Microbial Ecology

Genetic Diversity of Plankton Community as Depicted by PCR-DGGE Fingerprinting and its Relation to Morphological Composition and Environmental Factors in Lake Donghu

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Abstract

To collect information about the genetic diversity of the plankton community and to study how plankton respond to environmental conditions, plankton samples were collected from five stations representing different trophic levels in a shallow, eutrophic lake (Lake Donghu), and investigated by PCR-DGGE fingerprinting. A total of 100 bands (61 of 16S rDNA bands and 39 of 18S rDNA bands) were detected. The DGGE bands unique to any single station accounted for 38% of the total bands, whereas common bands detected at all five stations accounted for only 11%. Using UPGMA clustering and MDS ordination of DGGE fingerprints, stations I and II were found to initially group together into one cluster, which was later joined by station V. Stations III and IV were isolated into two separate groups of one station each. Some differences in grouping relationships were found when analysis was completed on the basis of chemical characteristics and morphological composition, with zooplankton composition showing the greatest variability. However, the most similar stations (I and II) were always initially grouped into one cluster. Moreover, stations that exhibited the same or similar trophic level (stations III and IV), but different concentrations of heavy metals, were further differentiated by the DGGE method. Results of the present study indicated that PCR-DGGE fingerprinting was more sensitive than the traditional methods, as other studies suggested. Additionally, PCR-DGGE appears to be more appropriate for diversity characterization of the plankton community, as it is more canonical, systematic, and effective. Most importantly, fingerprinting results are more convenient for the comparative analyses between different studies. Therefore, the use of the described fingerprinting analysis may provide an operable and sensitive biomonitoring approach to identify critical, and potentially negative, stress within an aquatic ecosystem.

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Introduction

Plankton, the small, free-floating organisms in aquatic ecosystems, is generally considered to be composed of both the phytoplankton and zooplankton, but also includes bacteria. Planktonic organisms are considered good indicators of water quality and aquatic ecosystem health because many are highly sensitivity to physical and chemical perturbations and ecosystem changes are reflected in relatively rapid density and diversity shifts associated with short life spans [1, 2, 20]. However, taxonomic identification has historically been a difficult task (even for the seasoned taxonomist) due to the lack of distinguishing features, especially for many nondescript, yet abundant, organisms. Technical developments in molecular biology have found extensive applications in the areas of community structure and function [6, 17, 29]. Recently, different fingerprinting techniques have been developed and applied successfully to analyze bacterial groups and, more recently, picoplankton communities and eukaryotic diversity. Advanced techniques that have been successfully employed in aquatic ecology include the use of denaturing gradient gel electrophoresis (DGGE) in microbial ecology [15] and single strand conformation polymorphism (SSCP), which has been used to study the natural bacterial communities in aquatic ecosystems [10]. Liu et al. [13] employed

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terminal-restriction fragment length polymorphism (T-RFLP) to characterize the structure of microbial communities. Yu et al. [28] explored the feasibility of DNA fingerprinting to community-level analysis and Yan et al. [27] applied random amplified polymorphic DNA (RAPD) to investigate DNA polymorphism of plankton communities. These DNA-based community-level studies, together with others, have significantly increased our understanding of community diversity [14, 21, 29], which, in turn, makes it possible to elucidate the manner in which molecular-level actions influence ecosystem changes. Among all of the available fingerprinting approaches, PCR-DGGE is one of the most commonly used methods to investigate the diversity and spatial–temporal dynamics of communities [26]. However, most of the studies to date have focused on the analysis of prokaryotic 16S ribosomal RNA (rRNA) genes; comparatively, little is known about the eukaryotic communities.

In the present study, the genetic diversity of the whole plankton community (including both prokaryotic and eukaryotic organisms) in a shallow Chinese eutrophic lake was investigated using PCR-DGGE fingerprinting. Results of the fingerprinting analysis were then compared to community composition based on morphological characteristics, as well other environmental factors. The aims of this study were to: (1) supplement existing information with regard to genetic diversity at the community level, (2) explore how a plankton community responds to different environmental conditions, and (3) determine the applicability of this or similar genetic analyses as a means of monitoring changes in environmental stress.

Materials and Methods

Study Sites and Sample Collection. Lake Donghu (30°33′ N, 114°23′ E) is a shallow, freshwater lake with an average depth of 2.5 m. It is located near the middle reaches of the Yangtze River, about 5 km removed from the river itself. Lake Donghu is composed of several basins separated by artificial dikes, with a total surface area of 32 km. The lake has experienced increasingly serious eutrophication since the 1960s due to heavy discharge of sewage water, which has dramatically increased nutrient and organic matter loading to the system. It is currently dominated by two planktivorous filter-feeding fishes (silver carp and bighead carp) [23, 24]. Sampling was conducted at five stations (Fig. 1) that exhibited different trophic status in March 2006. Planktonic organisms were collected using horizontal surface tows with a 64 μm mesh net; community genomic DNA was extracted within 12 h of sample collection. The samples for morphological analysis were immediately fixed in 4% (final concentration) formalin, and live plankton samples were also collected for qualitative study and/or for confirming the taxonomic status of certain species. Polypropylene buckets were used to collect surface water for chemical analysis, and

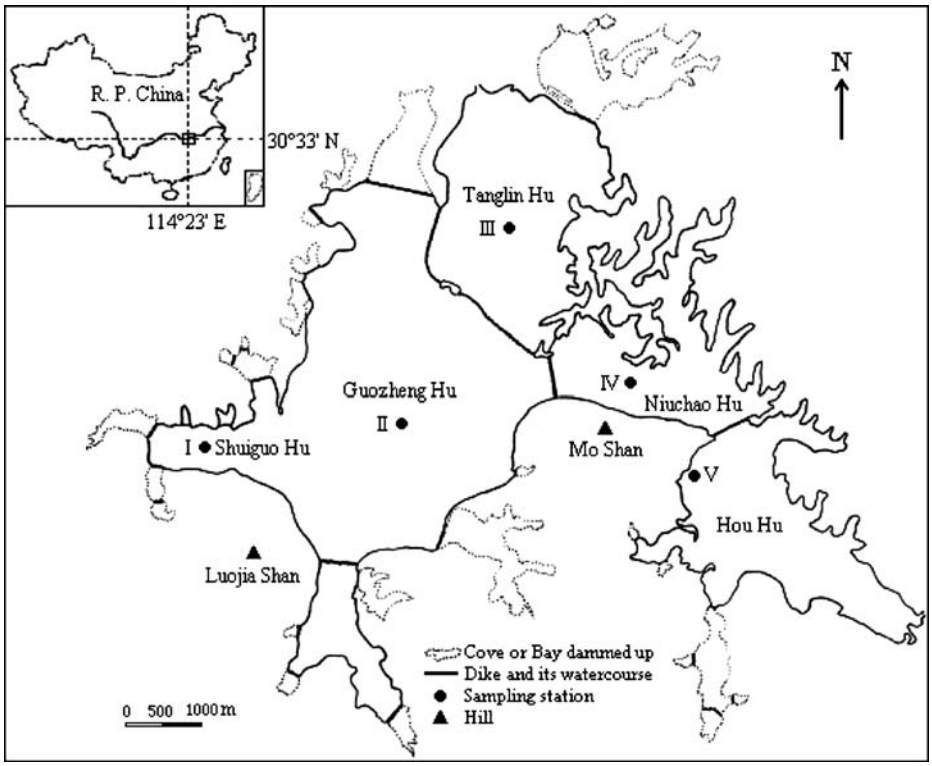

Figure 1. Map of five sampling stations in Lake Donghu, Wuhan, China.

the water samples were pretreated according to standard methods [8].

Chemical Analysis and Morphological Identification. One liter of lake water was collected at each site for chemical characterization. Alkalinity, hardness, chemical oxygen demand (COD), NH_4–N, NO_2–N, total nitrogen (TN), PO_4–P, total phosphorus (TP), SiO_2, Cl^-, and Ca^{2+} were measured according to standard methods [8]. The concentrations of heavy metals (Cr, Cd, Pb, As, and Cu) were determined by graphite furnace atomic absorption spectrophotometry (GFAAS) using a Perkin–Elmer AAnalyst 800 graphite furnace atomic absorption spectrometer (Perkin–Elmer, CT, USA). Zooplankton identification was performed under an Axioplan 2 Imaging microscope (Zeiss, Jena, Germany) according to Hu et al. [7], Shen et al. [19], Wang [22], Chiang and Du [4], and the Research Group of Carcinology [16].

DNA Extraction and PCR Amplification. To 1.5 mL of each water sample, two drops of 100% alcohol were added. The sample was then centrifuged to concentrate planktonic organisms, which were then rinsed with sterile distilled water and centrifuged three additional times. Genomic DNA was extracted using the standard phenol–chloroform method [9]. The primers used for amplification of the 16S ribosomal RNA (rRNA) genes were F357GC (5′-CGCCCGCCGCGCCCCGCGCCCGGCCC GCCGCCCCCGCCCCCCTACGGGAGGCAGCAG-3′), which contains a GC-rich clamp and is specific for most bacteria, and the universal primer R518 (5′-ATTACCG CGGCTGCTGG-3′) [15]. Polymerase chain reaction (PCR) conditions for each 50-μl reaction mixture were 1× PCR buffer, 2 mM $MgCl_2$, 3.0 U of Taq DNA polymerase, 80 μM of each deoxynucleotide (Fermentas Inc. Hanover, USA), 0.3 μM of each primer, and approximately 40 ng of template DNA. PCR cycling was performed in a GeneAmp PCR System 9600 thermal cycler (Perkin Elmer Cetus, USA) at the following temperatures: initial denaturation at 94°C for 5 min, followed by 10 cycles of 0.5 min at 94°C, annealing at 67–58°C (in the first cycle annealing was performed at 67°C, the temperature was then decreased by 1°C each cycle) for 0.5 min, and extension at 72°C for 1 min. This procedure was followed by 20 cycles of 0.5 min at 94°C, 0.5 min at 57°C and 1 min at 72°C. Finally, a primer extension at 72°C for 10 min was performed. The primers used for amplification of the 18S rRNA genes were F1427GC (5′-CGCC CGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGC CCCTCTGTGATGCCCTTAGATGTTCTGGG-3′) and R1616 (5′-GCGGTGTGTACAAAGGGCAGGG-3′) [21]. Both of them are specific to eukaryotic microorganisms. The reaction conditions were the same as described above. The PCR program included an initial denaturation at 94°C for 5 min and 10 touchdown cycles of denaturation at 94°C for 0.5 min, annealing at 69–60°C (with the temperature decreasing 1°C each cycle) for 0.5 min, and extension at 72°C for 1 min. Then 18 cycles of 94°C for 0.5 min, 59°C for 0.5 min, 72°C for 1 min, followed and terminated by an extension step consisting of 72°C for 10 min. To control the correct size of the PCR products, they were resolved on 1.5% agarose gels stained with ethidium bromide. A negative control was prepared in the same manner as the samples except that the DNA was excluded.

DGGE Analysis. DGGE was performed with an INGENYphorU-2 system (INGENY International BV, Leiden, The Netherlands) using a 9% (*w*/*v*) polyacrylamide (acrylamide:bisacrylamide ratio of 37.5:1) in 1×TAE buffer (40 mM Tris acetate, 40 mM acetic acid, and 1.0 mM EDTA [pH 7.6]). PCR products containing approximately equal amounts of DNA of similar sizes were separated on a gel containing a linear gradient of the denaturants urea and formamide. The concentration of the denaturants increased from 40% at the top of the gel to 60% at the bottom, for separation of the 16S rDNA fragments, and from 30 to 65% for separation of the 18S rDNA fragments (100% denaturant was defined as 7 M urea and 40% [*v*/*v*] formamide). Electrophoresis was performed at 60°C, with 120 V applied to the gel for 16 h. After electrophoresis, gels were stained in 1×TAE

Table 1. Chemical measurements taken at each station in Lake Donghu

	Station I	*Station II*	*Station III*	*Station IV*	*Station V*
Alkalinity (mg/L)	120.120	120.120	115.115	115.115	110.110
COD (mg/L)	4.909	4.909	4.275	4.275	4.909
NH_4–N (mg/L)	0.447	0.366	0.257	0.215	0.649
TN (mg/L)	1.175	0.823	0.796	0.620	1.683
PO_4–P (mg/L)	0.052	0.040	0.023	0.016	0.022
TP (mg/L)	0.110	0.087	0.054	0.054	0.059
SiO_2 (mg/L)	4.355	4.404	2.723	0.594	4.675
Ca^{2+} (mg/L)	48.713	49.539	49.539	37.154	42.934
Cl^- (mg/L)	41.048	45.446	45.446	38.849	43.247
Hardness (dH)	9.704	9.820	9.358	8.664	9.473
NO_2–N (μg/L)	15.33	6.07	6.59	8.15	26.27
Cr (μg/L)	1.14	2.57	1.12	0.64	1.22
Cd (μg/L)	0.08	0.15	0.13	0.06	0.08
Pb (μg/L)	1.17	1.90	1.62	1.28	3.78
As (μg/L)	1.81	2.70	1.63	1.01	0.51
Cu (μg/L)	13.79	13.62	99.81	71.74	22.04

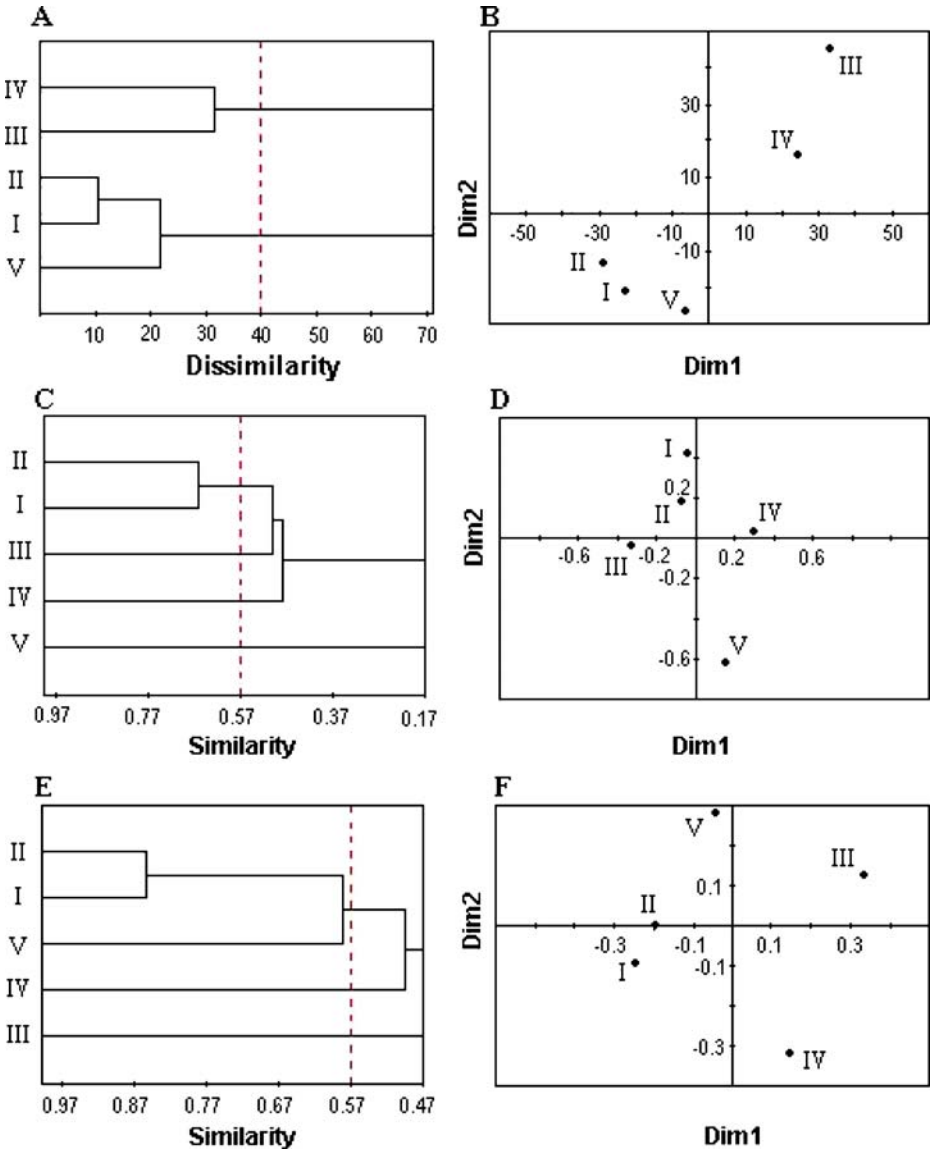

Figure 2. Group relationships of the five sampling stations. UPGMA clustering on the basis of the chemical characteristics (A), zooplankton composition (C), and DGGE fingerprints (E); MDS ordination on the basis of the chemical characteristics (B), zooplankton composition (D), and DGGE fingerprints (F).

buffer containing 1×SYBR Gold (Molecular Probes Europe BV, Leiden, The Netherlands) and then photographed using a UVP Imaging System (UVP Inc. CA, USA). The gel images were further processed using Adobe Photoshop 8.01 to maximize image contrast.

Data Analysis. DGGE profiles were scanned with LabWorks software (UVP Inc. CA, USA), and the banding patterns were carefully checked manually. The presence or absence of comigrating bands, independent of intensity, was converted to a binary (0/1) matrix. The Dice similarity coefficient (S_D), which represents similarities between pairs of samples, was calculated using the following equation: $S_D = 2n_{AB}/(n_A + n_B)$, where n_{AB} is the number of bands common to both samples, n_A is the number of bands in sample A, and n_B is the number of bands in sample B. The Dice similarity matrix was used to run the unweighted pair-group method using arithmetic averages (UPGMA) clustering and multidimensional scaling (MDS) with XLSTAT-Pro 2006 software (Addinsoft, NY, USA). UPGMA clustering and MDS were also applied to search for groups among sampling stations on the basis of zooplankton composition and water chemistry.

Results

Chemical Characters. Chemical characterization of the five stations is summarized in Table 1. Overall, the five stations exhibited similar water quality. COD was somewhat higher at stations I, II, and V; NH_4–N and TN were lower at stations III and IV. Both PO_4–P and TP were lower at stations III–V, relative to the other stations. The most notable difference among stations was the Cu

concentration, which was several times higher at stations III (99.81 μg/L) and IV (71.74 μg/L) relative to the other three locations. The concentrations of the remaining metals were generally similar. UPGMA clustering and MDS analysis indicated that the environmental conditions of station I were most similar to station II; station III and IV were more similar to each other (Fig. 2A, B).

Morphological Composition. A total of 36 zooplankton taxa were identified in the five investigated samples (Table 2). Station I had the maximum number of taxa (18) and station V had the least number (9). Only one species (*Brachionum angularis*) was detected at all five stations, whereas 58.3% of the taxa were restricted to single station. The UPGMA clustering and MDS ordination (Fig. 2C, D) showed that the zooplankton communities at stations I and II were the most similar to each other and station V was the most dissimilar to any other station. As for the phytoplankton, *Peridinium umbonatum* was the dominant species at stations I, II, and III; *Symedra* sp. and *Navicula* sp. were the dominant algae at stations IV and V, respectively.

DGGE Profiles and Plankton Community Structure. A total of 100 bands (61 of the 16S rDNA fragments and 39 of the 18S rDNA fragment) were detected (Fig. 3), which suggests that there were more taxa present than were actually identified based on external morphological features alone. Each sample produced a complex fingerprint composed of a large number of bands: 25 to 36 (mean 31.8) bands were obtained with the prokaryotic primer set, and 9 to 24 (mean 15.8) bands for the eukaryotic primer set. The DGGE bands unique to a single station were the greatest, accounting for 36.1% of the prokaryotic bands and 41.0% of the eukaryotic bands. The common bands accounted for only 14.75 and 5.13%, respectively (Table 3).

The UPGMA clustering and MDS ordination based on the Dice similarity matrix (Table 4) of DGGE fingerprints showed that the planktonic communities of

Table 2. Zooplankton taxa identified morphologically at each station in Lake Donghu

Organism identified	*Station I*	*Station II*	*Station III*	*Station IV*	*Station V*
Centropyxis aculeata		+			
Chlamydomonas cingulata	+				
Chlamydomonas simplex	+	+		+	
Chroomonas acuta	+				
Coleps hirtus	+	+			
Cryptomonas marssonii	+				
Cyphoderia ampulla			+		
Difflugia globulosa		+		+	+
Difflugia oblonga curvicaulis					+
Difflugia sp.			+		
Dinobryon sociale			+	+	
Euglena sp1.	+	+	+		
Euglena sp2.					+
Mallomonas elongata	+				
Peridinium umbonatum	+	+	+		
Phacus longicauda				+	
Pleuromonas jaculans					+
Tetrahymena priformis					+
Tintinnopsis wangi	+	+	+	+	
Trepomonas agilis					+
Vorticella convallaria					+
Brachionus angularis	+	+	+	+	+
Brachionus calyciflorus				+	
Brachionus urceus	+			+	
Epiphanes senta	+				
Filinia maior	+				
Keratella americana			+		
Keratella quadrata			+	+	+
Keratella ticinensis	+	+	+	+	
Keratella valga			+	+	
Polyarthra trigla	+	+		+	
Resticula sp.	+	+	+		
Rotaria sp.	+				
Daphnia cucullata				+	
Tropocyclops sp.				+	
Unidentified nauplius	+	+	+	+	

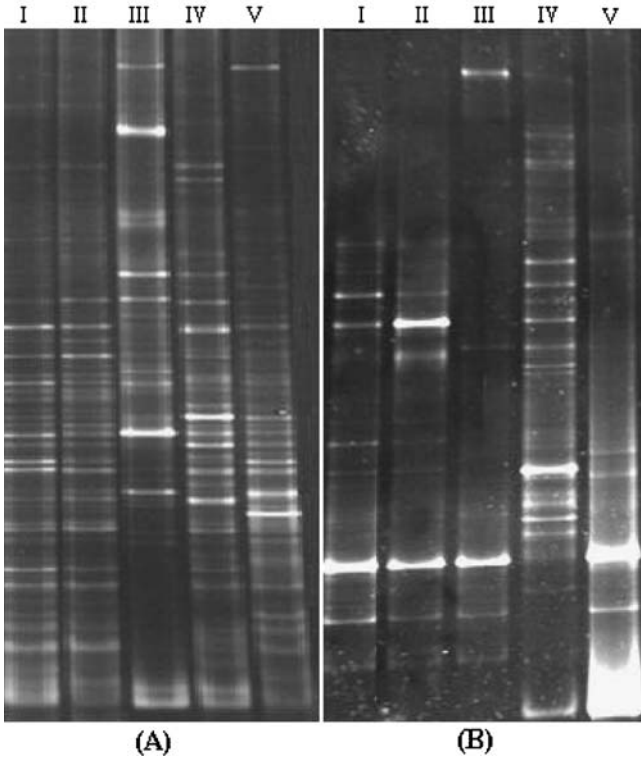

Figure 3. DGGE profiles of prokaryotic (A) and eukaryotic (B) rRNA genes amplified from plankton community DNA.

stations I and II were first grouped into a cluster, and then grouped together with station V; stations III and IV were divided into two single groups (Fig. 2E, F). The Dice similarity coefficient between stations I and II was the highest (0.857), indicating a high degree of similarity. These two stations were also the most similar relative to water quality characteristics. Similar results can be observed when the DGGE fingerprints of the two primer sets were considered separately (similarity between stations I and II was 0.862 and 0.848, for 16S rDNA and 18S rDNA, respectively).

Discussion

The rRNA genes contain both conserved regions, which can be used for primer design, and variable regions, which can be used to distinguish differences in the sequences. Therefore, comparative analysis of the ubiquitous rRNA genes, regardless of organisms' developmental stage, can be universally applied to infer relationships among the organisms. The use of rRNA genes to examine relationships among organisms is relatively new, Giovannoni et al. [5] having applied the method to study PCR-amplified 16S rRNA genes from natural environments. PCR-DGGE analysis of the microbial community produces a complex profile, which can be quite sensitive to spatial–temporal dynamics, and is one of the most commonly used fingerprinting methods in community characterization. However, most of the extant studies have focused on the prokaryotic microorganisms (e.g., bacterioplankton), with much less attention being paid to eukaryotes.

In the present study, the 16S rRNA and 18S rRNA genes were analyzed complementarily to reveal genetic diversity in the plankton community. Several more prokaryotic operational taxonomic units (OTUs, one band-one OTU in principle) than eukaryotic OTUs were detected in the DGGE profiles. Only 11% of the total OTUs were common to all five stations, whereas 38% of the OTUs were restricted to a single station. Similarly, 58.3% of the identified zooplankton taxa were restricted to a single station, and only one taxon was common to the five stations. This suggests that the plankton community composition in Lake Donghu differed significantly among stations. The observed differences may be due to variability in water chemistry, including the nutrient levels in different lake areas. Stations I, II, and III are generally considered to be hypertrophic, eutrophic, and mesotrophic, respectively. Station V appears to be situated between mesoeutrophic and eutrophic, while station IV trophic characteristics place it in the same category as station III, that is, mesotrophic [12]. Lindström [11] suggested that nutrient levels influenced the genetic structure of the bacterioplankton community. Although one band-one OTU is not always accurate, all the samples investigated were analyzed with the same procedure (including sampling collection, DNA extraction, PCR amplification, DGGE, and data processing). Therefore, any bias introduced into the study should have been the same across all samples and the comparisons remain valid. This kind of community screening makes assessment of the spatial–temporal dynamics particularly convenient, and it could also be used as a monitoring tool to identify significant environmental changes.

If comparable water samples collected from different locations harbor similar plankton communities, then the DGGE banding patterns are expected to be similar. The UPGMA clustering of the DGGE fingerprints showed that the plankton communities collected from the five stations were clustered into three groups at the position of S_D=0.57 (Fig. 2E). The composition of the first group, which included stations I, II, and V, was identical to the

Table 3. PCR-DGGE bands of the 16S rDNA and 18S rDNA

Primer identification	*Station I*	*Station II*	*Station III*	*Station IV*	*Station V*	*Total bands*	*Unique bands*	*Common bands*
F357GC R518	34	31	25	33	36	61	22	9
F1427GC R1616	19	14	9	24	13	39	16	2

UPGMA clustering of the environmental conditions (Fig. 2A). However, stations III and IV, which were generally considered to be at the same trophic level (mesotrophic) [12] and clustered into one group on the basis of environmental conditions (Fig. 2A), were subdivided into two groups (Fig. 2E). Other factors, therefore, could be influencing the cluster analysis.

Heavy metal contamination may be a critical factor affecting the plankton community in Lake Donghu. Based on the chemical characterizations conducted in this survey, stations III and IV were grouped together, which agreed with the nutrient-based classification as described above. However, the concentrations of the trace metals analyzed in this study, that is Cr, Cd, Pb, As, and Cu were lower at station IV than at station III. The environmental conditions of station IV may have been more favorable for a healthy plankton community than at station III. This possibility is supported by the fact that more 16S and 18S rDNA bands were detected at station IV than at station III (Table 3). In addition, more zooplankton taxa were identified at station IV than at station III (Table 2). Biomonitoring, therefore, can be considered as an important addition to physicochemical analyses in the evaluation of environmental conditions within an aquatic ecosystem.

Based on morphological characteristics, the zooplankton taxonomic compositions of stations I and II were less similar (0.667, Table 5) than when considering DGGE fingerprints (0.857). Stations III, IV, and V were classified as three separate groups when using S_D=0.57 as the differentiating criterion (Fig. 2C). However, station V should probably be grouped with the stations I and II on the basis of water chemical characteristics and the DGGE fingerprints (Fig. 2E, F). Additionally, the trophic level of station V (intermediate between mesoeutrophic and eutrophic) can also be regarded as similar to station II (eutrophic) [12]. These data indicate that evaluating relationships based on zooplankton morphological composition alone may be insufficient to fully recognize and understand community structure in a shallow, eutrophic lake. Using additional, and more subtle techniques such as DGGE fingerprinting provides greater resolution of relationships within the natural community. However, the most similar stations (station I and II) were always grouped first into one cluster (Fig. 2). One possible reason for the discrepancy may be that the DGGE method is more sensitive than the traditional methods. Intuitively, large populations of minute prokaryotic organisms that were difficult to identify on a morphological basis were detected in the DGGE analysis. Except for station III, more eukaryotic OTUs were detected using DGGE fingerprinting than the number of identified zooplanktonic taxa. The grouping relationships depicted by DGGE fingerprints of both 16S and 18S RNA genes appeared to be more reflective of actual community structure, whereas traditional methods could distinguish only those environments with higher similarity (e.g., stations I and II in the present study). Boon et al. [3] suggested that the DGGE approach seemed more sensitive than the physicochemical approach for characterizing habitat homogeneity. Yan et al. [25] proposed that RAPD fingerprinting appeared to be more sensitive than morphological classification. With the comparison of the plankton diversity measured by the morphological method and 18S rRNA genes, Savin et al. [18] suggested that molecular techniques also appeared to be better suited for revealing unidentified, but possibly ubiquitous, organisms.

Data from the present study suggests the DNA-based methods provide more definitive information (diversity, evolution, ecology, etc.) on natural communities. DNA-based community level analysis is a fast, easy, reliable, and inexpensive method to obtain scientifically sound results. In contrast to more traditional method, multiple samples can be analyzed simultaneously with the canonical and uniform procedures, making it convenient to perform comparative analyses between different studies. A molecular-based monitoring system, being sensitive to the dynamics of the aquatic ecosystem, could be valuable in identifying deleterious perturbations. All methods, however, have shortcomings and it might be worthwhile for future studies to combine fingerprinting techniques with other methods.

Table 4. Dice coefficient similarity matrix generated from DGGE fingerprints

	Station I	*Station II*	*Station III*	*Station IV*	*Station V*
Station I	1.000				
Station II	0.857	1.000			
Station III	0.437	0.506	1.000		
Station IV	0.509	0.490	0.440	1.000	
Station V	0.569	0.596	0.506	0.491	1.000

Table 5. Coefficient similarity matrix on the basis of the zooplankton composition

	Station I	*Station II*	*Station III*	*Station IV*	*Station V*
Station I	1.000				
Station II	0.667	1.000			
Station III	0.452	0.560	1.000		
Station IV	0.424	0.519	0.500	1.000	
Station V	0.074	0.190	0.182	0.250	1.000

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