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Characterization of Prokaryotic and Eukaryotic Microbial Community in Pacific White Shrimp Ponds

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Abstract

This research profiles the community compositions and dominant taxonomies of prokaryotic and eukaryotic microbes in 30 samples of the water from 5 different Pacific white shrimp ponds. The V4 region of the 16S rRNA gene and 18S rRNA gene were sequenced by high-throughput sequencing technology. Total of 1,387,317 16S rRNA and 1,612,056 18S rRNA gene fragments were selected for classification, including 3,841 prokaryotic Operational Taxonomic Units (OTUs) and 990 eukaryotic OTUs. It's observed that all of the 16S rRNA sequences were affiliated with at least 47 bacteria divisions and 18S rRNA sequences were affiliated with 50 eukaryotic divisions, respectively. Among all 30 samples, the dominant prokaryotic and eukaryotic community at phylum level shared considerable similarity in composition but not in abundance. The dominant prokaryotic community included Actinobacteria, Proteobacteria, Cyanobacteria, Planctomycetes, Verrucomicrobia, Bacteroidetes, Chlorobi, Chloroflexi, Firmicutes and Spirochaetes. Cercozoa, Chlororhyta, Arthropoda, Stramenopiles-unidentified, Fungi-unidentified, Prymnesiophyceae, Ciliophora, Mollusca, Choanomonada and Jakobida were the dominant compositions of the eukaryotic. Similarly, significant difference existed at the genus level among the 30 samples. Results of richness and diversity showed that prokaryotic and eukaryotic microbes possessed complex community compositions in 5 ponds. While in different periods and different ponds, the value of Chao, Ace, Shannon and Simpson index were not significantly different (P >0.05).

Keywords: Eukaryotic community; Prokaryotic community; High-throughput sequencing technology; Shrimp

Introduction

Shrimp is one of the most important products of fishery trading commodities [1]. During 2008 to 2013, the total world shrimp production was in the range of 3,400~4,450 kt [2]. China is the largest producer of shrimp, whose contribution to the total world shrimp production was approximately 40% [2]. The Pacific white shrimp, *Litopenaeus vannamei*, has become one of the most profitable aquaculture species and accounts for about 85% of total shrimp production in mainland of China [3].

In China, the fast growth rate and high density cultivation of aquaculture was based on the larger amount of energy and nutrition inputs, which caused the water eutrophication and made the microbial communities change easily. Water eutrophication may lead to algae blooms, such as Cyanobacteria bloom. In aquatic ecosystem, Cyanobacteria bloom can restrict light penetration, deplete oxygen levels, and decrease the numbers of submerged plants, killing of aquatic animals and modifying the food web dynamics [4,5]. Thus, it is of great significance to investigate the community compositions of prokaryotic and eukaryotic microbes in aquaculture ponds. A recent study [6] shows that season changes have more effects on the bacterial community than what the stocking density does in the pond water of tilapia. And the most communities were affected by the nutrient, except phylum Cyanobacteria was also affected by the feed control. Another study [7] on phytoplankton community in shrimp ponds indicates that the changes in physical factors and nutrient levels contribute to the dominant species changes from week to week, and Diatom shows dominancy in almost every week during the cultivation period. Besides, the study [8] in shrimp ponds water shows that the bacterial community structure in the intensive ponds differs from those in the extensive ponds and the bacterial community structure in the intensive ponds are variable depending on the water treatment system. These results suggest that profiles of bacterial community structure may become a biological indicator to evaluate the water constituents in the aquaculture ponds.

In a typical aquatic ecosystem, microbial communities play important roles in food net, being the producer, consumer and decomposer at the same time. Previous studies used to focus on the bacterial community in the shrimp ponds, sediment and intestinal extractive [7-10]. Traditional methods studying microbial community like microscopic identification and plate cultivation hold apparent short comings, including the disability in detecting a mass of uncultured species. Some biochemical methods, such as phospholipid fatty acids (PLFA) [11,12], denaturing gradient gel electrophoresis (DGGE) [9] and clone library analysis [13], have also been used to characterize the microbial community compositions, which have certain limitations because they tend to underestimate the overall diversity and it is difficult to profile a comprehensive community in complex environments. Fortunately, the high-throughput sequencing

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technology has become a powerfully tool to analyze microbial community of environmental samples [14,15], which could generate reliable and sufficient information on community compositions through the amplification and identification of 16S rRNA and 18S rRNA genes. Generally, the high-throughput sequencing technology is an ideal method for microbial community analyses.

Although various studies reported the bacterial community in aquaculture ponds, the relatively complete community compositions of prokaryotic and eukaryotic microbes in shrimp ponds have not been reported. Thus, the aim of our study was to investigate prokaryotic and eukaryotic community by the high-throughput sequencing technology in order to enlarge our understanding of microbial communities in shrimp ponds.

Materials and Methods

Sample collection

Water samples were collected from five shrimp ponds, which located in Maoming, Guangdong Province, China (21.68°N, 110.88°E). The surface area of the ponds was approximate 2,600 m^2 and the average depth was 1.5 m. Water was pumped from the nearby seawater whose salinity was approximate 10‰. Shrimp larvae were from Guangdong Huanqiu Aquaculture Company and 200,000 shrimp larvae were cultured in each pond. The feed was from Guangdong Haida Group Co. Water samples were collected every 15 days and finally 30 samples were gained. For simplification, the 5 ponds from which the samples were collected were named as A, B, C, D and E. Samples from the same pond were named 1, 2, 3, 4, 5 and 6, based on the collected date. Thus, 30 samples were named as A1~A6, B1~B6, C1~C6, D1~ D6 and E1~E6.

1.5L of water was taken for the following experiments [6]. The water temperature, pH value and salinity were monitored *in situ* with an YSI handheld multiparameter instrument (Model YSI 556, YSI, USA). 0.5 L water was taken to determine the concentration of sulfate, orthophosphate and dissolved inorganic nutrients (NO_2-N, NO_3-N and NH_4-N) by automatic discrete analyzer (Model CleverChem 200, DeChem-Tech, Germany). 1.0 L mixture water was filtered the biomass with 0.22 μm and 0.45 μm filter for the prokaryote and eukaryotes, respectively. The filter membranes were stored at -80°C before DNA extraction.

DNA extraction and sequencing

The filter membranes were put into a 50 mL centrifuge tube containing sterile glass beads and 10 mL PBS buffer was added. The tube was vortex thoroughly for 3 min and centrifuged at 10,000 g for 1 min, reducing the influence of unknown material. Genome DNA was extracted using the Water DNA Kit (Omega Bio-tek, USA) according to the manufacturer's directions. The concentration and purity of genome DNA were determined by the NanoVuePlus Spectrophotometer (GE Healthcare, USA). DNA was diluted to 1ng/μL using sterile water. The V4 hypervariable region of 16S rRNA gene was amplified with the primers 515F (5'-GTGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTA AT-3') [16]. The V4 hypervariable region of 18S rRNA gene was amplified with the primer pair 528F (5'-GCGGTAATTCCAGCTCCAA-3') and 706R (5'-AATCCRAGAATTTCACCTCT-3') [17]. The PCR program was as follows: 95°C for 5 min; 94°C for 30s; 50°C for 10s; 72°C for 20s. Run for 35 cycles. All PCR reactions were carried out with Phusion High-Fidelity PCR Master Mix (New England Biolabs, UK) PCR products were mixed in equidensity ratios. Sequencing libraries were generated using TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on Qubit 2.0 Fluorometer (Thermo Scientific, USA). The library was sequenced by Illumina Hiseq2500 system (Illumina, USA), conducted by Novogene Bioinformatics Technology Co. Ltd (Beijing, China).

Data analysis

Raw data generated from the Hiseq2500 system were merged with FLASH (Version 1.2.7, http://ccb.jhu.edu/software/FLASH/) [18]. In order to control the quality process, raw tags were then filtered to obtain the high-quality clean tags by the QIIME (Version 1.7.0, http://qiime.org/index.html) [19,20]. To detect and remove the chimera sequences, the tags were compared with the Gold database (http://drive5.com/uchime/uchime_download.html) using UCHIME algorithm (http://www.drive5.com/usearch/manual/uchime_algo.html) [21] and finally the effective tags were obtained. The effective tags analysis was completed by Uparse (Version 7.0.1001) to produce OTUs. Sequences with more than 97% similarity were assigned as the same OTU. The Green Gene Database (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) [22] was used to annotate taxonomic information for each representative sequence with the usage of RDP classifier (Version 2.2, http://sourceforge.net/projects/rdp-classifier) [23]. To figure out the phylogenetic relationship of different OTUs and the dominant species in different samples, multiple sequence alignment were conducted and UPGMA tree was made by Muscle (Version 3.8.31, http://www.drive5.com/muscle) [24]. OTUs abundance information was normalized with a standard of sequence number which was correspond to the sample that contained the least sequences [25]. The following analysis was based on the output normalized data. The relative abundance of individual taxa within each sample can be obtained by comparing the number of total sequences and sequences can be assigned to a specific taxon [26]. Alpha diversity was calculated of Chao index, Ace index, Shannon index and Simpson index value by QIIME (Version 1.7.0).

Results

Environmental parameters

The water temperature was relatively stable at approximately 30°C. *pH* value ranged from 7.5 to 8.61. Irregular changes in NH_3-N, NO_2-N, NO_3-N, PO_4^{3-} and SO_4^{2-}, were in range of 0.0089~1.1095 mgL^{-1}, 0.0022~0.9869 mgL^{-1}, 0.0323~3.3007 mgL^{-1}, 0.0171~0.3131 mgL^{-1} and 0.0012~0.3777 mgL^{-1}, respectively.

Community of prokaryotic and eukaryotic at phylum level

Total of 1,387,317 16S rRNA and 1,612,056 18S rRNA gene fragments were selected for classification. 3,841 prokaryotic OTUs and 990 eukaryotic OTUs were gained. The dominant length distributions were approximately 253 bp and 310 bp, respectively.

All of the 16S rRNA sequences were affiliated with at least 47 bacteria divisions (Figure 1a). Most of all sequences belonged to the following ten phyla, included Actinobacteria, Proteobacteria, Cyanobacteria, Planctomycetes, Verrucomicrobia, Bacteroidetes, Chlorobi, Chloroflexi, Firmicutes and Spirochaetes. The major phyla were always more than 90% proportion among all samples. The highest Actinobacteria proportions were exhibited in sample D1 and D6. The proportions of Cyanobacteria were more than 10% except in 6 samples (A5, C5, D1, D4, D5 and D6). The highest proportions were in sample A3 (36.38%) and B6 (36.94%). The highest and lowest Proteobacteria proportions were in sample C5 and E2, 45.72% and 17.81% respectively. Chlorobi always existed in all samples.

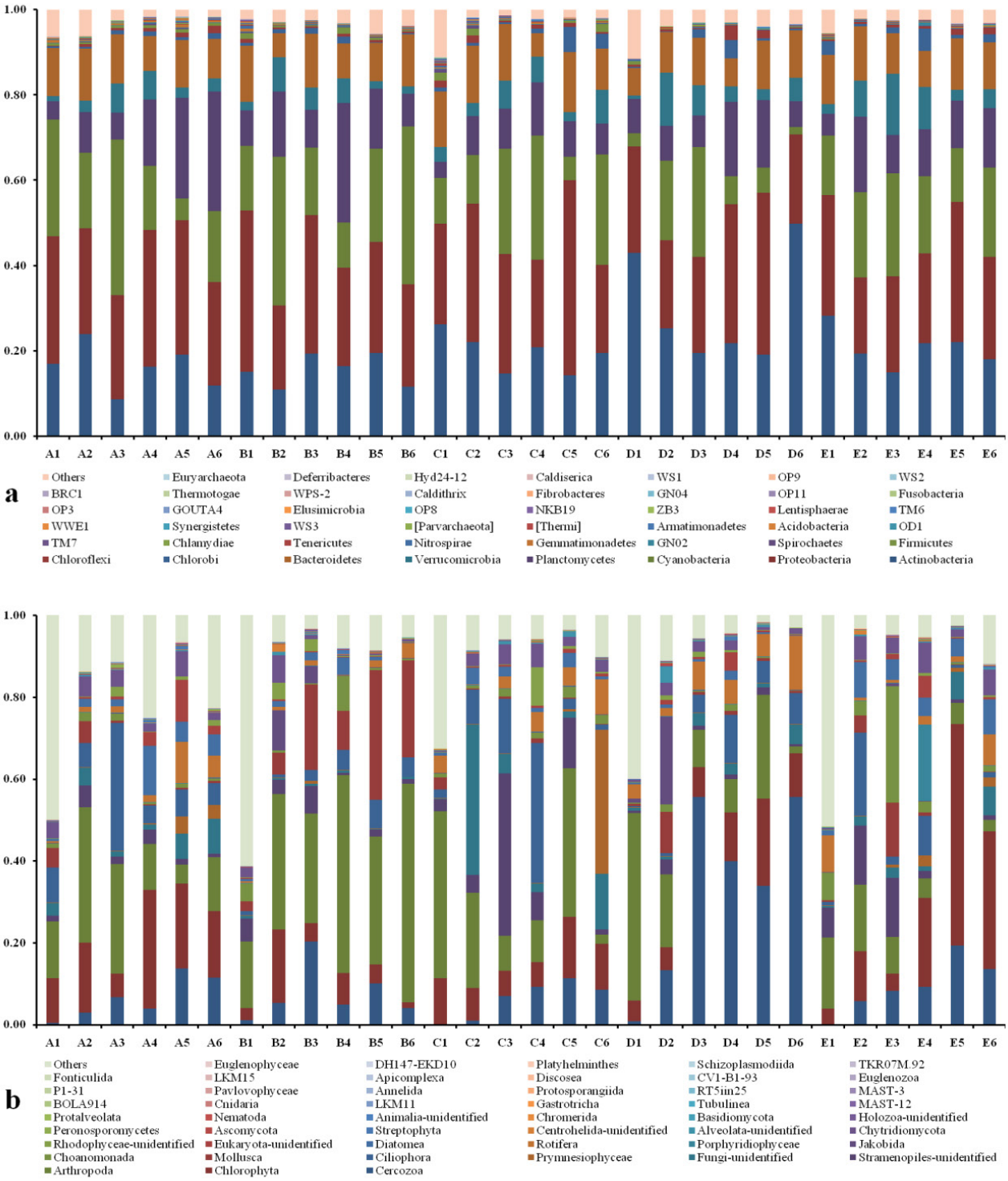

Figure 1: Phylum level relative abundance and community compositions of prokaryotic (a) and eukaryotic (b) obtained by 16S rRNA and 18S rRNA sequencing in 30 samples. The phylum level distribution presented is based on the 80% similarity clusters OTUs. Sequences whose relative abundance was lower than 1% and unclassified were assigned as "others".

All of the 18S rRNA sequences were affiliated with at least 50 divisions at phylum level (Figure 1b), and minority divisions were identified as phytoplankton. The majority of all eukaryotic community belonged to the following ten divisions: Cercozoa, Chlororhyta, Arthropoda, Stramenopiles-unidentified, Fungi-unidentified, Prymnesiophyceae, Ciliophora, Mollusca, Choanomonada and Jakobida. Cercozoa proportions were over 55% in sample D3 and D6. More than half of all samples, Chlororhyta and Arthropoda proportions were higher than 10%. Ciliophora and Mollusca always existed in all samples, which were highest in sample C4 (34.17%) and

B5 (31.69%), respectively. In sample E3, Choanomonada proportion was well far above those of other samples. *Diatomea*, Cladocera and Copepods are usually considered conducive to shrimp growth as the important source of food. *Diatomea* existed in all samples, which were the highest in sample A4 (12.14%), E2 (8.71%) and E6 (8.54%), respectively. *Diatomea* proportions were higher than 1% of 17 samples. Cladocera and Copepods were not detected.

Genus level on prokaryotic and eukaryotic community

The clustering heatmap was based on the top 35 abundant prokaryotic and eukaryotic community at the genus level (Figure 2). The most abundant prokaryotic at genus level were *Microcystis*, *Candidatus Aquiluna*, *Oceanibaculum*, *Gemmatimonas*, *Bdellovibrio*, *Bacteriovorax*, KSA1, *Rhodobacter*, *Candidatus Xiphinematobacter*,

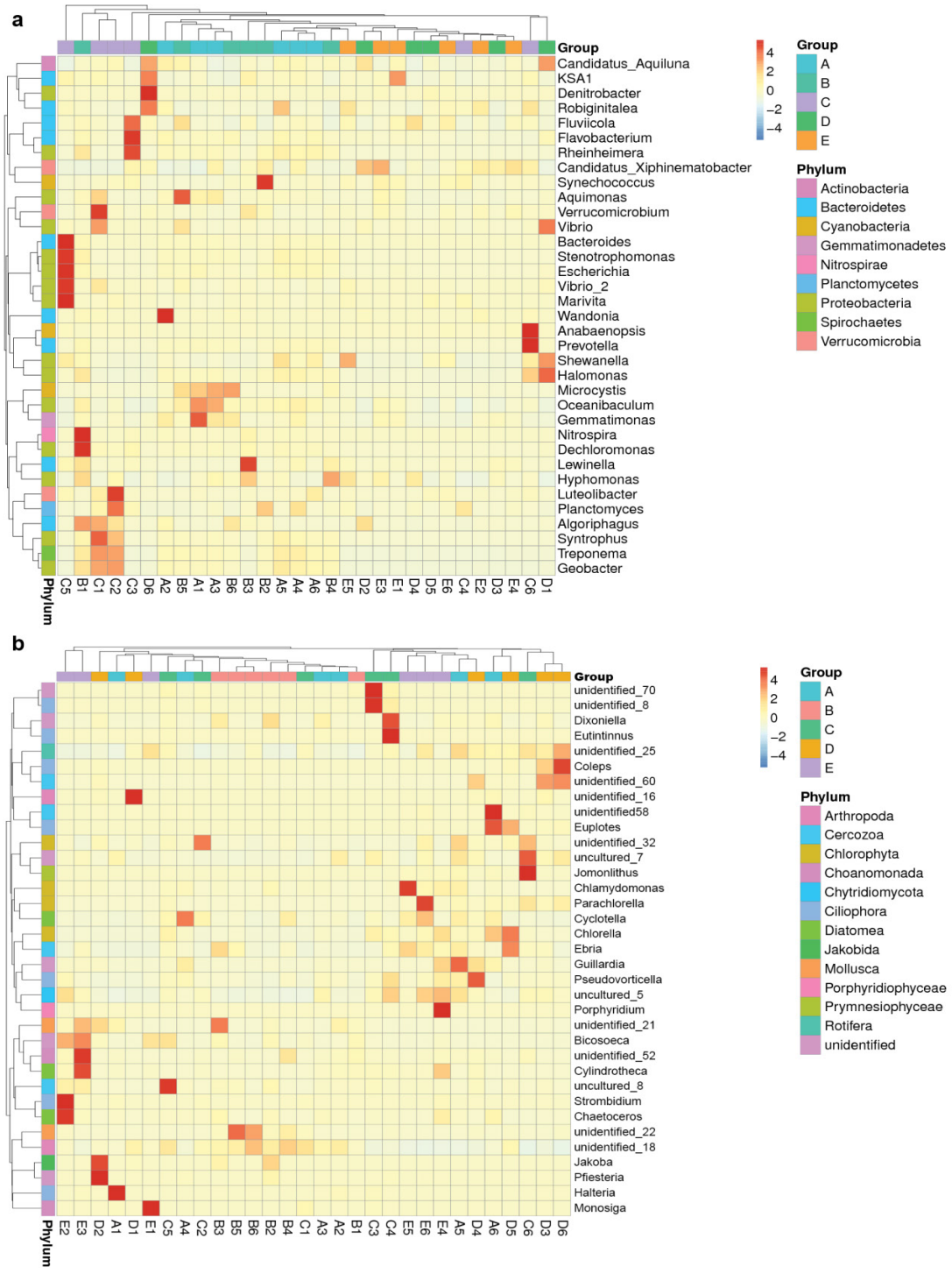

Figure 2: Heatmap analysis of prokaryotic (a) and eukaryotic (b) community at the genus level based on the 16S rRNA and 18S rRNA sequencing profiles. In the figure, the longitudinal is the phylogentic analysis based on Unweighted Pair Group Method with Arithmetic (UPGMA), the transverse is the Q-type Cluster Analysis based on the abundance of different genus of each sample.

Aquimonas, *Hyphomonas* and *Synechococcus*. *Microcystis* were most abundant in 4 samples, including sample A1, A2, B5 and B6, whose proportions were 24.90%, 30.36%, 20.98% and 32.21%, respectively. Besides, *Microcystis* is several or dozens times higher than other samples. *Oceanibaculum* proportions were relatively stable at approximately 0.16%. *Synechococcus* were always found in all samples and particularly abundant in sample B2 (14.12%). The *Vibrio* and *Pseudomonadaceae*, are major dangerous pathogens, their proportions were less than 0.01%. *Bacillus*, *Lactobacillus* and Yeast were used as probiotics in aquaculture. The proportion of *Bacillus* was less than 0.01% in 19 samples and not detected in 11 samples. In all samples, *Lactobacillus* and *Yeast* were not detected. The most abundant eukaryotic were *Cercozoa*-unidentified, *Maxillopoda*-unidentified, *Chlamydomonas*, *Stramenopiles*-unidentified, *Jomonlithus*, *Gastropoda*-unidentified, *Ebria* and *Salpingoecidae*-unidentified. *Cercozoa*-unidentified proportions were quite high in sample D3, D4, D5 and D6, even achieved 54.73% in sample D6. Two thirds of all samples, the proportions of *Maxillopoda*-unidentified were over 10%, and the highest abundance was in sample B6 (53.32%). *Chlamydomonas*, *Stramenopiles* - unidentified, *Jomonlithus*, *Gastropoda*-unidentified, *Ebria*, *Salpingoecidae*-unidentified, *Bicosoeca*, *Parachlorella*, *Cyclotella* and *Chlorella* were found in all samples with low proportions, and several of them were more than 10%.

Richness, diversity and similarity analysis

The prokaryotic and eukaryotic richness of all samples from five ponds was indicated by Chao index and Ace index (Figure 3). There was no significant difference in Chao index and Ace index value of different ponds (P>0.05). The highest Chao index and Ace index value of prokaryotic community richness were in sample A5, B1, C6, D5 and E5, while the highest value of eukaryotic community richness were in sample A2, B2, C4, D2 and E2. Moreover, the prokaryotic and eukaryotic diversity of the water samples from five ponds was indicated by Shannon index and Simpson index (Table 1). These results suggested that the value of Shannon index and Simpson index showed no significant difference between different periods and different ponds (P>0.05). The highest Shannon index and Simpson index value of prokaryotic community diversity were in sample A4, B1, C1, D5 and E5. While the highest value of eukaryotic community diversity were in sample A6, B2, C4, D4 and E4.

To show their similarity, all samples were compared using Non-Metric Multi-Dimensional Scaling (NMDS) (Figure 3). For prokaryotic community, 6 samples from the same pond were dispersed to different quadrant, but 5 ponds were not separated from each other. Likewise, eukaryotic community of different samples from the same pond was separated from each other, except 5 samples (including B2, B3, B4, B5 and B6) clustered together within pond B.

The prokaryote results showed that the class of Deltaprotebacteria and Betaprotebacteriathe were the specific bacterial taxa in pond A; the class of Cytophagia and Oscillatoriophycideae, the order of Chroococcales and Cytophagales, the family of Microcystaceae, the genus of *Microcystis* and *Synechococcus* were the specific bacterial taxa in pond B; the genus of *Marivita* was the specific bacterial taxa in pond C; the phylum of Actinobacteria, the class of Acidimicrobiia and the order of Acidimicrobiales were the specific bacterial taxa in pond D. And the eukaryote results showed that the class of Mediophyceae was the specific bacterial taxa in pond A; the phylum of Mollusca, the class of Gastropoda and Maxillopoda were the specific bacterial taxa in pond B; the phylum of uncultured-phytoplankton and Stramenopiles, the family of Euplotia and the genus of *Euplotes-rariseta* were the specific bacterial taxa in pond C; the phylum of uncultured- eukaryote and the order of NOR26 were the specific bacterial taxa in pond D; the phylum of *Diatomea* and the class of Craspedida were the specific bacterial taxa in pond E. In conclusion, the NMDS plots suggested that prokaryotic and eukaryotic community of different samples from the same pond were relatively dispersed.

Discussion

High-throughput sequencing was one of the most effective techniques to determine the identification and quantification of microbial community, which can generate a profile of the whole community in aquatic ecosystem [27,28]. Studies on prokaryotic and eukaryotic community compositions by 16S rRNA and 18S rRNA sequencing were seldom reported in the water of Pacific white shrimp ponds. The study on prokaryotic and eukaryotic community is very important for understanding the structure and function of the ecosystem for shrimp ponds.

Previous research has shown that large amount of nutrient input and high temperature may lead to the Cyanobacteria bloom [29], and high phosphate concentration usually encourages the growth of Cyanobacteria [30]. In shrimp culture period, the phosphate level was

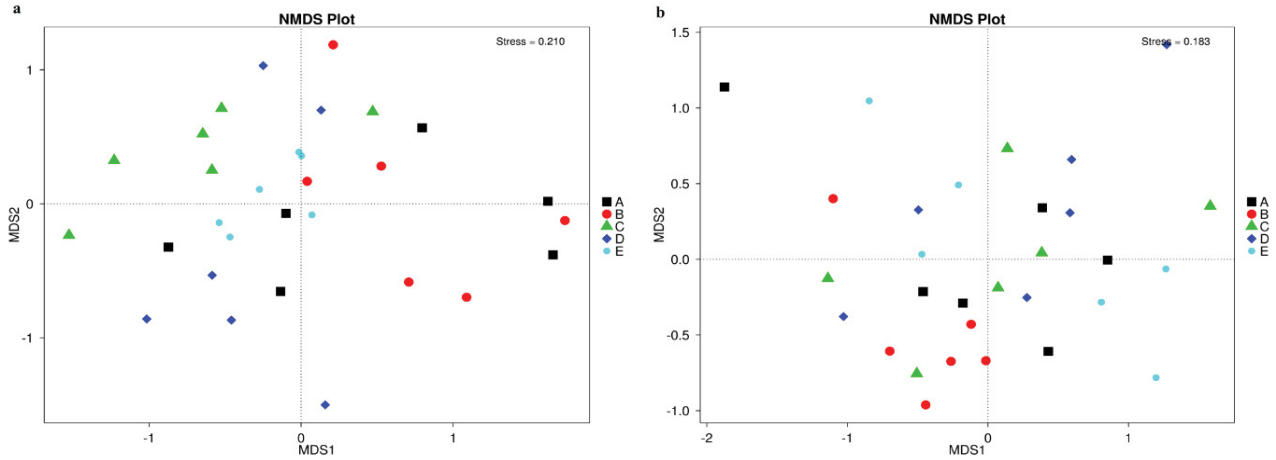

Figure 3: Grouping of prokaryotic (a) and eukaryotic (b) community according to taxonomic compositional similarity using by Non-Metric Multi-Dimensional Scaling analysis. Each symbol represents one sample, corresponding to a particular pond (color).

		A1	A2	A3	A4	A5	A6	B1	B2	B3	B4	B5	B6	C1	C2	C3	C4	C5	C6	D1	D2	D3	D4	D5	D6	E1	E2	E3	E4	E5	E6
a	shannon	6.33	6.76	6.15	7.42	7.72	7.06	8.12	6.3	7.58	6.71	6.29	5.71	7.67	7.64	7.18	6.61	7.12	6.97	6.59	6.40	6.23	6.70	7.26	6.70	7.39	6.82	7.20	7.38	7.78	7.50
	simpson	0.93	0.97	0.90	0.98	0.98	0.96	0.98	0.95	0.98	0.97	0.95	0.89	0.99	0.99	0.98	0.97	0.98	0.97	0.97	1	0.95	0.97	0.98	1	0.99	0.96	0.98	0.98	0.99	0.99
	chao1	1285	1589	1776	1990	1980	1751	1975	1262	1660	1566	1342	1127	1593	1599	1207	1095	1328	1603	1328	947	1063	1129	1414	882	1240	1227	1229	1325	1633	1381
	ACE	1365	1687	1812	2097	2048	1871	2098	1284	1734	1633	1462	1203	1578	1685	1247	1134	1365	1710	1347	956	1126	1138	1452	876	1274	1201	1231	1354	1620	1367
b	shannon	3.37	5.11	5.01	4.71	5.72	5.73	3.32	4.67	4.37	3.85	3.85	2.99	3.62	4.02	4.57	4.90	5.16	4.30	4.14	5.00	4.15	5.11	4.29	3.50	3.38	5.19	4.69	5.79	3.16	5.31
	simpson	0.76	0.89	0.88	0.90	0.95	0.96	0.70	0.89	0.88	0.79	0.81	0.69	0.83	0.82	0.84	0.88	0.92	0.86	0.89	0.90	0.77	0.88	0.85	0.70	0.75	0.93	0.90	0.97	0.72	0.95
	chao1	356	688	610	539	552	496	400	581	534	424	572	380	398	409	554	569	621	414	350	596	478	485	464	399	380	533	399	478	286	392
	ACE	372	632	625	560	539	504	417	542	517	387	507	392	399	429	536	535	637	409	346	559	465	507	488	404	400	533	419	485	297	407

Table 1: Richness (the Chao, Ace index) and diversity (the Shannon, Simpson index) of prokaryotic (a) and eukaryotic (b) community in five ponds. Higher values of Chao and Ace index represent more richness. A higher value of Shannon index represents more diversity, while represents less diversity of Simpson index. There were no significantly different between different ponds for all samples of Chao, Ace index, Shannon and Simpson value (P>0.05).

high due to the large amount of nutrition inputs including feeding and fertilization. Thus, we need to control phosphate input, thereby reduce the occurrence of Cyanobacteria bloom. In this study, the dominant prokaryotic bacteria, Actinobacteria, Proteobacteria and Cyanobacteria were found in the water of shrimp ponds. The results are not consistent with those of other methods. The sequencing results by 454 pyrosequencing technique [31] suggested that the major phylum in the shrimp ponds were Proteobacteria, Flavobacteria and Actinobacteria. DGGE [9] sequencing results showed that most species from fresh tropical shrimps (*Penaeus notialis)* ponds and the surrounding brackish water belonged to Firmicutes, followed by Proteobacteria and Actinobacteria. Except for regional difference, the discrepancy among the shrimp aquaculture water was mainly caused by the high percentage of uncultured clones and different methods used in the study. In addition, above results conflicted with the previous reports that Cyanobacteria was never the dominant phylum in shrimp ponds, whose proportions were normally less than 5% [9,11,32,33]. However, at present study, Cyanobacteria proportions were higher than 10% among 24 samples.

Results in our study also showed that *Microcystis* and *Synechococcus* were the dominant genera among Cyanobacteria. It has been reported that high temperature is the main driving factor on the growth of *Microcystis* [34-36], and *Microcystis* survives better in high pH [37,38] and high phosphate level environments [39-41]. Consistent with the previous reports, the abundance of *Microcystis* was greatly increased in high temperature, pH and phosphate level circumstance in this research. Moreover, the temperature performed relatively as a stable environmental factor, while the high pH and high phosphate level were main effective factors on the growth of *Microcystis*. *Synechococcus* survived and propagated easily, acting as the main participants in the global carbon cycle and the major contributors to primary productivity [42], which is particularly abundant in offshore waters [43]. *Bdellovibri* was exhibited in all samples. As the obligate gram-negative predatory bacteria, *Bdellovibri* was always found in Cyanobacteria bloom. It was previously reported that *Bdellovibrio* can split *Microcystis* through breakdown of cell structures [44]. As the type of strictly anaerobic and photoautotrophic bacteria, *Chlorobi* may utilize sulfide or thiosulfate as an electron donor for CO_2 accumulation [45]. Chlorobi were found in various types of aquaculture water [6,46], in accordance with our results that Chlorobi was detected in all samples, which might result from significant hypoxia phenomena in 5 shrimp ponds. In order to ameliorate aquatic, effective microorganism (EM, include Yeast, *Bacillus*, *Lactobacillus*) was added to aquaculture ponds, frequently [39]. Yeast and *Lactobacillus* were not found in all samples, and *Bacillus* was also scarce only detected in 11 samples. These results indicated that the effective microorganism might have enormous pressure for survival in the water of shrimp ponds.

Compared with estuary and marine ecosystem [27,28], eukaryotic community compositions remains less understood in aquaculture water ecosystem. Unlike other aquatic ecosystem [47,48], the eukaryotic community in our study was dominated by Cercozoa, Chlorophyta and Arthropoda. Generally, zooplankton community was dominated by *Arthropoda* and *Rotifera* in a prawn (*Macrobrachium rosenbergii)* farm based on traditionally morphology [49]. While in this study, Cercozoa and Arthropoda were the highest abundance zooplankton community. Cercozoa is abundant in any protest phyla of every marine habitat, and act as a quantitatively significant player in carbon cycles and food webs by preying on bacteria and Diatom [50,51]. Our results showed that the Cercozoa proportion was limited during the early shrimp culture period, which might be caused by the changes of living environment, from offshore to the ponds with defined artificial treatments. *Pseudodiaptomus annandalei* and *Apocyclopsroyi* were the dominant species among Arthropoda. For Arthropoda, only *Pseudodiaptomus annandalei* existed in all samples except sample A1, whose predominant species was *Apocyclopsroyi*. Ammonium is the major nitrogenous waste excreted by Crustacea, including shrimps and zooplankton [52]. *Chlorophyta, Stramenopiles, Haptophyta* and *Rhodophyceae* were the most abundant phytoplankton community. Compared with other studies [53-55*], Diatomea* was not the dominant species in 5 ponds. *Diatomea* is considered conducive to shrimp growth as the important source of food. As a result most shrimp farm managers prefer a high proportion of *Diatomea* in phytoplankton community [56]. High nitrate concentration level and high N/P ratio will encourage the growth of diatom [57,58]. Thus, the low N/P ratio accounts partly for the low *Diatomea* proportion in this study. Besides, the number of zooplankton species was larger than phytoplankton, which was consistent with the previous research [27,59]. In general, previous studies focusing on phytoplankton community composition and function in aquaculture ecosystems, paid not enough attention on zooplankton [5,37,53,54].

Conclusion

In summary, we generate a profile of the relatively complete prokaryotic and eukaryotic community in shrimp ponds. Our results showed that the abundance of microbial community is more linked to environmental factors (unpublished data). Hence, more intention should be paid to the effects of environmental factors on the diversity and distribution of microbial community, and the relationship between species and environmental factors in the ecosystem of shrimp ponds.

Acknowledgement

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Author's Contributions

Dongwei Hou, Shaoping Weng, Zhijian Huang and Jianguo He conceived of the study, and participated in its design and coordination. Dongwei Hou drafted the manuscript, and extracted DNA. Shenzheng Zeng participated in the design of the study and extracted DNA. Jian Liu collected the samples. Muting Yan helped to draft the manuscript. All authors read and approved the final manuscript.

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