. 3 – Variations of biomass in phytoplankton community composition at the two sampling sites: (a) Relative ratio of different phyla; (b) Cyanophyta and Bacillariophyta from July 2008 to June 2009.

Cyclotella was the main genus (>40% and >80% at Sites A and B, respectively).

Fig. 3b displays the variations in biomass of Cyanophyta and Bacillariophyta at both sites during the sampling period. Biomass of Cyanophyta maintained at a high level (>2 mg L^{-1}) from July 2008 to January 2009 at both Sites A and B, and the peak values occurred in October with 129.7 mg L^{-1} and November with 17.1 mg L^{-1}, respectively. From February 2009 biomass of Cyanophyta dropped quickly and remained at a low level (<1 mg L^{-1}) until June. In general, more serious cyanobacterial bloom occurred at Site A than at Site B. At Site A, biomass of Bacillariophyta ranged from 0 to 1.6 mg L^{-1} and reached a peak in early spring. While at Site B, biomass of Bacillariophyta remained at a low level from July to November, followed by a sharp increase in December and gradually decreased until April. Finally, the highest values occurred in May with 6.5 mg L^{-1} and dropped to 2.5 mg L^{-1} in June.

3.4. *Seasonal variations in diversity of phytoplankton and bacterioplankton communities*

Seasonal variations in the Shannon–Wiener diversity index of phytoplankton and bacterioplankton community are shown in Fig. 2h. Variations in the Shannon–Wiener diversity index of phytoplankton community revealed a similar pattern at both sites. The index remained at a low level during most time of the cyanobacterial blooms and remarkably increased from December. It reached a peak in February and gradually decreased afterwards. The variation trend in Shannon–Wiener diversity index in bacterioplankton community was somewhat similar to that of phytoplankton community. At Site A, the value gradually increased from August, reached its peak in December, and then continuously declined until June. The value was relatively stable and high at Site B ($p < 0.05$).

3.5. *Composition of bacterioplankton community*

DGGE profiles of 16S rRNA gene fragments from the two sites (24 samples in total) show visible changes in relative brightness and position of DGGE banding pattern in various seasons (Fig. 4a). The cluster analysis (UPGMA) dendrograms of BCC also revealed remarkable seasonality (Fig. 4b). The bacterioplankton communities in various months from Site A (Fig. 4b) were grouped into 5 defined clusters. The October sample (*Microcystis* biomass reached a peak with 128.7 mg L^{-1})

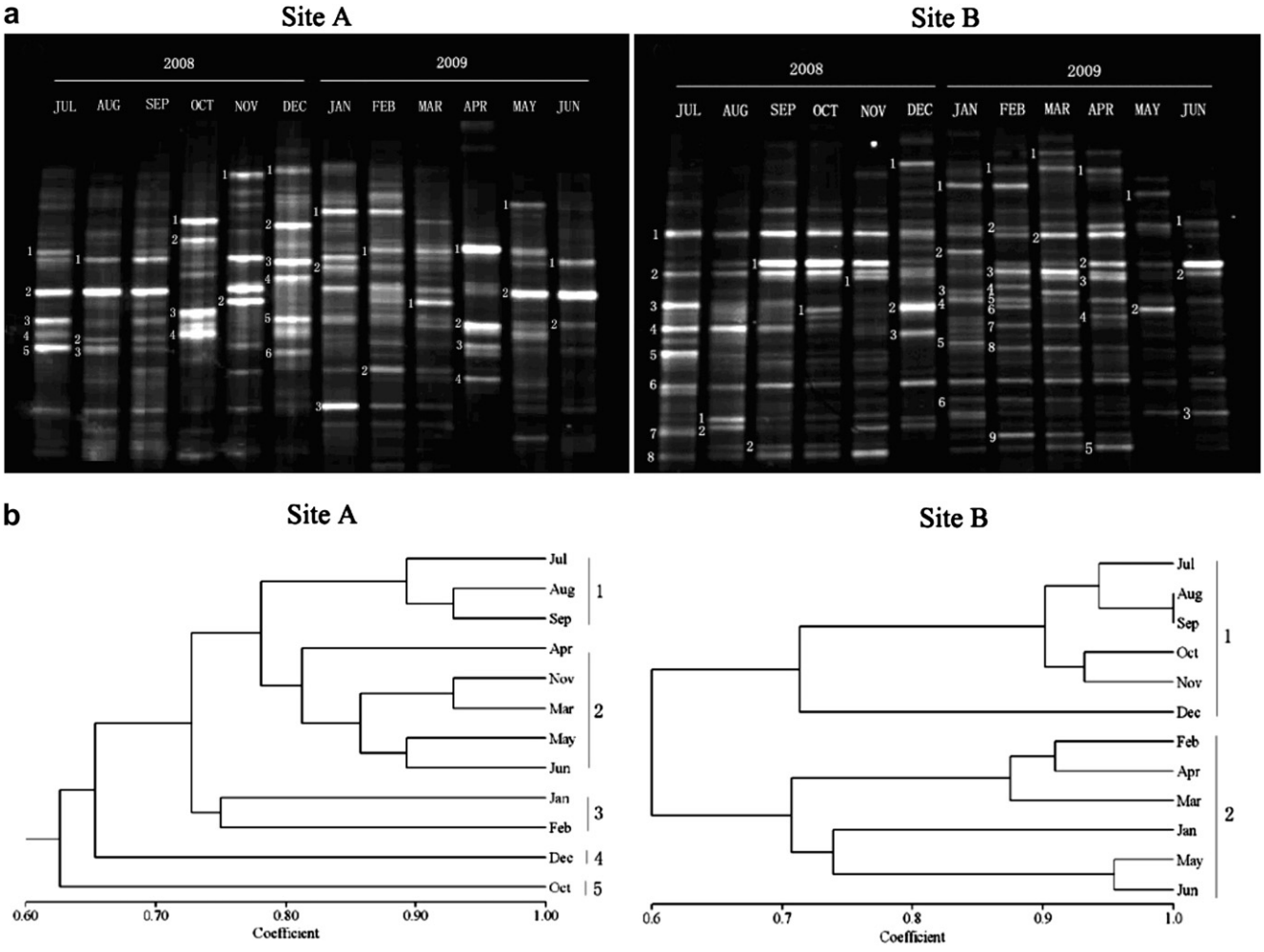

Fig. 4 – (a) DGGE profiles of 16S rRNA gene fragments from the two sampling sites (b) Cluster analysis of BCC based on DGGE profiles of the two sampling sites from July 2008 to June 2009. The first band obtained from July at Site A in Gonghu Bay was indicated by GH-A-JUL1.The other bands were named in the same manner.

at Site A separately formed cluster 5 (Fig. 4b) since it was very dissimilar to the other samples. Samples from Site B distinctly formed two separate clusters (Fig. 4b). Cluster 1 included the samples taken in 2008 and Cluster 2 was composed of samples collected from 2009. Combined with Fig. 3, we found that BCC was in close association with succession of phytoplankton community.

Bands from different months at the same vertical position in a gel were assumed to have identical sequences (Riemann et al., 1999). Thirty-four and forty-four bands from different vertical positions in the DGGE profiles from Sites A and B, respectively, were excised, reamplified, purified and sequenced, of which 4 sequences belonging to Cyanobacteria (bands GH-A-JUL5, GH-B-JUL4, GH-B-FEB3 and GH-B-JUN1) were rejected from further analysis since we focused on heterotrophic bacteria. In the DGGE profiles, the first band obtained from July at Site A was indicated by GH-A-JUL1.The other bands were named in the same manner. Phylogenetic affiliation of clones obtained from the DGGE profiles at Sites A and B is shown in Table 2. The majority of the sequences were affiliated with phylogenetic groups commonly found in freshwater: *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes* and *Actinobacteria*. A total of 78 clones were classified into 50 OTUs , where 12 OTUs were belonging to *Bacteroidetes*, followed by *Actinobacteria* (8 OTUs), *Alphaproteobacteria* (7 OTUs) and *Betaproteobacteria* (6 OTUs). Taxonomic descriptions of the 78 bands obtained from the DGGE profiles of the two sampling sites are shown in Table S1.

It had been suggested that band intensity was related to relative abundance of the corresponding phylotype in the template mixture (Murray et al., 1996; Riemann et al., 1999; Fromin et al., 2002), thus bands with relatively high intensities in a lane were assumed to be dominated. During the *Microcystis* bloom phase (from July to December), *Actinobacteria* (bands GH-A-JUL2 and GH-B-SEP1), *Alphaproteobacteria* (band GH-A-AUG1), *Betaproteobacteria* (bands GH-B-JUL1 and GH-B-JUL8) and *Bacteroidetes* (band GH-B-JUL2) were the most prominent. October sample from Site A was quite different with dominance of *Firmicutes* (bands GH-A-OCT2 and GH-A-OCT3), *Bacteroidetes* (band GH-A-OCT1) and *Alphaproteobacteria* (band GH-A-OCT4). During most of the diatom-dominated period, samples were more diverse and there was no obviously predominant band. Remarkably, a considerable number of bands affiliated with the family *Flavobacteriaceae*, *Bacteroidetes* (bands GH-A-DEC5, GH-A-FEB1, GH-B-JAN1, GH-B-FEB1, GH-B-FEB4, GH-B-APR1, GH-B-APR2 and GH-B-APR3, see Fig. S1d) appeared in this period. May and June samples were dominated by *Alphaproteobacteria* (bands GH-A-APR1, GH-A-APR2, GH-A-APR4 and GH-B-MAY2). In addition, cluster STA2-30, *Actinobacteria* (bands GH-A-JAN3 and GH-B-JUL6) was seen almost throughout the whole sampling stage at both sites.

Table 2 – Phylogenetic affiliation of clones obtained from the DGGE profiles at the two sampling sites.

Taxon	No. of clones	
	Site A	Site B
Alphaproteobacteria	6	8
Betaproteobacteria	4	6
Gammaproteobacteria	1	2
Deltaproteobacteria	1	1
Bacteroidetes	9	9
Actinobacteria	4	10
Firmicutes	6	1
Thermomicrobia	0	1
Fibrobacteres	0	1
Verrucomicrobia	2	2
Cyanobacteria	1	3
Total	34	44

3.6. CCA

Correspondence canonical analysis (CCA) based on DGGE data and environmental variables were carried out separately for the two sites. According to the results of CCA (Fig. 5a), the differences in BCC are related to the two most important environmental variables (biomass of Cyanophyta and water temperature, $p < 0.05$) at Site A. The two axes explained 33% of the observed variation in BCC. The first axis was positively related with the biomass of Cyanophyta ($r = 0.98$) and the second axis was positively related with water temperature ($r = 0.89$). Similarly, results of CCA (Fig. 5b) illustrated that the differences in BCC were related to the three most important environmental variables (biomass of Cyanophyta and Bacillariophyta, and water temperature, $p < 0.05$) at Site B. The three variables and the two axes explained 53% and 42% of the observed variation in BCC, respectively. The first axis was positively related with the biomass of Cyanophyta and water temperature ($r = 0.92$ and 0.47 respectively), and the second axis was positively related with the biomass of Bacillariophyta ($r = 0.93$).

CCA biplot revealed that samples from Sites A and B formed three and four clusters respectively (Fig. 5). Samples collected at Site A from July to September 2008 and from April to June 2009 formed one cluster, while the remaining samples (except October) formed the second cluster. October sample was quite different from the others due to its high biomass of Cyanophyta. Samples collected at Site B were grouped into 4 clusters. Cluster 1 consisted of samples from January to April 2009. Cluster 2 contained samples from July to November 2008. Cluster 3 was composed of samples from May and June 2009 whereas December sample of 2008 formed a separate cluster. These results are similar to cluster analysis of BCC based on DGGE profiles (Fig. 4b).

4. Discussion

In recent years, the *Microcystis* blooms have expanded from the northern bays to the center and the northern as well as western regions of the Lake Taihu are now regularly covered by thick blooms from late spring into autumn (Xu et al., 2010), whereas East Bay is covered by a submersed macrophyte community composed mainly of *Potamogeton* spp. (Wu et al., 2007a). Previous studies indicated that nutrients (Xing and Kong, 2007), submersed macrophytes (Wu et al., 2007a) and phytoplankton community (Tian et al., 2009) have been

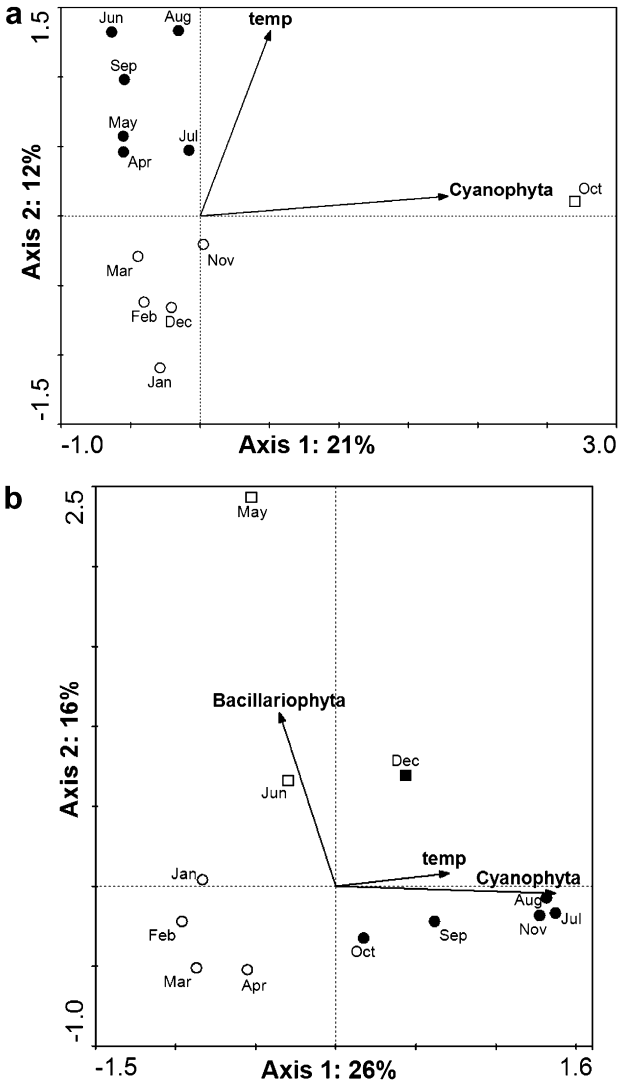

Fig. 5 – Correspondence canonical analysis (CCA) biplot based on DGGE data and environmental variables of Sites A (a) and B (b) from July 2008 to June 2009.

suggested to play important roles in shaping BCC in Lake Taihu. However, our results further explained the relationship between predominant phytoplankton groups and specific bacterioplankton taxa. DGGE banding patterns and subsequent cluster analysis intuitively revealed that BCC had a remarkable seasonality which was closely related to phytoplankton community succession. CCA analyses confirmed the key role of phytoplankton community in shaping BCC. By sequencing all visible DGGE bands with phylogenetic analysis, we also found specific bacterioplankton taxa (cluster ML-5-51.2 and family *Flavobacteriaceae*) that coupled to predominant phytoplankton taxonomic groups (*Microcystis* spp. and diatom, respectively) at different stages.

4.1. *Environmental (abiotic and biotic) factors regulating BCC*

The CCA results of the present study revealed that biomass of Cyanophyta and Bacillariophyta, as well as water temperature, were the three most influential factors on BCC, responsible for a major part of the observed variation in BCC.

Our results agree with the observation of several studies that temperature can significantly influence the seasonal variation in BCC (Crump and Hobbie, 2005; Kan et al., 2007; Shade et al., 2007). As one of the main seasonal factors, water temperature may exert direct and indirect influences on abundance and composition of bacterioplankton community. First, it is always a potentially limiting factor since every bacterial phylotype has minimal, maximal and optimal temperature characteristics (Pomeroy and Wiebe, 2001). Consequently, abundance of each bacterial phylotype probably changes as the water temperature fluctuates, which results in variation of BCC. Second, in particular, temperature should be viewed as an interactive factor because it affects all chemical and biochemical processes (Pomeroy and Wiebe, 2001). It is well known that water temperature has a pronounced impact on phytoplankton and zooplankton compositions, and several studies also indicated that phytoplankton and zooplankton strongly influence bacterioplankton diversity via bottom-up and top-down regulations (Langenheder and Jürgens, 2001; Muylaert et al., 2002; Pinhassi et al., 2004; Rooney-Varga et al., 2005). As a consequence, temperature-mediated succession of plankton community likely contributed to the variations in BCC. Nutrient concentration and composition may directly influence BCC through affecting on growth of bacteria (Pinhassi and Hagstrom, 2000; Haukka et al., 2006). However, none of them was significantly related to BCC ($p > 0.05$) in our study. TN:TP ratio is very important in the development of phytoplankton populations and cyanobacteria may dominate in the phytoplankton under low TN:TP ratio conditions (Smith, 1983; Sekar et al., 2002). It is possible that they may have an indirect impact on BCC via influencing the phytoplankton populations.

Our study indicated that succession of phytoplankton community was the most important influential factor on BCC. Specially, the biomass of Cyanophyta explained over 20% of the observed variation in BCC independently at both sampling sites, indicating that cyanobacterial blooms may be the major shaping force in Gonghu Bay, Lake Taihu. In addition, the biomass of Bacillariophyta at Site B was significantly related to BCC ($p < 0.05$) and explained 17% of the observed variation in BCC independently. It has been reported that phytoplankton plays a key role in regulating BCC in the mesocosms and natural systems (Höfle et al., 1999; Pinhassi et al., 2004; Rooney-Varga et al., 2005; Tian et al., 2009). In some mesocosms experiments, specific bacterioplankton taxa that were in association with different phytoplankton taxonomic groups were also found (Pinhassi et al., 2004; Li et al., 2011). The release of dissolved organic matter (DOM) by phytoplankton has long been recognized as an important source of high quality carbon to bacterial (Cole et al., 1982) and it has been demonstrated that changes in dissolved organic carbon (DOC) can drive changes in BCC (Eiler et al., 2003; Jones et al., 2009). The DOM is rapidly consumed and remineralized by the bacterial community (Jensen, 1983; Obernosterer and Herndle, 1995). Thus, differences in the quality of organic matter produced by different dominant phytoplankton communities probably result in changes in the composition of bacterioplankton community utilizing this organic matter (van

Table 3 – Distribution of three specific taxons (clusters STA2-30, ML-5-51.2 and family *Flavobacteriaceae*) in the present study.

Taxon	Site	Sampling Month											
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
STA2-30	A	+	+	+	−	+	−	+	+	+	−	+	+
	B	+	+	+	+	+	+	+	+	+	+	+	+
ML-5-51.2	A	+	+	+	−	+	−	−	−	−	−	−	−
	B	+	+	+	+	+	+	−	−	−	−	−	−
Flavobacteriaceae	A	−	−	−	−	−	+	+	+	+	−	−	−
	B	−	−	−	−	−	−	+	+	+	+	+	+

Hannen et al., 1999a). van Hannen et al. (1999a) showed that the green algal detritus and cyanobacterial detritus can affect the structure of the microbial community. Combining these results, we can infer that the diverse DOM originated from cyanobacterial detritus, diatomic detritus and Cryptophyta detritus (three dominant phyla in our study, see Fig. 3) might have markedly influenced the composition of bacterioplankton community. High total bacterial abundance was always companied with the occurrence of cyanobacterial bloom, indicating that since the strengthened photosynthesis of cyanobacteria can increase the amount of organic substrates for bacteria, excessive primary production accelerates the growth of heterotrophic bacteria (Wu et al., 2007b). To this end, different phytoplankton regimens might support development of specific bacterial groups or species in Lake Taihu.

Table 4 – Information of the cyanobacterial bloom-related sequences that were 100 identical to bands GH-A-JUL2 or GH-B-SEP1 (both affiliated with cluster ML-5-51.2).

Clone	GenBank accession no.	Location	Reference
Uncultured bacterium clone xyhfb1-22[a]	HM050912	Lake Xingyunhu, China	Bacterial community composition in four eutrophic shallow lakes (unpublished data)
Uncultured bacterium clone xyhfb1-19[a]	HM050909		
Uncultured bacterium clone dcfb4-60[a]	HM050642	Lake Dianchi, China	
Uncultured bacterium clone dcfb4-48[a]	HM050630		
Uncultured bacterium clone chfb1-50[a]	HM050479	Lake Chaohu, China	
Uncultured bacterium clone chfb1-35[a]	HM050467		
Uncultured bacterium clone dcfb4-80[b]	HM050661	Lake Dianchi, China	
Uncultured actinobacterium clone WA0.2-0d-46[a]	HM153628	Lake Taihu, China	Li et al., 2011
Uncultured actinobacterium clone WA0.2-0d-80[b]	HM153640		
Uncultured actinobacterium clone WA0.2-0d-75[b]	HM153638		
Uncultured actinobacterium clone WA0.2-0d-33[b]	HM153619		
Uncultured actinobacterium clone WA0.2-0d-31[b]	HM153618		
Uncultured actinobacterium clone WA0.2-0d-26[b]	HM153615		
Uncultured actinobacterium clone WA0.2-0d-20[b]	HM153611		
Uncultured actinobacterium clone TH1-97[a]	AM690889		Wu et al., 2007a
Uncultured bacterium clone ML-9-97.2[a]	DQ520197		Wu et al., 2007b
Uncultured bacterium clone ML-5-51.2[b]	DQ520165		
Uncultured actinobacterium clone CYN-1-50[a]	EF158354	Lake Samsonvale, Australia	Pope and Patel, 2008

a sequences that were 100 identical to band GH-A-JUL2.
b sequences that were 100 identical to band GH-B-SEP1.

Muylaert et al. (2002) showed that composition of bacterial community was related to phytoplankton biomass, and found no evidence for top-down regulation of bacterial community composition in the turbid lakes characterized by the occurrence of phytoplankton blooms and high-nutrient. Similarly, in our study, abundance of crustacean zooplankton was not significantly related to BCC ($p > 0.05$). It is possible that bottom-up (resources) regulation could be more important compared to top-down (predation) regulation in the eutrophic lake.

4.2. *Taxonomic groups and their distribution associated with phytoplankton community*

In the present study, the majority of the non-cyanobacterial sequences obtained from the two sampling sites were affiliated with the divisions *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Firmicutes* (Table 2). Clones affliated with *Thermomicrobia*, *Fibrobacteres*, and *Verrucomicrobia* were also found in low numbers (<5 sequences per division).

Actinobacteria were regarded as one of the most significant divisions in this study. In various freshwater habitats *Actinobacteria* comprise large fractions of the total bacterioplankton (Glöckner et al., 2000; Warnecke et al., 2004; Allgaier and Grossart, 2006). A few studies have suggested that *Actinobacteria* are resistant to flagellate predation (Pernthaler et al., 2001; Hahn et al., 2003), which could be one possible explanation for their wide distribution and high abundances in freshwater systems. Clusters ACK-M1 and STA2-30, which are detected in almost every freshwater habitat (Zwart et al., 2003; Eiler and Bertilsson, 2004), were also identified in the present study. Interestingly, cluster STA2-30 (bands GH-A-JAN3 and GH-B-JUL6 at Sites A and B, respectively) was found as a dominant phylotype almost across the sampling time at the two study sites (Table 3). In accordance with previous studies in Lake Taihu, cluster STA2-30 was detected across all five open water areas (Wu et al., 2007a) and it was the only one of the two groups containing OTUs from all the four libraries (March, May, July, and September) obtained from Meiliang Bay (Wu et al., 2007b). Consequently, cluster STA2-30 may be one of the most important dominant phylotypes in the shallow eutrophic Lake Taihu. In the present study, we found that the cluster ML-5-51.2 (includes bands GH-A-JUL2, GH-A-JUL3, GH-B-SEP1 and GH-B-OCT1) appeared as the most prominent phylotype only during the *Microcystis* bloom period (Table 3; Fig. 4a). According to the BLAST analyses of bands GH-A-JUL2 and GH-B-SEP1 (a single-base difference between them), considerable part of the sequences that were 100 identical to bands GH-A-JUL2 or GH-B-SEP1 were closely related to the cyanobacterial bloom (Table 4). Most clones in Table 4 were originated from the four eutrophic shallow lakes in China (Lake Xingyunhu, Dianchi, Chaohu and Taihu), whereas the uncultured actinobacterium clone CYN-1-50 was detected in Lake Samsonvale, Australia, where the toxin-producing cyanobacteria dominate the planktonic blooms (Pope and Patel, 2008). Specially, of the eighteen clones listed in Table 4, three were found in the September libraries (Clone TH1-97, Wu et al., 2007a, ; Clone ML-9-97.2, Wu et al., 2007b) or May library (Clone ML-5-51.2, Wu et al., 2007b) in Meiliang Bay, Lake Taihu during the cyanobacterial bloom period and seven were detected when Li et al. (2011) studied the short-term dynamics of bacterial community composition in response to *Microcystis* bloom in Lake Taihu. As a consequence, it is conceivable that cluster ML-5-51.2 is a typical phylotype associated with the occurrence of *Microcystis* bloom and may regard as an indicator in eutrophic shallow lakes at least in Lake Taihu.

It was reported that *Bacteriodetes* was the most representative division in the clone libraries of four Swedish lakes associated with cyanobacterial blooms (Eiler and Bertilsson, 2004). This division was also present with high frequency in this study (Fig. S1d). Particularly, the family *Flavobacteriaceae* appeared with a higher proportion in the *Bacteroidetes* phylum (9 of 18) in the upper region of the DGGE profiles. Pinhassi et al. (2004) found that *Flavobacteriaceae* phylotypes were characteristic of the diatom-dominated community in their seawater microcosm experiments and the consistent response to diatom bloom conditions of a few *Flavobacteriaceae* phylotypes could indicate that some bacteria in this family harbor physiological or ecological traits that make them tightly coupled to diatom species. Subsequent phylogenetic analyses of published *Bacteroidetes* 16S rRNA gene sequences confirmed that members of the *Flavobacteriaceae* are remarkably responsive to algal blooms in seawater ecosystems (Pinhassi et al., 2004). According to a pervious study in Meiliang Bay, Lake Taihu (Wu et al., 2007b), as much as 70% of the *Flavobacteriaceae* phylotypes in the four clone libraries (March, May, July, and September) in Meiliang Bay (among a total of 10 sequences) were detected in March library (diatom-dominated period) whereas no clone was found in July and September when the *Microcystis* bloom happened. In accordance with the above-mentioned studies, in our field study the family *Flavobacteriaceae* was only detected from December 2008 to July 2009, when diatom began to flourish and dominated phytoplankton communities (Table 3). Our results indicated that some *Flavobacteriaceae* phylotypes may be closely linked to the diatom bloom and play particularly important roles in the processing of organic matter during the diatom blooms in Lake Taihu. Therefore, we suggest that there is coupling between members of the *Flavobacteriaceae* phylotypes and diatom blooms both in seawater and freshwater ecosystems.

In the present study, a total of 29 clones were affliated with the *Proteobacteria*, and most of these clones were from the subdivisions α-*Proteobacteria* and β-*Proteobacteria* (Fig. S1a and b). The majority of the α-*Proteobacteria* sequences fell into two distinct families, *Sphingomonadaceae* and *Rhodobacteraceae*. In this study, 4 typical freshwater clusters of α-*Proteobacteria* defined by Zwart et al. (2002) were detected, GOBB3-C201, *Brevundimonas intermedia*, CR-FL11, and LD12. In addition, α-*Proteobacteria* (bands GH-A-MAY2, GH-A-JUN1 and GH-A-JUN2) seemed to be predominant in early summer at Site A. Clones affiliated with the genus *Rhodoferax* of the β-*Proteobacteria* (BAL47 freshwater cluster; Zwart et al., 2003; Fig. 6b), which were abundant in all four cyanobacterial bloom libraries in Sweden (Eiler and Bertilsson, 2004), were also detected in our study. A mere five clones grouped in the γ- *Proteobacteria* and δ-*Proteobacteria* subdivisions (Fig. S1e).

Firmicutes are seldom discussed in previous studies of microbial ecology due to their low frequencies in the libraries (Eiler and Bertilsson, 2004; Wu et al., 2007b). Nevertheless, 6 and 1 clones were detected in our study at Sites A and B, respectively, and they all fell into the class Bacilli (Fig. S1f).

Three clones (bands GH-A-APR1, GH-A-APR2 and GH-A-APR4) were detected in April at Site A and they seemed to dominate the bacterioplankton community reflected by the DGGE profile (Fig. 4a). Bands GH-A-OCT2 and GH-A-OCT3 appeared in October, when the *Microcystis* bloom was very severe (*Microcystis* biomass reached a peak with 128.7 mg L^{-1}).

The left 6 of the 74 non-cyanobacterial clones were not affliated with the relatively abundant divisions discussed above. These clones were grouped into *Thermomicrobia*, *Fibrobacteres* and *Verrucomicrobia* (Fig. S1g).

4.3. *Diversity of phytoplankton and bacterioplankton community*

In the present study, variation trend of the Shannon–Wiener diversity index in bacterioplankton community was similar to that of phytoplankton community at both sites. The values were relatively low in cyanobacterial bloom period and high in winter or spring (Fig. 2h). Höfle et al. (1999) speculated that the diverse spring phytoplankton bloom releases more different substrates that provide increased niches for the more diverse bacterioplankton observed than does the phytoplankton bloom of a single alga in late-summer. We can conclude that the heavy cyanobacterial bloom consisted of *Microcystis* spp. resulted in low bacterial diversity while the diverse spring phytoplankton bloom (mainly consisted of Bacillariophyta and Cryptophyta) led to more diverse bacterioplankton community.

5. Conclusion

A detailed field survey on relationships between specific bacterioplankton taxa and phytoplankton communities shows that biomass of Cyanophyta and Bacillariophyta, as well as water temperature, were the three most influential factors on variation of BCC in Lake Taihu of China. *Microcystis* bloom resulted in low bacterial diversity while the diverse spring Bacillariophyta and Cryptophyta blooms led to more diverse bacterioplankton community. The family *Flavobacteriaceae* tightly coupled to diatom bloom while the cluster ML-5-51.2 dominated the bacterioplankton communities during *Microcystis* bloom. Conclusively, succession of phytoplankton community played key roles in shaping BCC. These may facilitate to understand the microbial loop and ecosystem functioning in eutrophic lake, although the mechanisms on interaction between phytoplankton and bacterioplankton communities need further investigations.

Acknowledgments

We thank the editor and two anonymous reviewers for their constructive suggestions and professional editing. This study was jointly supported by National Basic Research Program of China (2008CB418101), Lake water quality, water quantity and biological resources investigation in China (2006FY110600) and State key laboratory of freshwater ecology and biotechnology (2008FBZ01). We are grateful to Dr Xiaoxue Sun, Jing Zhang for zooplankton identification, Meng Zhang for the help on sample collection.

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.watres.2011.05.022.

REFERENCES

Allgaier, M., Grossart, H.P., 2006. Diversity and seasonal dynamics of actinobacteria populations in four lakes in northeastern Germany. Applied and Environmental Microbiology 72 (5), 3489–3497.

Chiang, S.C., Du, N.S., 1979. Fauan Sinica, Crustacea, Freshwater Cladocera. Science Press, Academia Sinica, Beijing (In Chinese).

Caron, D.A., 1994. Inorganic nutrients, bacteria, and the microbial loop. Microbial Ecology 28 (2), 295–298.

Cole, J.J., Likens, G.E., Strayer, D.L., 1982. Photosynthetically produced dissolved organic carbon: an important carbon source for planktonic bacteria. Limnology and Oceanography 27 (6), 1080–1090.

Cole, J.J., Findlay, S., Pace, M.L., 1988. Bacterial production in fresh and saltwater: a cross-system overview. Marine Ecology-Progress Series 43 (1–2), 1–10.

Crump, B.C., Hobbie, J.E., 2005. Synchrony and seasonality in bacterioplankton communities of two temperate rivers. Limnology and Oceanography 50 (6), 1718–1729.

Dorigo, U., Volatier, L., Humbert, J.F., 2005. Molecular approaches to the assessment of biodiversity in aquatic microbial communities. Water Research 39 (11), 2207–2218.

Eiler, A., Langenheder, S., Bertilsson, S., Tranvik, L.J., 2003. Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. Applied and Environmental Microbiology 69 (7), 3701–3709.

Eiler, A., Bertilsson, S., 2004. Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environmental Microbiology 6 (12), 1228–1243.

Fromin, N., Hamelin, J., Tarnawski, S., Roesti, D., Jourdain-Miserez, K., Forestier, N., Teyssier-Cuvelle, S., Gillet, F., Aragno, M., Rossi, P., 2002. Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. Environmental Microbiology 4 (11), 634–643.

Glöckner, F.O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A., Amann, R., 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. Applied and Environmental Microbiology 66 (11), 5053–5065.

Hahn, M.W., 2006. The microbial diversity of inland waters. Current Opinion in Biotechnology 17 (3), 256–261.

Hahn, M.W., Lünsdorf, H., Wu, Q.L., Schauer, M., Höfle, M.G., Boenigk, J., Stadler, P., 2003. Isolation of novel ultramicrobacteria classified as Actinobacteria from five freshwater habitats in Europe and Asia. Applied and Environmental Microbiology 69 (3), 1442–1451.

Haukka, K., Kolmonen, E., Hyder, R., Hietala, J., Vakkiainen, K., Kairesalo, T., Haario, H., Sivonen, K., 2006. Effect of nutrient loading on bacterioplankton community composition in lake mesocosms. Microbial Ecology 51 (2), 137–146.

Höfle, M.G., Haas, H., Dominik, K., 1999. Seasonal dynamics of bacterioplankton community structure in a eutrophic lake as determined by 5S rRNA analysis. Applied and Environmental Microbiology 65 (7), 3164–3174.

Hu, H.J., Wei, Y.X., 2006. The Freshwater Algae of China. Science and Technology Press, Beijing (In Chinese).

Jensen, L.M., 1983. Phytoplankton release of extracellular organic carbon, molecular weight composition, and bacterial assimilation. Marine Ecology-Progress Series 11 (1), 39–48.
Jin, X.C., Tu, Q.Y., 1990. The Standard Methods for Observation and Analysis of Lake Eutrophication, second ed. China Environmental Science Press, Beijing, China (In Chinese).
Jones, S.E., Newton, R.J., McMahon, K.D., 2009. Evidence for structuring of bacterial community composition by organic carbon source in temperate lakes. Environmental Microbiology 11 (9), 2463–2472.
Kalff, J., Knoechel, R., 1978. Phytoplankton and their dynamics in oligotrophic and eutrophic lakes. Annual Review of Ecology and Systematics 9, 475–495.
Kan, J., Suzuki, M.T., Wang, K., Evans, S.E., Chen, F., 2007. High temporal but low spatial heterogeneity of bacterioplankton in the Chesapeake Bay. Applied and Environmental Microbiology 73 (21), 6776–6789.
Langenheder, S., Jürgens, K., 2001. Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnology and Oceanography 46 (1), 121–134.
Li, H.B., Xing, P., Chen, M.J., Bian, Y.Q., Wu, Q.L., 2011. Short-term bacterial community composition dynamics in response to accumulation and breakdown of *Microcystis* blooms. Water Research 45 (4), 1702–1710.
Lindström, E.S., 2000. Bacterioplankton community composition in five lakes differing in trophic status and humic content. Microbial Ecology 40 (2), 104–113.
Lindström, E.S., 2001. Investigating influential factors on bacterioplankton community composition: results from a field study of five mesotrophic lakes. Microbial Ecology 42 (4), 598–605.
Lindström, E.S., Kamst-van Agterveld, M.P., Zwart, G., 2005. Distribution of typical freshwater bacterial groups is associated with pH, temperature, and lake water retention time. Applied and Environmental Microbiology 71 (12), 8201–8206.
Murray, A.E., Hollibaugh, J.T., Orrego, C., 1996. Phylogenetic compositions of bacterioplankton from two California estuaries compared by denaturing gradient gel electrophoresis of 16S rDNA fragments. Applied and Environmental Microbiology 62 (7), 2676–2680.
Muylaert, K., Van der Gucht, K., Vloemans, N., Meester, L.D., Gillis, M., Vyverman, W., 2002. Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. Applied and Environmental Microbiology 68 (10), 4740–4750.
Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59 (3), 695–700.
Muyzer, G., Smalla, K., 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek 73 (1), 127–141.
Muyzer, G., 1999. DGGE/TGGE a method for identifying genes from natural ecosystems. Current Opinion in Microbiology 2 (3), 317–322.
Obernosterer, I., Herndle, G.J., 1995. Phytoplankton extracellular release and bacterial growth: dependence on the inorganic N: P ratio. Marine Ecology-Progress Series 116 (1–3), 247–257.
Pernthaler, J., Posch, T., Simek, K., Vrba, J., Pernthaler, A., Glöckner, F.O., Nubel, U., Psenner, R., Amann, R., 2001. Predator-specific enrichment of Actinobacteria from a cosmopolitan freshwater clade in mixed continuous culture. Applied and Environmental Microbiology 67 (5), 2145–2155.
Pinhassi, J., Hagstrom, A., 2000. Seasonal succession in marine bacterioplankton. Aquatic Microbial Ecology 21 (3), 245–256.
Pinhassi, J., Sala, M.M., Havskum, H., Peters, F., Guadayol, Ò., Malits, A., Marrasé, C., 2004. Changes in bacterioplankton composition under different phytoplankton regimens. Applied and Environmental Microbiology 70 (11), 6753–6766.
Pomeroy, L.R., Wiebe, W.J., 2001. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquatic Microbial Ecology 23 (2), 187–204.
Pope, P.B., Patel, B.K.C., 2008. Metagenomic analysis of a freshwater toxic cyanobacteria bloom. FEMS Microbiology Ecology 64 (1), 9–27.
Poter, K.G., Feig, Y.S., 1980. The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography 25 (5), 943–948.
Qin, B.Q., Xu, P.Z., Wu, Q.L., Luo, L.C., Zhang, Y.L., 2007. Environmental issues of Lake Taihu, China. Hydrobiologia 581, 3–14.
Riemann, L., Steward, G.F., Fandino, L.B., Campbell, L., Landry, M.R., Azam, F., 1999. Bacterial community composition during two consecutive NE monsoon periods in the Arabian Sea studied by denaturing gradient gel electrophoresis (DGGE) of rRNA genes. Deep Sea Research 46 (8–9), 1791–1811.
Rooney-Varga, J.N., Giewat, M.W., Savin, M.C., Sood, S., LeGresley, M., Martin, J.L., 2005. Links between phytoplankton and bacterial community dynamics in a coastal marine environment. Microbial Ecology 49 (1), 163–175.
Saitou, N., Nei, M., 1987. The Neighbor-Joining method—a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4 (4), 406–425.
Sekar, R., Nair, K.V.K., Rao, V.N.R., Venugopalan, V.P., 2002. Nutrient dynamics and successional changes in a lentic freshwater biofilm. Freshwater Biology 47 (10), 1893–1907.
Sheng, J.R. (Ed.), 1979. Fauna Sinica, Crustacea, Freshwater Copepoda. Science Press, Academia Sinica, Beijing In Chinese.
Shade, A., Kent, A.D., Jones, S.E., Newton, R.J., Triplett, E.W., McMahon, K.D., 2007. Interannual dynamics and phenology of bacterial communities in a eutrophic lake. Limnology and Oceanography 52 (2), 487–494.
Smith, V.H., 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221 (4611), 669–671.
Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24 (8), 1596–1599.
ter Braak, C.J.F., 1987. The analysis of vegetation–environment relationships by canonical correspondence analysis. Vegetatio 69 (1–3), 69–77.
ter Braak, C.J.F., Verdonschot, P.F.M., 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquatic Sciences 57 (3), 255–289.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22 (22), 4673–4680.
Tian, C., Tan, J., Wu, X., Ye, W.J., Liu, X.L., Li, D.T., Yang, H., 2009. Spatiotemporal transition of bacterioplankton diversity in a large shallow hypertrophic freshwater lake, as determined by denaturing gradient gel electrophoresis. Journal of Plankton Research 31 (8), 885–897.
van Hannen, E.J., Mooij, W., Kamst-van Agterveld, M.P., Gons, H.J., Laanbroek, H.J., 1999a. Detritus-dependent development of the microbial community in an experimental system: qualitative analysis by denaturing gradient gel electrophoresis. Applied and Environmental Microbiology 65 (6), 2478–2484.
van Hannen, E.J., Veninga, M., Bloem, J., Gons, H.J., Laanbroek, H.J., 1999b. Genetic changes in the bacterial community structure associated with protistan grazers. Archiv für Hydrobiologie 145 (1), 25–38.

Warnecke, F., Amann, R., Pernthaler, J., 2004. Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. Environmental Microbiology 6 (3), 242–253.

Wu, Q.L., Zwart, G., Wu, J.F., Kamst-van Agterveld, M.P., Liu, S.J., Hahn, M.W., 2007a. Submersed macrophytes play a key role in structuring bacterioplankton community composition in the large, shallow, subtropical Taihu Lake, China. Environmental Microbiology 9 (11), 2765–2774.

Wu, X., Xi, W.Y., Ye, W.J., Yang, H., 2007b. Bacterial community composition of a shallow hypertrophic freshwater lake in China, revealed by 16S rRNA gene sequences. FEMS Microbiology Ecology 61 (1), 85–96.

Xing, P., Kong, F.X., 2007. Intra-habitat heterogeneity of environmental factors regulating bacterioplankton community composition in Lake Taihu, China. Aquatic Microbial Ecology 48 (2), 113–122.

Xu, H., Paerl, H.W., Qin, B.Q., Zhu, G.W., Gao, G., 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnology and Oceanography 55 (1), 420–432.

Zwart, G., Crump, B.C., Kamst-van Agterveld, M.P., Hagen, F., Han, S.K., 2002. Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquatic Microbial Ecology 28 (2), 141–155.

Zwart, G., van Hannen, E.J., Kamst-van Agterfeld, M.P., Van der Gucht, K., Lindström, E.S., Van Wichelen, J., Lauridsen, T., Crump, B.C., Han, S.K., Declerck, S., 2003. Rapid screening of freshwater bacterial groups by using reverse line blot hybridization. Applied and Environmental Microbiolog 69 (10), 5875–5883.