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Genetic diversity patterns of microeukaryotic plankton communities in Shenhu Bay, southeast China

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ABSTRACT

Microeukaryotic plankton is an abundant and diverse component of marine environments and plays an important role in microbial food webs. However, few studies have been conducted on the genetic diversity of microeukaryotes in the subtropical bays of China. In the present study, we investigated the microeukaryotic plankton in the Shenhu Bay by using denaturing gradient gel electrophoresis (DGGE) and sequencing of prominent bands. Our results indicated that Copepoda and Dinophyceae were the most diverse groups, and that the microeukaryotic communities varied significantly between summer and autumn, with the autumn communities exhibited a higher diversity than summer communities. Furthermore, the community composition and diversity from both surface and bottom waters showed more significant differences in summer than in autumn. Environmental parameters also displayed obvious seasonal patterns. Redundancy analysis (RDA) showed that temperature was the most significant environmental factor shaping the seasonal patterns of the microplanktonic members in the Shenhu Bay. Community-level molecular techniques such as DGGE appear as useful tools to detect the presence of red tide causing species and to guide the management of coastal water mariculture.

1. Introduction

Microeukaryotic plankton are a fundamental component of marine ecosystems and account for a considerable percentage of marine biomass and activity (Logares et al., 2014). Dinoflagellates, diatoms and some metazoan species are regarded as the most abundant eukaryotes in the world (Caron et al., 1999; Moon-van der Staay et al., 2001; Taylor and Cunliffe, 2014; Liu et al., 2015). More importantly, microorganisms contribute significantly to primary productivity in marine ecosystems (Stockner, 1988; Cheung et al., 2010) and play a vital role in global mineral and carbon cycles, particularly in oligotrophic sea areas (Fogg, 1995). Some eukaryotic plankton assemblages can be used as biomonitors for assessing aquatic ecosystem health or the effect of physicochemical agents, because they are sensitive to environmental deterioration and pollutants (Sun et al., 2010; Lv et al., 2013). Additionally, understanding microplankton ecosystems is critical for predicting ecosystem function as global environments change (Margules and Pressey, 2000; Worm et al., 2006). So it is very important to study the microplankton community structure and diversity in different marine ecosystems.

Over the past decade, molecular fingerprinting techniques have revealed insights into microeukaryotic communities efficiently complementing classical identifications based on morphology, and they have proved effective methods to assess the composition and structure of microeukaryotic communities (Van Hannen et al., 1998; Díez et al., 2001a; Lovejoy et al., 2006; Caron et al., 2012; Liu et al., 2015). Among all of the available fingerprinting methods, DGGE has been most successfully applied for microeukaryotic communities based on sequencing of 18S rDNA fragments (Savin et al., 2004; Liu et al., 2013b; Yu et al., 2014, 2015; Grattepanche et al., 2014). For example, Liu et al. (2013b) had found that both bacterial and microbial eukaryotic communities in a reverine ecosystem varied significantly in time and were spatially structured from the upper stream, middle-lower stream and estuary. Yu et al. (2015) examined the community composition and genetic diversity of the microeukaryotic plankton in Xiamen offshore water and compared the DGGE and amplicon high-throughput sequencing (Illumina MiSeq sequencing). They found that the Illumina MiSeq sequencing revealed a much higher species richness than DGGE, but there were no significant statistical difference between the two methods.

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In the past decades, harmful red tide species have attracted great attention along offshore areas all over the world due to the multiple influences on the balance of marine ecosystem (Anderson et al., 2012). In summer 2010, a red tide was observed for the first time in the Meilin sea area of the Shenhu Bay caused by a bloom of dinoflagellates *Karenia mikimotoi*, which is the third most common red-tide forming species in the East China Sea (Wang and Wu, 2009). This red tide lasted for five days, causing great loss for the marine ecosystem and aquaculture. For example, a large number of juvenile fish died during and after the red tide as reported by local newspapers. After that, the red tide occurred occasionally in the Shenhu Bay because of the deterioration of marine environmental quality and mariculture pollution. In this context, the harmful algae species are of a special concern because they may decrease the aquatic biodiversity and damage the ecosystem function (Smayda, 2007). The better understanding of harmful algal blooms can assist policymakers to design approximate strategies, since the management of harmful algal blooms is time-consuming and costly along the large coastal area of China.

Although the Shenhu Bay is an important subtropical natural coastal harbor, only a few studies about ecology of microplankton have been reported so far. Luo and Huang (2002) based on two environmental surveys in Shenhu Bay during the period of May, September 1998, they analyzed the species composition of copepods and its relation to the environmental factors. Wang et al. (2014) had investigated the spatial distribution and seasonal variation of large tintinnids during three seasons of 2012. So it is necessary to have a better understanding of the diversity in microeukaryotic plankton communities in Shenhu Bay. We hypothesized that microeukaryotic communities in autumn would shift away from the summer, and this shift would be related to temperature and water stratification conditions. The aims of our research were (1) to investigate patterns of microeukaryotic plankton communities across spatial and seasonal scales in the Shenhu Bay and (2) to determine which factors are most strongly affect the spatiotemporal pattern of microeukaryotic plankton communities.

2. Materials and methods

2.1. Study area and sampling

The Shenhu Bay is located at the southeast part of the Jinjiang Peninsula, Quanzhou, Fujian Province, southeast of China. The bay has an area of 68 km^2 with 30.8 km intertidal zone and it is characterized by a large open mouth connecting with the Taiwan Strait in the east. Two field cruises were carried out in the Shenhu Bay (118.65–118.70°E, 24.61–24.69°N) in summer (August) and late autumn (November) 2012. In total, seven sampling stations were selected to cover most of the area of the bay including the northern mariculture region, eastern open sea region and southwest sea region (Fig. 1). For microeukaryotic plankton analysis, 800 ml water was sampled using Niskin bottles from both the surface (upper 50 cm) and bottom waters (1.0 m above the bottom sediment). Seawater samples were pre-screened on a 200-μm sieve to remove debris, macro- and meso-zooplankton, then filtered through a 0.22-μm-pore polycarbonate membrane (Millipore, Billerica, MA, USA). All membranes were immediately frozen and stored at -80 °C until DNA extraction.

2.2. Environmental data collection

In this study, we measured water depth and eleven surface seawater physicochemical factors. Depth, temperature, DO (dissolved oxygen), pH and salinity were determined in situ by a Hydrolab DS5 Multi-Parameter Water Quality Meter (Hach Company, Loveland, CO, USA). In the laboratory, suspended solids, NH_4-N (ammonium nitrogen), NO_3-N (nitrate nitrogen), NO_2-N (nitrite nitrogen), COD (chemical oxygen demand), AP (active phosphate) and petroleum were measured based on standard methods of the Offshore Marine Chemical Survey Technical Regulations (Wang et al., 2014).

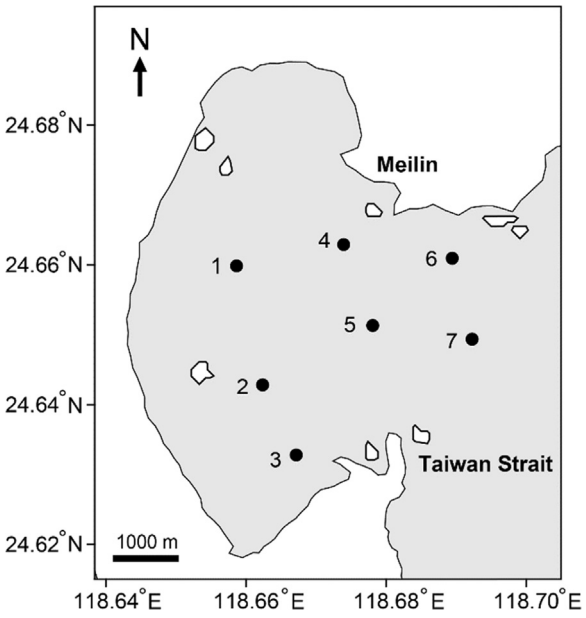

Fig. 1. Map of the Shenhu Bay showing the location of seven sampling stations.

2.3. DNA extraction and PCR-DGGE

The total genomic DNA was extracted with the FastDNA spin kit (Bio101, Carlsbad, CA, USA). Briefly, each membrane was cut into small pieces and stored in Lysing Matrix E tube adding 0.5 mg of beads. After that, 978 μL sodium phosphate buffer and 122 μL MT buffer were transferred into the tube. The tubes were vortexed in a FastPrep® instrument for 40 s at the speed setting 6.0. Then the samples were processed according to the manufacturer protocol. All DNA extracts were eluted with 40 μL TE buffer and stored at −20 °C until used. DNA concentration and quality were determined with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The amplification of partial 18 S rRNA gene was done by the touchdown PCR using the universal primer pair Euk516r-GC and Euk1A (Díez et al., 2001b). The protocol has been slightly modified as our previous study described (Yu et al., 2015). That is total 50 μL PCR mixtures contained 10×reaction buffer, 200 μM dNTP, about 40 ng target DNA, 0.3 μM of forward-reverse primer, 2.5 U *Ex-Taq* polymerase (Takara, Otsu, Shiga, Japan) and 1.5 mM $MgCl_2$. 'Touch-down' PCR process was as follows: an initial 10 min denaturing step at 94 °C, then ten times touchdown steps including denaturation at 94 °C for 30 s, then 30 s annealing starting at 67 °C (with the annealing temperature decreasing 1 °C each cycle), and extension step at 72 °C for 1 min. Then, 25 additional cycles including 94 °C for 30 s, 57 °C for 30 s and 72 °C for 1 min were carried out. A final extension step was carried out at 72 °C for 10 min. Our PCRs were run in a Mastercycler (Eppendorf AG, Hamburg, Germany).

We used the Universal Mutation Detection System (BioRad, Hercules, CA, USA) to analyze DGGE profiles. A volume of 25 μL of each PCR product mixed with loading dye (5:1) were loaded into each lane of 6% (w/v) polyacrylamide gels (37.5:1 acrylamide:bisacrylamide) in 1×TAE buffer with a 25%-45% denaturing gradient (a 100% denaturing gradient is defined as 7 mol/L urea and 40% (v/v) formamide). We chose additional AS1 as the marker (MAS1 lane) to standardize DGGE gels.

The electrophoresis was run at 60 °C and 100 V for 16 h. After that, gels were stained with SYBR Green I for about 20 min in the dark, then carefully washed by distilled water, and finally photographed by Ettan DIGE Imager (GE Healthcare, Piscataway, NJ, USA). Two more DGGE replicates were run for all samples in order to estimate the reproducibility of the DGGE profiles.

Prominent DGGE bands were carefully isolated from the gel, transferred into aseptic tubes with 30 μL sterilized distilled water, and stored at 4 °C overnight. A volume of 6 μL of eluted DNA was subjected to a new amplification with the same primers Euk1A and Euk516r (without GC clamp). Both *Escherichia coli* DH5α competent cells (Takara, Otsu, Shiga, Japan) and pGEM-T Easy Vector (Promega, Madison, WI, USA) were used for cloning. Finally, three well inserted plasmids for each band were manually picked and sequenced unidirectionally with an automated sequencer (Applied Biosystems, Foster City, CA, USA). After that, sequences were assigned to operational taxonomic unit (OTU) at a 97% sequence similarity threshold in MOTHUR v.1.33.3 (Schloss et al., 2009). To further confirm the OTUs and taxa classified information, each sequence was identified by using blastn in the GenBank database, and the most similarities known species sequences was choose as a reference for phylogenetic analysis. The sequences dataset was realigned and manually edited with the Clustal X aligner, and the Maximum likelihood (ml) tree was constructed in MEGA 6.0 (Tamura et al., 2013) with 1000 bootstrap replicates. Additional, Bayesian phylogenetic analysis was implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Two simultaneous Markov chain Monte Carlo (MCMC) chains were carried out for 2,000,000 generations, by the end of which the standard deviation of split frequencies was below 0.01. Sampling frequency was one every 100 generations and the burn-in value was set at 25%.

2.4. Data analysis

We analyzed DGGE profiles using Quantity One image analysis (BioRad, Hercules, CA, USA). The software could automatically detect and match bands which can also be manually corrected. DGGE bands profile was transformed into binary code where '1' and '0' indicated 'presence' and 'absence', respectively. The heat maps showing the bands profile was generated in the pheatmap package of R (R Core Team, 2015). Then a presence/absence-based non-metric multidimensional scaling (MDS) ordination was used to detect differences in microeukaryotic communities (Yu et al., 2015). MDS is a multidimensional ordination of microeukaryotic samples brought down to a two-dimensional plot. The quality of the MDS plot is measured by stress value: values < 0.2 corresponds to a potentially useful two-dimensional picture, values < 0.1 indicates a good ordination, and values < 0.05 shows a perfect representation (Clarke and Gorley, 2001). Significant differences between groups were evaluated using the randomization permutation procedure analysis of similarities (ANOSIM). The global R statistic ranging from 0 to 1 represents separation degree between site groups, and no separation is showed by R = 0, while R = 1 indicates complete separation.

Preliminary detrended correspondence analysis (DCA) on the microeukaryotic data showed that the longest gradient length was shorter than 3.0, demonstrating that the major species displayed linear response to environmental variation. Therefore, redundancy analysis (RDA) was used to explore the relationships between microeukaryotic plankton communities and environmental variables. Environmental parameters were transformed to avoid skewed data distributions, and all selected physicochemical factors were square-root transformed, except pH. The ability of environmental factors to explain the community data variance in RDA was assessed by Monte Carlo simulation with 999 permutations. In order to avoid collinearity among factors, explanatory environmental factors with the highest variance inflation factor (VIF) were eliminated until all VIF values were lower than 20. A forward manual selection RDA was used to select a minimal subset of environmental variables that explained significant proportions of the variations in the community data ($P < 0.05$) (Ter Braak and Smilauer, 2002). To evaluate the relationship between taxa and environmental variables, Spearman correlation coefficient r value and *P*-value were calculated in the 'psych' package in R software. *P*-values were corrected by implementing false discovery rates (q-values) that were kept below 5% with the Benjamini Hochberg procedure (Benjamini and Hochberg, 1995). Network analysis was constructed and graphically edited in Cytoscape (Shannon et al., 2003). The Shannon-Wiener index (H') was calculated for the microeukaryotic diversity of DGGE profiles using the following equation: $H' = -\Sigma P_i \ln P_i$. The term P_i was calculated as follow: $P_i = n_i/N$, where n_i is the number of *i*th band and N is the sum of the band number in a sample. Analysis of variance (ANOVA) was applied to examine differences of band numbers and Shannon-Wiener indices between each group samples. In addition, we performed Scheffe's *F* multiple-comparison test to check for significant difference among the different groups. Independent-samples *T* test was performed to detect significant differences between two groups. All data analyses were processed by the CANOCO 4.5 (Ter Braak and Smilauer, 2002), the SPSS 19.0 (SPSS Inc. Crawfordville, FL, USA) and the PRIMER 5.0 (Clarke and Gorley, 2001).

2.5. Accession numbers

The 18S rRNA gene sequences from DGGE bands in this study were deposited in the GenBank database under the accession numbers KM277371 to KM277401 and KR138544 to KR138588.

3. Results

3.1. Physicochemical parameters

Water depth and eleven physical and chemical variables of the surface water are given in Table S1. Most variables displayed a clear seasonal difference between summer and autumn. The average values of water temperature, pH and salinity were higher in summer than those in autumn (*t*-test, $P < 0.05$), whereas the average values of suspended solids, DO, COD, active phosphate, NO_2-N and NO_3-N in summer were lower than those in autumn (*t*-test, $P < 0.05$). However, the concentration of NH_4-N remained relatively constant throughout two seasons.

3.2. Microeukaryotic community composition

Our DGGE banding patterns generated a total of 652 bands representing 55 distinct, reoccurring bands in the 28 samples (Fig. S2). On average, each sample displayed 23 bands. Only one band (Band 23) was prevalent at all sites, which was shown to be a Dinophyceae species (*Pentapharsodinium tyrrhenicum*) by using blastn against the GenBank database (Fig. 2 and Table S2). There was only one band (Band 44) that was only present at one site (AS4), unfortunately this band was too weak to be isolate for sequencing. The sample AB1 had the greatest number of bands (37), whereas samples SS2 and SB4 displayed the lowest band number (15).

In total, 28 prominent DGGE bands were successfully re-amplified to obtain further information (Fig. S1 and Fig. S2), and 84 clones were chosen for sequencing. Finally, 76 successful sequences belonging to 23 OTUs were achieved based on 97% sequence similarity (Fig. 2). Copepoda, Dinophyceae, Chlorophyta, Ciliophora and Euglenozoa were identified, and their species (OTU) number and sequences number

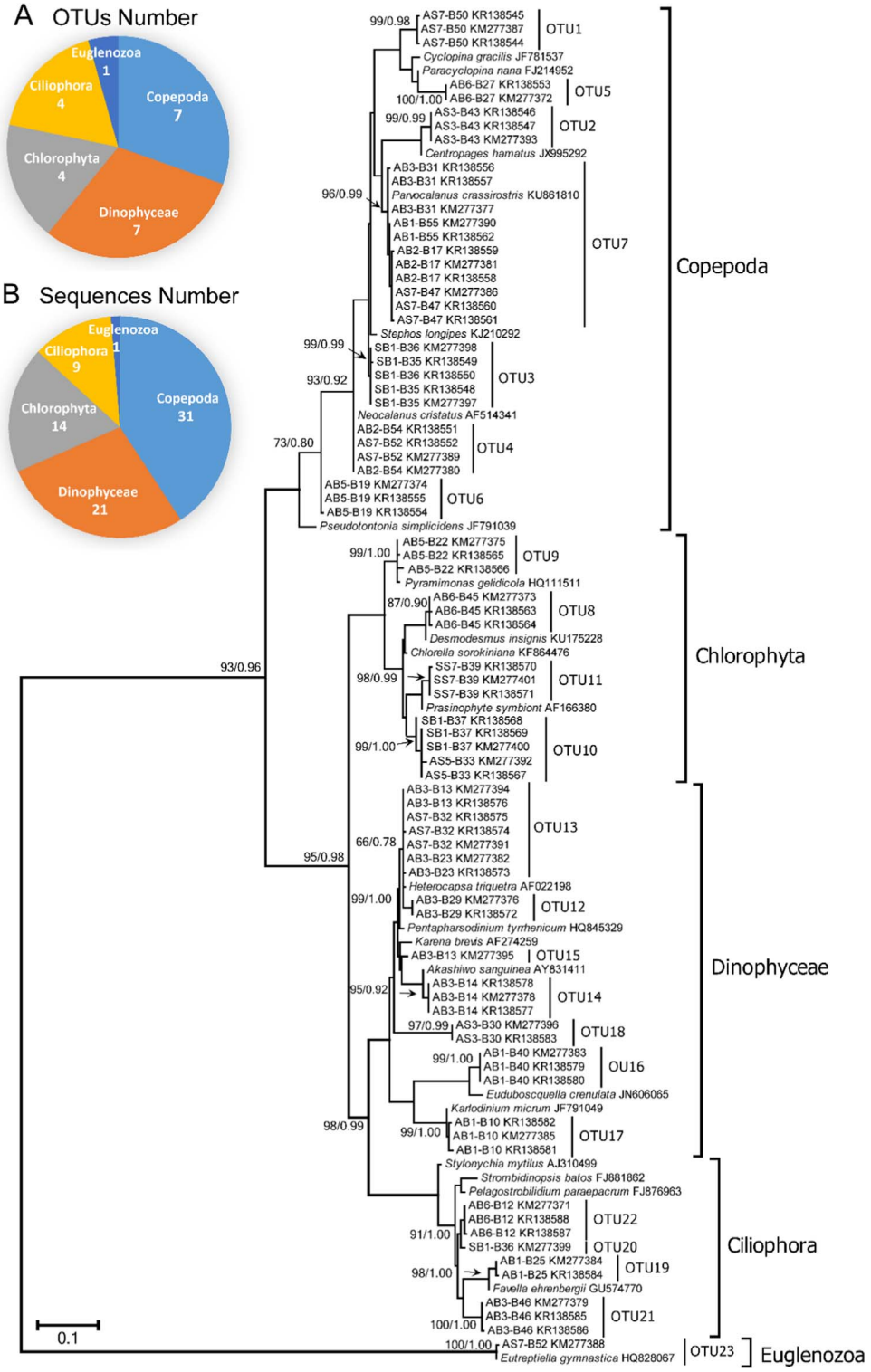

Fig. 2. Phylogenetic tree of the 18S rRNA gene from the Shenhu Bay. The tree was rooted using the Maximum likelihood and Bayesian inference methods. The bootstrap support values and posterior probabilities at the major nodes are showed (lower than 50% or 0.50 are omitted). A and B showing the OTUs number and sequences number of the five phylum group, respectively.

were respectively 7 (31 sequences), 7 (21 sequences), 4 (14 sequences), 4 (9 sequences) and 1 (1 sequences) (Fig. 2).

On average, 66% of the bands from each sample were identified, samples SS4 and SS6 had the greatest number of bands identified (76%), while the sample AS4 had the lowest number of bands identified (50%). Based on these identified information, we compared the community composition of our samples and found that Copepoda was the most diverse group (average occupied 22.2% species number),

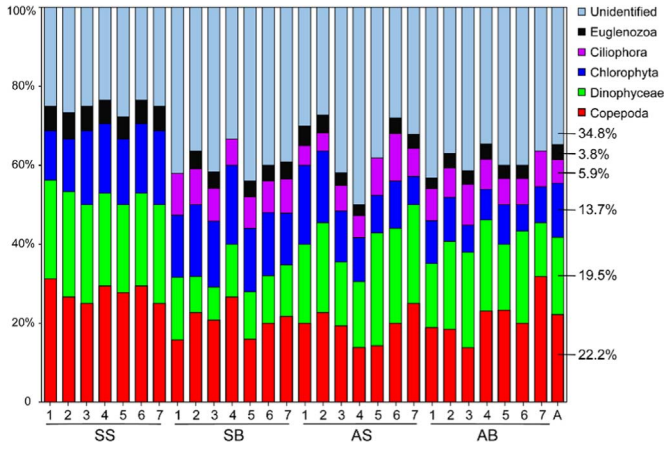

Fig. 3. Microeukaryotic community composition of major group in the Shenhu Bay. SS, SB, AS and AB indicate summer surface, summer bottom, autumn surface, and autumn bottom samples, respectively. "A" indicate the average OTU percentage of each group in all samples.

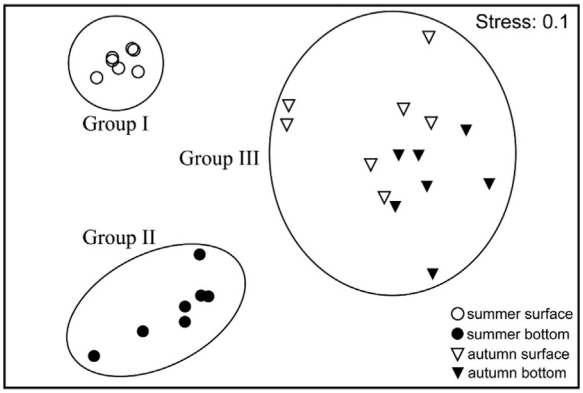

Fig. 4. Non-metric multidimensional scaling (MDS) ordination of microeukaryotic communities based on the DGGE profile.

and the Dinophyceae was the second most diverse group (average occupied 19.5% species number) (Fig. 3). We also observed that two bands (SB1-B36, AS7-B52) harbored two different taxa, and five OTUs (OTU3, OTU4, OTU7, OTU10 and OTU13) were found from more than two different positions on the DGGE gel (Table S2).

3.3. Spatiotemporal patterns of microeukaryotic plankton communities

The DGGE profile displayed great variations in band number and position among 28 samples (Fig. S1 and S2). Microeukaryotic plankton communities in different seasons and layers were clearly distinguished by non-metric multidimensional scaling (MDS) ordination with stress values = 0.1 indicating a good ordination (Fig. 4). Group I was composed of seven summer surface samples, while Group II consisted of all summer bottom samples. Group III included both surface and bottom samples in autumn. This was also supported by the ANOSIM analysis, revealing a strong separation of the microeukaryotic community composition (R = 0.966, P = 0.001). The DGGE band number and Shannon-Wiener index ranged from 15 to 37 and 2.692 to 3.565, respectively. More importantly, our multiple-comparison test results showed a significant difference in DGGE band number and Shannon-Wiener index among the SS (summer surface), SB (summer bottom), AS (autumn surface) and AB (autumn bottom) samples (Fig. 5).

At temporal scale, the summer groups (Group I and Group II) were significantly different from the autumn group (Group III). ANOSIM analysis revealed a strong separation of the microeukaryotic community composition between Group I and Group III (R = 0.959, P = 0.001), and between Group II and Group III (R = 0.954, P = 0.001). Among Group III, the surface and bottom groups were not significantly separated (R = 0.293, $P > 0.05$). Further, the band number and Shannon-Wiener index in the summer samples were significantly lower than in the autumn samples in both surface and bottom waters, respectively (Fig. 5).

At the spatial scale, our MDS showed that the microeukaryotic communities from surface (SS) and bottom (SB) waters were completely separated (R = 1.000, P = 0.002) in summer. In autumn, however, both surface and bottom communities (AS, AB) were mixed together (R = 0.293, P = 0.007). Interestingly, both band number and Shannon-Wiener index of SS were significantly lower than those of SB, whereas no significant difference was observed between surface and bottom samples in autumn (Fig. 5).

3.4. Relationship between environmental factors and microeukaryotic communities

Network analysis showed that all of the five phylum taxa (Copepoda, Dinophyceae, Chlorophyta, Ciliophora and Euglenozoa) were positively correlated to special environmental variables (q value

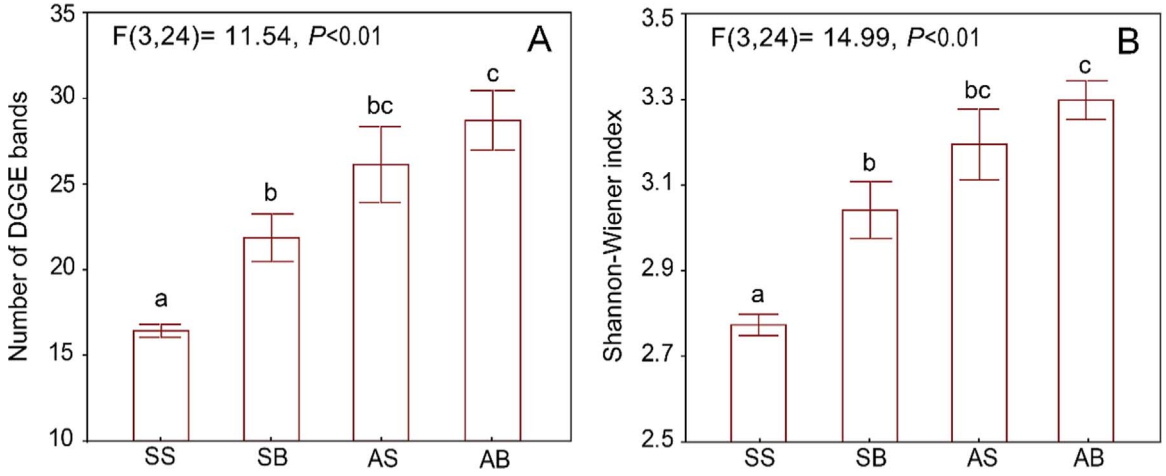

Fig. 5. DGGE band number (A) and Shannon–Wiener index (B) based on DGGE profiles of microeukaryotic communities from the Shenhu Bay. Data are mean ± SE (n = 7). SS, SB, AS and AB indicate summer surface, summer bottom, autumn surface and autumn bottom, respectively. Significant differences between groups are indicated by different letters ($P < 0.05$).

< 0.05, Fig. S3). Among them, Euglenozoa was affected by the greatest number environmental variables (eleven), while the Dinophyceae was only affected by seven environmental variables. To the total community, forward selection procedures showed that the Temperature, pH, NH_4-N and COD were the most significant proportions associate with the changes of the microeukaryotic communities in Shenhu Bay, among of them, surface waters temperature was the most significant factor ($P < 0.05$). The axes 1 and 2 of the RDA ordination explained 52.4% and 5.4% of the community variability, respectively (Fig. 5). In addition, the RDA ordination showed an obvious temporal variability, since the surface samples were clearly separated into summer and autumn groups.

4. Discussion

4.1. Spatiotemporal variation in microeukaryotic communities

Previous studies on microeukaryotic communities of the Shenhu Bay were based on traditional microscopy methods and mainly focused on horizontal and temporal variation in certain limited microeukaryotic groups such as copepods and ciliates (Luo and Huang, 2002; Wang et al., 2014). In our study, the whole microeukaryotic communities including zooplankton and phytoplankton were investigated based on universal eukaryotic primers in two dimensions: temporal and spatial scales.

Temporal variation in water temperature usually results in large gradient changes in physical and chemical factors of aquatic ecosystems (Lv et al., 2013; Liu et al., 2015), and ultimately changes in microeukaryotic community composition. In our study, both DGGE band number and Shannon-Wiener index of surface and bottom samples in the summer were significantly lower than those in the autumn, displaying a significant difference between two seasons (Fig. 5). This was largely due to the temporal variation in environmental factors. In our dataset, it was apparent that the environmental variables such as temperature, salinity, DO, suspended solids, pH, active phosphate and NOx-N changed significantly between two seasons in surface waters (t-test, $P < 0.05$). More importantly, water temperature was significantly related to variation in microeukaryotic communities (Fig. 6). In fact, the summer water had high temperature, salinity, and pH, while autumn water showed high DO, suspended solids and macro-nutrient concentrations (nitrate and phosphate).

The north part of the Shenhu Bay (Meilin sea area) has a long history of aquaculture for fish, molluscs and seaweed (Luo and Huang, 2002; Song, 2013). The concentration of NO_3-N and NH_4-N were very high around Meilin sea area (site 4) in both summer and autumn compared to other sites (Table S1). Eutrophication indicated by high nutrient concentrations is very common in intensive mariculture systems and often causes an increase in phytoplankton biomass. Phytoplankton are normally used as live foods by all growth stages of molluscs, as well as by larval stage of some fish and crustaceans, and by zooplankton used in mariculture food chains (Zheng et al., 1984). In our study, AS4 (autumn surface station 4) was the second most diverse station with a total of 36 bands. As to zooplankton, Copepoda were the most diverse group in the Meilin sea area especially in summer (Fig. 3). Planktonic copepods are important to the composition of zooplankton species in coastal bays and estuaries and play a vital role in coastal food webs. Indeed, planktonic copepods prey on marine protist, and they are the main prey for many fish of economic importance, both larval and juvenile, therefore dense area of copepods can form the feeding grounds (Zheng et al., 1984). Furthermore, the relative percentage of OTUs of copepods was higher in summer than that in autumn, particularly the summer surface was significantly higher than that of autumn surface (t-test, $P < 0.05$), this is largely due to the higher water temperature in summer surface layer (t-test, $P < 0.05$). Past studies showed that the growth rate of copepods was accelerated with the increase of water temperature within a certain range (Zheng et al., 1984). Salinity was another environmental factor proved to impact the growth of copepod species (Milione and Zeng, 2008). The copepods in the Shenhu Bay mainly belonged to warm water and tropic ocean taxa, and their species number and diversity would increase with the higher temperature and salinity (Wang et al., 2012). Proper temperature, salinity, nutrients and diverse microeukaryotic plankton made the Meilin sea area an ideal feeding ground for molluscs and fish. The study on the distribution of planktonic copepods can not only provide the scientific basis for the development of shallow marine aquaculture, but has important practical significance for comprehensive exploitation and utilization of marine biological resources.

Ciliated protozoa are an important component of the microplankton communities and play a crucial role in functioning of the microbial food web in most aquatic ecosystems (Jiang et al., 2007). Four ciliate species were detected in our study. It is surprising for us that no ciliate species were detected in summer surface samples (Fig. 3). They escaped from surface layer mainly due to the very high water temperature in summer, another reason could be the escape from predators (mainly by copepods) (Johansson et al., 2004). Indeed, because of their short life cycles and delicate membranes, they generally respond more rapidly to environmental stress than metazoa (Jiang et al., 2011; Xu et al., 2011).

Our MDS ordination clearly indicated a significant difference of microeukaryotic communities between surface and bottom layers of the Shenhu Bay in summer (Fig. 4, R = 1.000, P = 0.002). The reason is that the obvious thermal stratification in summer has a strong influence on the abundance and composition of microeukaryotic communities. A previous study has found that the thermal stratification in the Shenhu Bay was caused by surface heating from the hot weather inside of the bay or upwelling-forced cold water intrusions driven by southwest wind from the Taiwan Strait in summer (Gill, 1982). Environmental factors can be driven by the stratification pattern, thereby showing distinct vertical characteristics and having different effects on microeukaryotic communities from surface and bottom layers. Contrarily, MDS showed that the microeukaryotic communities were highly similar between autumn surface and bottom (Fig. 4, Group III). The reason is that the water column of the Shenhu Bay was well-mixed by the combined effects of atmospheric cooling and northeast wind in autumn (Jan et al., 2002; Tian and Xu, 2012). In our study, the suspended solids in autumn were significantly higher than in summer (Table S1), these might be caused by the water mixing. Additionally, we found that the vertical variation of microeukaryotic community composition was significantly lower in autumn than in summer. Therefore,

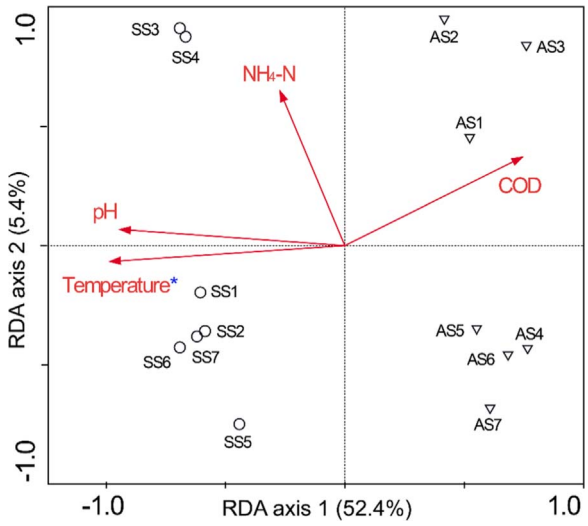

Fig. 6. RDA ordination showing the microeukaryotic community composition in relation to the environmental factors the Shenhu Bay. The statistically significant variable is marked with an asterisk (*) according to a Monte Carlo permutation test ($P < 0.05$).

we consider that the similarity between the surface and bottom microeukaryotic communities is related to the mixing during the autumn in the Shenhu Bay.

4.2. DGGE use for detect red tide forming species

Some common dinophyceae species are well-known for forming harmful algal blooms along the Fujian coastal seas, thereby threatening marine organisms and negatively impacting aquatic ecosystem health (Lan et al., 2004; Yu et al., 2015). In the Shenhu Bay, suitable water temperature and nutrients could greatly contribute to the growth of the dinophyceae cells. In our dataset, dinophyceae was the second most diverse group as revealed by DGGE band even though they accounted for a larger proportion in autumn than in summer (Fig. 3). In total, seven dinophyceae species including *Heterocapsa niei*, *Akashiwo sanguinea*, *Karena brevis*, *Pentapharsodinium tyrrhenicum, Eudubosquella crenulata, Karlodinium micrum* and *Heterocapsa triquetra* were observed in our sequence (Table S2), all of them are well known for forming harmful blooms and red tides (Sato et al., 2002; Cho et al., 2009). These species have not been reported to bloom in Shenhu Bay, however, they were frequently forming red tide around the East China Sea (Liu et al., 2013a; Lou and Hu, 2014; Yang et al., 2012). Indeed, harmful algal blooms in the East China Sea have been reported every year in the last decade (Yang et al., 2012), and the red tide outbreaks are mainly caused by dinophyceae (Liu et al., 2013a). This illustrates that DGGE was a useful tool for red tide forming species detection. In fact, previous studies had showed that DGGE is an efficient technique for investigating the microeukaryotic community dynamics in marine ecosystems (Díez et al., 2001a; Yu et al., 2015). Therefore, the introduction of molecular methods should help us gain a comprehensive understanding of microeukaryotic communities in this subtropical bay. However, it should be noted that this method have some bias. For example, we occasionally found that one DGGE band contained more than one species (Fig. S2). A previous study has corroborated this finding by retrieving multiple sequences representing different microorganisms associated with a single band position (Sekiguchi et al., 2001). Additionally, the replicate samples run at the same DGGE gel but the bands did not look exactly the same (Fig. S1, sample AS1 and MAS1). Furthermore, we could not re-amplify all the bands because some of them were not intense or strong enough to retrieve enough DNA (Massana et al., 2004). Therefore, it is suggests that the resolution of DGGE for quantifying species richness is limited. The sequencing for a limited number of rDNA clones cannot eliminate the possibility that some minority phylotypes may escape being surveyed (Zuendorf et al., 2006). Molecular fingerprinting techniques can only retrieve the 10–50 most DNA abundant taxa (based on PCR) from a sample, thus deep sequencing is necessary to elucidate completely the patterns of microeukaryotic communities in the future (Logares et al., 2014; Yu et al., 2015).

5. Conclusion

In the present study, we have showed that Copepoda and Dinophyceae were the most diverse groups in the Shenhu Bay, and that the microeukaryotic communities have a significant variation between summer and autumn, with the autumn communities exhibiting a higher diversity than summer communities. Community composition and diversity from both surface and bottom waters showed more significant differences in summer than in autumn. Environmental parameters also displayed obvious seasonal patterns. RDA analysis indicated that temperature was the most significant environmental factor shaping the seasonal patterns of the dominant microplanktonic members in the Shenhu Bay. Our study suggested that community-level molecular techniques such as DGGE appear as useful tools to detect the presence of red tide causing species and to guide the management of coastal water mariculture.

Compliance with ethical standards

The authors have declared that no competing interests exist. No specific permissions were required for these activities. Informed consent was obtained from all participants and this article did not contain any studies with human participants or animals performed by any of the authors.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csr.2017.05.005.

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