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Plankton community composition in the Three Gorges Reservoir Region revealed by PCR-DGGE and its relationships with environmental factors

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Abstract

To explore the relationships between community composition and the environment in a reservoir ecosystem, plankton communities from the Three Gorges Reservoir Region were studied by PCR-denaturing gradient gel electrophoresis fingerprinting. Bacterial and eukaryotic operational taxonomic units (OTUs), generated by DGGE analysis of the PCR-amplified 16S and 18S rRNA genes, were used as surrogates for the dominant "biodiversity units". OTU composition among the sites was heterogeneous; 46.7% of the total bacterial OTUs (45) and 64.1% of the eukaryotic OTUs (39) were identified in less than half of the sampling sites. Unweighted pair group method with arithmetic averages (UPGMA) clustering of the OTUs suggested that the plankton communities in the Xiangxi Rive sites were not always significantly different from those from the Yangtze River sites, despite clear differences in their environmental characterizations. Canonical correspondence analysis (CCA) was applied to further investigate the relationships between OTU composition and the environmental factors. The first two CCA ordination axes suggested that the bacterial community composition was primarily correlated with the variables of NO_3^--N, dissolved oxygen (DO), and SiO_3^{2-}-Si, whereas, the eukaryotic community was mainly correlated with the concentrations of DO, PO_4^{3-}-P, and SiO_3^{2-}-Si.

Key words: plankton community; environmental factors; denaturing gradient gel electrophoresis (DGGE); spatial pattern; Three Gorges Reservoir Region

Introduction

The Three Gorges Project on the Yangtze River has been a focus of international interest because of its possible effects on the ecosystem. After the closure of the dam, algal blooms began to occur frequently in the Xiangxi River and in some upstream tributaries (Kuang *et al*., 2005; Ye *et al*., 2006; Zeng *et al*., 2006). Since then, it has become increasingly clear that the aquatic ecosystems of the Yangtze River and Xiangxi River are going to be greatly impacted by the huge reservoir. This has generated increasing interest in the development of methods to monitor and predict the potential ecological effects. Planktonic organisms exhibit complex and sensitive responses to environmental stimuli that are manifested through changes at the individual, population, and community levels of organization. Therefore, the plankton community is generally considered to be a good indicator of water quality, and ecosystem changes are reflected in relatively rapid shifts in density and community composition (Bianchi *et al*., 2003; Cairns *et al*., 1993; Leppard and Munawar, 1992). However, the analysis of the community composition by classical taxonomic identification has historically been a difficult task because of the lack of distinguishing features and the small size of a multitude of planktonic microorganisms.

Fortunately, technical developments in molecular biology have led to new techniques and approaches to the study of community composition, diversity, and function in microbial ecosystems (Holben and Harris, 1995; Lee and Fuhrman, 1991; Santo-Domingo *et al*., 1998; Zehr and Voytek, 1999). As the metagenome can be used to define the environmental genome (Handelsman *et al*., 1998), culture-independent metagenomic approaches have opened a new avenue for ecological study (Handelsman, 2004). Fifteen years ago, denaturing gradient gel electrophoresis (DGGE) was introduced to microbial ecology (Muyzer *et al*., 1993). Since then, DGGE has been widely used to study natural community composition in numerous environments (Muyzer and Smalla, 1998). Although the DGGE banding patterns (OTU composition) may not reflect the community a hundred percent, it has become a reliable and popular method for the examination of differences and similarities in community composition (van Hannen *et al*., 1998; Lindström, 2000).

With respect to planktonic organisms, PCR-DGGE

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fingerprinting based on the 16S rRNA genes has been used to depict the structure of bacterioplankton community in rivers (Zwart *et al*., 2002), lakes (Lindström, 2000, 2001), and oceans (Rooney-Varga *et al*., 2005). DGGE analysis of 18S rRNA genes has also been applied to the exploration of the diversity of microbial eukaryotic communities (van Hannen *et al*., 1998; Savin *et al*., 2004). More recently, Yan *et al*. (2007) explored the genetic diversity of both the bacterial and the eukaryotic plankton communities (with DGGE analysis of the 16S rDNA and 18S rDNA) in a shallow freshwater lake. The present study was designed to extend the use of PCR-DGGE fingerprinting analysis in plankton ecology by exploring the possibility of using DNA fingerprinting to estimate the potential ecological effects of the Three Gorges Reservoir system. The relationships between genetic diversity and the environmental factors were also explored to determine the correlation of environmental factors with the target community.

1 Materials and methods

1.1 Area description

The Three Gorges Reservoir, the world's largest water control project, has greatly influenced the ecological conditions of the Yangtze River since water storage began on 1 June 2003. Hydrologic conditions of the Yangtze River have changed considerably. For example, the concentration of sediment in front of the dam has dropped from 0.578 to 0.155 g/L; the average flow velocity in the main channel has dropped from 0.85 to 0.20 m/s; and the residence time of the reservoir has been increased to 77 d or even more in backwater areas (Du *et al*., 2004). The Xiangxi River, which lies about 32 km upstream from the dam, is the nearest midsize tributary to the dam. With the filling of the reservoir, the lower reaches of the Xiangxi River have been transformed from a riverine to a lacustrine system. The water level of the Xiangxi River has increased about 40 m and the water flow velocity has dropped from 0.43–0.92 m/s to 0.0020–0.0041 m/s (Tang *et al*., 2004; Wang, 2005).

1.2 Sample collection and physico-chemical analysis

Water samples were collected from the Three Gorges Reservoir Region in April 2006. Sampling was conducted at five sites (A1–A5) along the Xiangxi River and six sites (B1–B6) along the Yangtze River (Fig.1). Planktonic organisms were collected from the surface using horizontal tows with No. 25 plankton nets; the samples were stored at 4°C until DNA extraction. The physico-chemical characteristics, including transparency, concentrations of dissolved oxygen (DO), NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}-P, and SiO_3^{2-}-Si, were analyzed according to the standard methods (Huang, 2000).

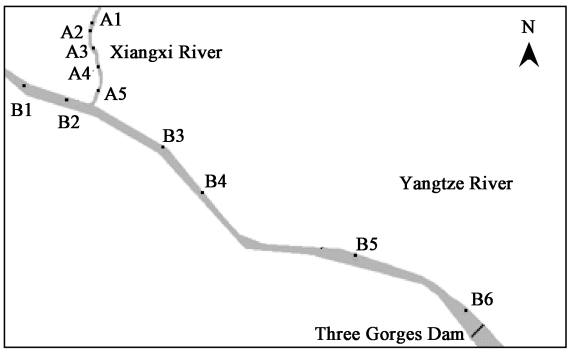

Fig. 1 Distribution of the sampling sites in the Three Gorges Reservoir Region.

1.3 DNA extraction and PCR amplification

DNA was extracted according to the method described previously (Yan *et al*., 2007). The 16S rRNA and 18S rRNA genes were amplified with bacterial primers (Muyzer *et al*., 1993) and eukaryotic primers (van Hannen *et al*., 1998), respectively. Polymerase chain reaction (PCR) conditions for each 50 μl reaction mixture were 1 × buffer, 2 mmol/L Mg^{2+}, 3.0 U of Taq DNA polymerase, 80 μmol/L of each deoxynucleotide (Fermentas Inc., USA), 0.3 μmol/L of each primer, and approximately 40 ng of template DNA. Touchdown PCR was performed on a GeneAmp PCR System 9600 thermal cycler (Perkin Elmer Cetus, USA) with an initial denaturation step at 94°C for 5 min. Then, each cycle was carried out at 94°C for 0.5 min, at the annealing temperature (Table 1) for 0.5 min, and extended at 72°C for 1 min. Finally, a primer extension at 72°C for 10 min was performed. A negative control was conducted in the same manner as the samples, except that the DNA was excluded.

1.4 DGGE analysis

DGGE was performed with the INGENYphorU-2 system (INGENY International BV, the Netherlands) using 9% polyacrylamide in 1 × TAE buffer. PCR products containing approximately equal amounts of DNA of similar size were separated on a gel containing a linear gradient of the denaturants. Denaturants 40%–60% were applied to separate the 16S rDNA, and 30%–65% for separation of the 18S rDNA (100% denaturant was defined as 7

Table 1 Primer sequences and annealing temperatures used in touchdown PCR

Primer[a]	Oligonucleotide sequence (5′–3′)	Annealing temperature	Reference
F357[b]	CCTACGGGAGGCAGCAG	67–58°C (10 cycles[c]),	Muyzer *et al*., 1993
R518	ATTACCGCGGCTGCTGG	then 57°C (20 cycles)	
F1427[b]	TCTGTGATGCCCTTAGATGTTCTGGG	69–60°C (10 cycles[c]),	van Hannen *et al*., 1998
R1616	GCGGTGTGTACAAAGGGCAGGG	then 59°C (18 cycles)	

[a] Primer set F357 and R518 for 16S rDNA, and primer set F1427 and R1616 for 18S rDNA; [b] a 40-nucleotide GC-clamp (5′-CGCCCGCCGCG CCCCGCGCCCGGCCCGCCGCCCCCGCCCC-3′); [c] with the temperature decreasing 1°C each cycle.

mol/L urea and 40% formamide). Electrophoresis was performed at 60°C, 120 V for 14 h. After electrophoresis, gels were stained in 1 × TAE buffer containing 1 × SYBR Gold (Molecular Probes Europe BV, the Netherlands), then photographed using UVP Imaging System (UVP Inc., USA). The gel images were further processed using Adobe Photoshop 8.01 to maximize image contrast. Each band was related to one single population and considered to be one operational taxonomic unit (OTU). The OTUs were used as surrogates for the predominant "biodiversity units".

1.5 Data analysis

DGGE profiles were scanned using LabWorks software (UVP Inc., USA), and the patterns were carefully checked manually. The presence or absence of comigrating bands, independent of intensity, was converted to a binary (0/1) matrix. Jaccard's coefficient of similarity (F) between two samples was calculated according to the equation:

$$F = n_{11}/(n-n_{00}) \quad (1)$$

where, n is the total number of bands in all samples; n_{11} represents the bands present in both samples, and n_{00} represents the bands absent in both samples. From here on, for convenience, the term "OTU" is used for each DGGE band. The dendrogram was then constructed from the F values using the unweighted pair group method with arithmetic averages (UPGMA) by the XLSTAT-Pro 2007 (Addinsoft, USA). The UPGMA was also employed to compare site groups on the basis of the physico-chemical parameters. Canonical correspondence analysis (CCA), originally developed to relate community composition to environmental factors (ter Braak, 1986), was used here to investigate the relationships between OTU composition and the environmental parameters. CCA was performed using the software program CANOCO (version 4.15). The data were log-transformed before CCA to eliminate the influence of extreme values on ordination scores. Additionally, the environmental factors with high partial correlation coefficients and variance inflation factors were eliminated from the final CCA.

2 Results

2.1 Plankton community composition

Forty-five different 16S rDNA OTUs (Fig.2a) and 39 different 18S rDNA OTUs (Fig.2b) were detected in the DGGE analysis. The number of OTUs detected per site ranged between 20 and 27, with an average of 23. Ten of the 45 bacterial OTUs (22.2%) were site-specific, and only four were common to all the investigated samples. No eukaryotic OTUs were common to the 11 samples,

Table 2 Physico-chemical characterization of the samples in the Three-Gorge Reservoir Region

	Xiangxi River site					Yangtze River site						p*
	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	B6	
Transparency (m)	2.40	1.20	1.80	1.40	2.80	4.40	4.20	3.80	4.60	4.40	3.80	0.000
DO (mg/L)	15.90	16.86	15.45	18.41	12.63	9.02	9.05	9.21	9.73	8.87	9.13	0.000
NH_4^+-N (μmol/L)	0.65	0.24	0.64	0.17	3.83	5.35	5.41	5.26	5.70	4.59	3.93	0.000
NO_3^--N (μmol/L)	29.43	32.11	50.9	56.19	76.93	111.55	103.85	78.99	112.72	117.84	116.22	0.000
NO_2^--N (μmol/L)	1.01	1.45	1.84	1.93	2.28	2.59	2.45	1.94	2.63	2.23	2.13	0.024
PO_4^{3-}-P (μmol/L)	2.95	1.67	1.10	1.28	1.19	0.90	0.72	0.80	0.86	1.46	1.29	n.s.
SiO_3^{2-}-Si (μmol/L)	85.88	85.73	86.12	85.77	85.57	87.09	87.61	86.67	73.92	87.11	86.94	n.s.

* The statistical significant level ($p < 0.05$) between the Xiangxi River sites and Yangtze River sites (n.s. is not significant).

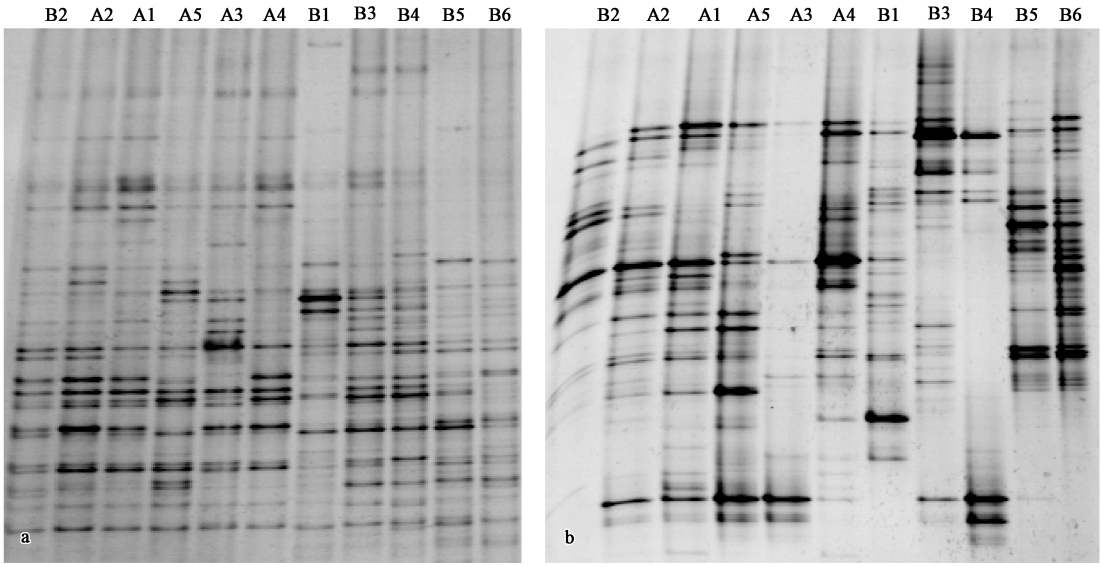

Fig. 2 DGGE profile of 16S DNA (a) and 18S DNA (b) fragments amplified from natural community DNA. Each band is considered to be an operational taxonomic unit (OTU), A1–A5 and B1–B6 indicate the sampling sites in Fig.1.

and only three (7.7%) were restricted to single samples. Additionally, 46.7% of the bacterial OTUs and 64.1% of the eukaryotic OTUs were detected in less than half of the samples. The similarity in the composition of bacterial/eukaryotic plankton communities, as visualized by UPGMA clustering, indicates that not all the communities in the Xiangxi River sites were significantly different from those in the Yangtze River (Figs.3b and 3c), despite their clearly different physico-chemical characteristics (Table 2, Fig.3a). At the dissimilarity level of 0.60 (shown as the dotted line), all the sites were clustered together when just the bacterial community was considered (Fig.3b), whereas, most sites separated at the same dissimilarity level (Fig.3c) when the analysis was based on eukaryotic data.

2.2 Environmental characters

Physico-chemical characterizations of the investigated samples are summarized in Table 2. The variables of DO, NH_4^+-N, NO_3^--N, NO_2^--N, and transparency in the Xiangxi River sites were significantly different from those of the Yangtze River sites at the significant level of $p < 0.05$ (ANOVA, Table 2). Briefly, the concentrations of DO in the Xiangxi River sites were higher than those of the Yangtze River sites, whereas, the concentrations of NH_4^+-N, NO_3^--N, and NO_2^--N were comparatively lower. The transparency in the Xiangxi River sites was also relatively lower than the Yangtze River sites. From the view of the UPGMA clustering, all sites of the Xiangxi River except A5 were clustered into one group, and all the Yangtze River sites, plus Xiangxi River A5 comprised of another group (Fig.3a).

2.3 Plankton community composition in relation to environmental variables

CCA analyses were performed using the environmental variables of DO, PO_4^{3-}-P, SiO_3^{2-}-Si, NO_3^--N, and NO_2^--N with either bacterial or eukaryotic OTU composition. The first two CCA ordination axes explained 41.0% of the bacterial OTU variation and 34.1% of the eukaryotic OTU variation. The 71.4% and 64.0% of the cumulative variance of OTU-environment relation was represented by the first two axes (Table 3). The CCA ordinations showed that the distribution of bacterioplankton was primarily correlated with NO_3^--N, DO, and SiO_3^{2-}-Si (Fig.4a). Briefly, NO_3^--N and DO were positively ($r = 0.8301$) and negatively ($r = –0.7622$) correlated with the first CCA axis, respectively. SiO_3^{2-}-Si was negatively ($r = –0.8605$) correlated with the second axis. With respect to the eukaryotic plankton, both DO ($r = –0.6361$) and PO_4^{3-}-P ($r = –0.5917$) were negatively correlated with the first CCA axis, and SiO_3^{2-}-Si was negatively ($r = –0.9276$) correlated with the second axis (Fig.4b). All the sites except B4 were generally separated on the first ordination axis (Fig.4). Additionally, the Xiangxi River sites generally distribute in the second and third quadrants, whereas, the Yangtze sites generally distribute in the first and fourth quadrants.

3 Discussion

DGGE bands are generally used as surrogates of the relative abundance of dominant populations, for example, abundance above 0.3%–0.4% of the total targeted cells

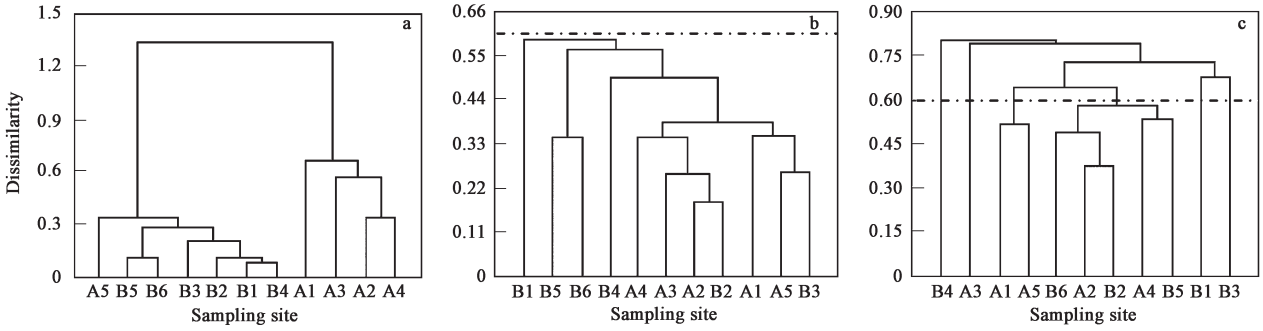

Fig. 3 UPGMA dendrograms of the sampling sites on the basis of the physico-chemical characteristics (a), bacterial DGGE fingerprints (b), eukaryotic DGGE fingerprints (c). Euclidean distance was used to calculate the dissimilarity in the physico-chemical analysis, and dissimilarity was calculated using the Jaccard's coefficient in the other two dendrograms.

Table 3 Summary results of the canonical correspondence analysis

Parameter	Bacterial OTU-environment		Eukaryotic OTU-environment	
	Axis 1	Axis 2	Axis 1	Axis 2
DO (mg/L)	–0.7622	–0.3009	–0.6361	–0.0282
SiO_3^{2-}-Si (μmol/L)	–0.0462	–0.8605	0.1790	–0.9276
NO_3^--N (μmol/L)	0.8301	0.2697	0.5062	0.0030
PO_4^{3-}-P (μmol/L)	–0.2150	–0.4015	–0.5917	–0.0971
NO_2^--N (μmol/L)	0.5185	0.4295	0.3715	0.0891
Eigenvalues	0.173	0.134	0.240	0.193
OTU-environment correlations*	0.952	0.965	0.921	0.968
CPV of OTU data	23.1	41.0	18.9	34.1
CPV of OTU-environment relation	40.3	71.4	35.5	64.0

* OTU-environment correlations describe the strength of the relationships between OTUs and environmental parameters for the axes; CPV: cumulative percentage variance.

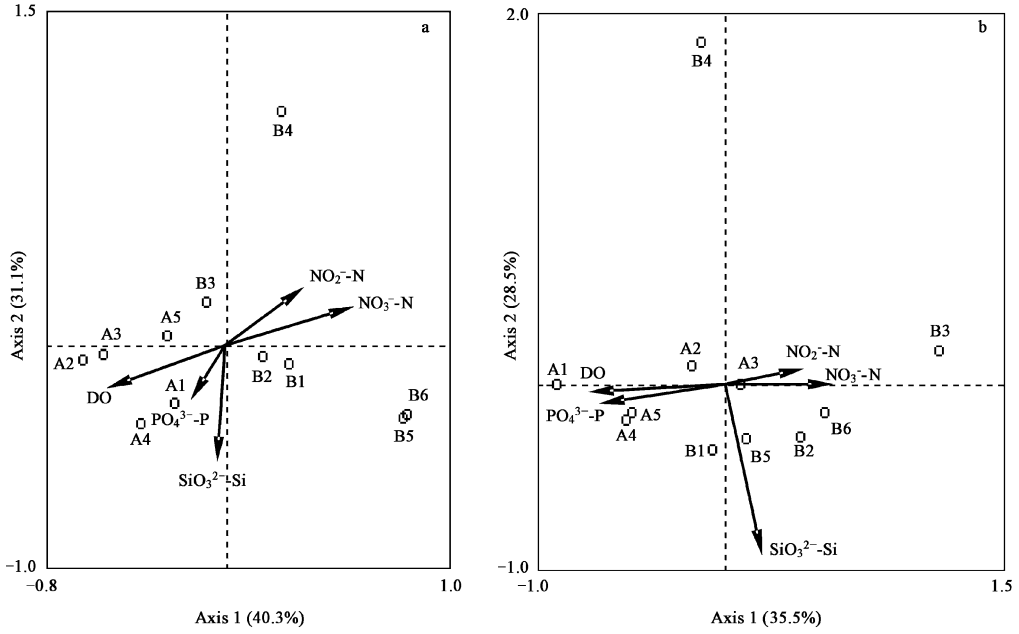

Fig. 4 Canonical correspondence analysis (CCA) ordination diagram of the bacterial community composition data (a) and eukaryotic community composition data (b) in relation to the five selected environmental variables.

(Casamayor *et al*., 2000, 2002). A large number of OTUs (46.7% of the bacterial and 64.1% of the eukaryotic OTUs) identified herein were present in less than half of the sampling sites, whereas, less than 5% of the total OTUs were common to all the samples. These indicate that the Three Gorges Reservoir Region has a comparatively high heterogeneity in the plankton community composition. From the perspective of biomonitoring, the environmental conditions differed among the sampling sites, so that one could reasonably assume that the plankton communities would as well. The samples derived from A3, which is the middle site along the Xiangxi River (Fig.1), revealed the minimum number of bacterial (20) and eukaryotic OTUs (7). This suggests that the environmental conditions at that point are less conducive to the development of a robust and diverse plankton community than elsewhere in the system. In fact, algal blooms in the downstream reaches of the Xiangxi River occurred frequently after the dam closure (Kuang *et al*., 2005; Ye *et al*., 2006; Zeng *et al*., 2006).

Most of the investigated physico-chemical parameters (i.e., DO, NH_4^+-N, NO_3^--N, NO_2^--N, and transparency) in the Xiangxi River were significantly different from those of the Yangtze River (ANOVA, $p < 0.05$, Table 2). From the clustering based on the investigated environmental variables, the sites generally fell into two groups according to their geographic locations (Fig.3a). Many references have reported the differences between the Xiangxi River and the Yangtze River after the dam closure, for example, the soluble nutrient concentrations (including NH_4^+-N, NO_3^--N, NO_2^--N, PO_4^{3-}-P, and SiO_3^{2-}-Si) in the Xiangxi River were significantly different from those of the Yangtze River (Zeng *et al*., 2006). The concentrations of phosphorous and nitrogen in the downstream of the Xiangxi River increased after dam closure (Fang *et al*., 2006), whereas, the annual average of TN in front of the Three Gorges Reservoir decreased from 2.38 to 1.62 mg/L, and TP decreased from 0.274 to 0.132 mg/L after the dam closure (Cao *et al*., 2006). These changes suggest that the water quality of the Xiangxi River may be degraded or, at least, reflect that the lake-like conditions are more conducive to algal blooms. By contrast, after the dam closure, the environmental conditions at the investigated Yangtze River sites seem to be more favorable than the Xiangxi River sites for the support of riverine planktonic organisms.

In traditional physico-chemical analysis, instantaneous effects (such as changes in water flow, extensive wind resuspension) are considered to be important factors that may make neighboring sites in a river system more homogeneous than they really are. As a reflection of the integrative ecological effects, biomonitoring methods are generally considered more acceptable than the physico-chemical methods, for estimating long-term environmental conditions. Moreover, genetic fingerprinting of a targeted community has been reported to be more sensitive than the physico-chemical analyses in characterizing the similarities or differences of environmental habitats (Yan *et al*., 2005, 2007; Boon *et al*., 2000). Therefore, the group relationships of the sampling sites herein were not simply the same as those calculated on the basis of the environmental parameters (Fig.3). Furthermore, the eukaryotic community showed comparatively higher discrepancy than the bacterial community to site grouping based on distance or instantaneous environmental measures.

CCA was applied to further explore the correlation between plankton communities and environmental factors. The obtained CCA ordination axes (based on community composition data) were linear combinations of environmental variables, assuming a unimodal species-environment relationship (ter Braak, 1986). Lindström (2000, 2001) applied this statistical method to look at how the bacterioplankton community composition, which was revealed by PCR-DGGE fingerprinting, correlated with

different environmental variables. In the present study, the correlations of the first two CCA axes (0.921–0.968, Table 3) indicated a strong relationship between the OTU composition and the selected environmental variables. The first CCA axis was found to be strongly correlated with the concentrations of DO and NO_3^--N (bacterial OTU-environment analysis), or DO and PO_4^{3-}-P (eukaryotic OTU-environment analysis, Table 3). The second axis was strongly correlated with SiO_3^{2-}-Si. However, all of the sites except B4 were generally separated on the first ordination axis (Fig.4). This suggested that the spatial pattern of the investigated plankton communities was primarily correlated with the factors of DO, PO_4^{3-}P, and NO_3^--N among the investigated parameters.

Results of the present research together with the previous studies (Yan *et al*., 2005, 2006, 2007; Yu *et al*., 2004) suggest that genetic fingerprinting of the plankton community is a convenient and useful method that reflects the environmental conditions. The current study also indicates that it is possible to apply DNA fingerprinting of the plankton community in the reservoir ecosystem to determine the effects of the reservoir on the aquatic ecosystem. As the method continues to be refined, it is expected to play an increasingly important role in ecological studies.

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