Journal of the Marine Biological Association of the United Kingdom, 2017, 97(1), 151–159.
doi:10.1017/S0025315416000205

Comparative study of the composition and genetic diversity of the picoeukaryote community in a Chinese aquaculture area and an open sea area

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Picoeukaryotes (<2–3 μm) perform key roles for the functioning of marine ecosystems, but little is known regarding the composition and diversity of picoeukaryotes in aquaculture areas. In this study, the Illumina MiSeq platform was used for sequencing the V4 variable region within the 18S rDNA gene to analyse genetic diversity and relative abundance of picoeukaryotic communities in the Qinhuangdao scallop cultivation area of the Bohai Sea. The community was dominated by three super groups, the alveolates (54%), stramenopiles (41%) and chlorophytes (3%), and three groups, dinoflagellates (54%), pelagomonadales (40%) and prasinophytes (3%). Furthermore, a contrasting station with open water away from the eutrophic aquaculture area was chosen. The communities collected from the two stations exhibited significant differences, with higher diversity in the aquaculture area. These results provide the first snapshot of the picoeukaryotic diversity in surface waters of the Qinhuangdao scallop cultivation area, and basic data for future studies on picoeukaryote community in an aquaculture region.

Keywords: environmental heterogeneity, genetic diversity, picoeukaryotes, MiSeq, 18S rDNA

Submitted 16 September 2015; accepted 26 January 2016; first published online 1 March 2016

INTRODUCTION

Picoeukaryotes (eukaryotes smaller than 2–3 μm in diameter) have key roles in marine ecosystems, particularly in primary production, nutrient cycling and for food-web dynamics (Caron *et al.*, 1999). They occur in aquatic environments worldwide at concentrations between 10^2 and 10^4 cells ml^{-1} in the photic zone (Massana, 2011). Marine picoeukaryotes belong to a wide range of different phylogenetic groups (Sherr & Sherr, 2008; Jardillier *et al.*, 2010; Caron *et al.*, 2012). In fact, nearly every algal phylum has picoplanktonic representatives (Thomsen, 1986). In the open oceans, most picoeukaryotes are coccoid or flagellated forms with (phototrophic) or without chloroplasts (heterotrophic), and with few morphologically distinct features (Thomsen, 1986; Simon *et al.*, 1994; Andersen *et al.*, 1996). This phytoplankton is mainly composed of phyla such as haptophytes, dinoflagellates, prasinophytes and many phylogenetic groups within these very broad phyla still lacking cytological analysis. However, the extent of the diversity, distribution and abundance of the different taxa *in situ* remain unknown (Partensky *et al.*, 1997). Over the past decade, 18S rDNA-based molecular approaches, such as Sanger-based sequencing of clone libraries, 454 pyrosequencing and Illumina MiSeq platform sequencing, have provided broad insights into picoeukaryotic diversity in many areas, such as in the hypoxic north-western coast of the Gulf of Mexico (Rocke *et al.*, 2013), in the South China Sea (Wu *et al.*, 2014) and in subtropical coastal waters of Hong Kong (Cheung *et al.*, 2010).

Aquaculture is a fast-growing industry because of significant increases in the demand for fish and seafood throughout the world (Naylor *et al.*, 2000). Marine aquaculture in China consists of four sea regions including the Bohai Sea, Yellow Sea, East China Sea and South China Sea. Qinhuangdao aquaculture area is an important aquaculture area in the Bohai Sea, currently extending to ~214,510 hectares (Cao *et al.*, 2007). The key shellfish crop in Qinhuangdao is devoted to intensive scallop cultivation in an area of ~37,300 hectares (Cao *et al.*, 2007).

In eutrophic waters of aquaculture areas, picoeukaryotes were abundant and the main food organism of shellfish (Muller-Feuga, 2000), and some species blooming could cause 'red tides' even 'brown tides', which occurred due to a picoplanktonic (~2–3 μm) alga in North America, Africa and Asia (Gobler & Sunda, 2012). Moreover, there are protozoa-like ciliates which are major consumers. They together participate in the energy flow and element cycling. So, to understand the complex ecosystem of aquaculture areas is very important, especially since the research on picoeukaryotes is still scant. In this study, we used

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Illumina's MiSeq platform sequencing V4 variable region within the 18S rDNA gene to analyse genetic diversity and relative abundance of picoeukaryotic communities in the Qinhuangdao scallop cultivation area.

MATERIALS AND METHODS

Microbial diversity analysis

SAMPLE COLLECTION

Surface seawater samples (1 L) were collected from two stations in June 2012 (Figure 1). Station F6 was in the Qinhuangdao scallop cultivation area. Located far from the eutrophic aquaculture area, Station B30 was a contrasting open water station in the North Yellow Sea. Each water sample was filtered first through a 3 μm and then through a 0.22 μm pore-sized polysulphone/polycarbonate filter (Whatman, Piscataway, NJ) using a gentle vacuum pump (<20 cm Hg). The 0.22 μm filter was then transferred to a 5 mL tube and covered with 2 mL of lysis buffer. Samples were immediately frozen in liquid nitrogen and stored at −80°C until DNA extraction. Seawater temperature and salinity were recorded with a SBE19-CTD profiler. Environmental parameters were measured simultaneously.

DNA EXTRACTION AND PCR AMPLIFICATION

DNA was extracted from the 0.22 μm filters using a modified phenol: chloroform extraction and alcohol precipitation procedure (Bostrőm *et al.*, 2004). The 18S rDNA fragments were amplified by polymerase chain reaction (PCR) (95°C for 2 min, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s and a final extension at 72°C for 10 min) using primers 3NDF 5′-barcode-GGCAAGTCT GGTGCCAG-3′ and V4 5′-ACGGTATCT(AG)ATC(AG)T CTTCG-3′ (Stoeck *et al.*, 2010), where the barcode was an eight-base sequence unique to each site. PCR reactions were performed in a triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA.

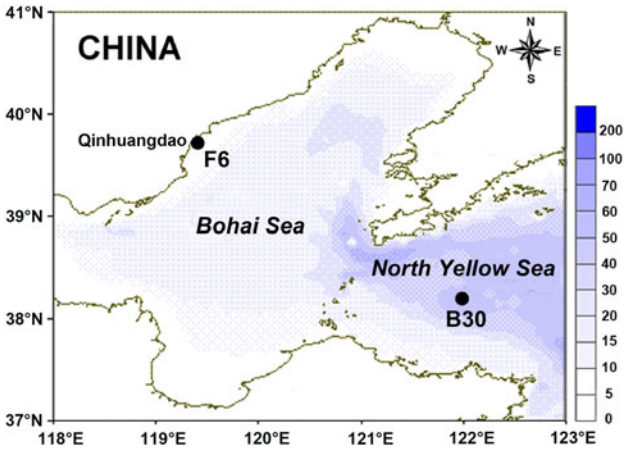

Fig. 1. Sampling sites. F6, Qinhuangdao aquaculture area; B30, contrasting water in the open sea area.

ILLUMINA MISEQ SEQUENCING

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA), according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (accession number: SRP056556).

PROCESSING OF SEQUENCING DATA

Raw fastq files were demultiplexed, quality-filtered using QIIME (version 1.17) with the following criteria: (i) The 300 bp reads were truncated for any site with an average quality score <20 over a 50 bp sliding window, discarding the truncated reads shorter than 50 bp. (ii) Exact barcode matching, 2 nucleotide mismatch in primer-matching and reads containing ambiguous characters were removed. (iii) Only sequences that overlapped by more than 10 bp were assembled according to their overlap sequence. Reads which could not be assembled were discarded.

Operational Taxonomic Units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1, http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The phylogenetic affiliation of each 18S rRNA gene sequence was analysed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115)18S rRNA database using a confidence threshold of 70% (Amato *et al.*, 2013).

Phylogenetic and statistical analysis

PHYLOGENETIC ANALYSES

We determined the phylogenetic relationships of the picoeukaryotes based on maximum likelihood analysis of the V4 area of 18S rDNA gene sequences from the Bohai Sea and Yellow Sea site. FastTree (version 2.1.3, http://www.microbesonline.org/fasttree/) was used to build a maximum-likelihood (ML) tree informed by the methods of Philippot *et al.* (2013), and using R language tools to draw the phylogenetic tree (Figure 2S). Metazoan OTUs were excluded.

STATISTICAL ANALYSES

Non-parametric species richness ACE, Chao1, and diversity indices (Shannon and Simpson) were calculated using the Mothur package (version v.1.30.1, http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity) (Schloss *et al.*, 2009). Rarefaction and Shannon–Wiener curves were also performed using Mothur, and using R language tools to create curve. SIMPER (similarity percentage analysis) test was performed using PAlaeontological STatistics (PAST) version 2.17 (Hammer *et al.*, 2001) based on the relative proportion of taxa abundances.

RESULTS

Environmental conditions

The environmental conditions of the two stations were determined by chemical analysis by the chemistry group. The

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seawater temperature and salinity were slightly higher in the aquaculture area water than the open water (Table 1) and the concentrations of ammonium (NH_4-N), nitrite (NO_2-N) and phosphate (PO_4-P) were slightly higher in F6 than those in B30. Notably, nitrate (NO_3-N) and silica (Si) concentrations were substantially elevated in the eutrophic aquaculture area compared with the contrasting station (Table 1). Furthermore, the satellites map (Supplementary Figure S1) showed the distribution of concentrations of chlorophyll, the primary productivity of the aquaculture area water were significantly higher than that of the open water. On the basis of these values, stations F6 and B30 were confirmed to be two different habitats.

Sequencing conditions

After removal of all low-quality, unassembled and potentially chimeric sequences, a total of 149,038 high-quality sequences were obtained from the two water samples. Following the exclusion of metazoan sequences, 74,845 and 2474 high-quality target tags from F6 and B30 were clustered into 234 OTUs (190 OTUs in F6 and 136 OTUs in B30) and these were used in the downstream genetic analyses. The average tag length was about 444.3 bp.

Picoeukaryotic communities between two habitats

The sequences we found were widely distributed across eight major eukaryotic supergroups: alveolates, stramenopiles, chlorophytes, hacrobia, rhizaria, opisthokonta, apusozoa and amoebozoa. The community captured by our approach was dominated by three super groups, the alveolates (54%), stramenopiles (41%) and chlorophytes (3%), and three groups comprising dinoflagellates (54%), pelagomonadales (40%) and prasinophytes (3%) in the aquaculture area. But in the open water, the dominated super groups were alveolates (60%), chlorophytes (19%), stramenopiles (4%) and opisthokonta (11%), the groups were dinoflagellates (47%), prasinophytes (19%) and ciliates (13%) (Figure 4 and Table 2).

A communality between the F6 and B30 stations was that the alveolata contributed more than half (54% and 60%) of the total community. Of these the overwhelming majority belonged to the dinoflagellates, which accounted for almost half of all the sequences (54% and 47%). But another group, ciliates were more abundant in B30 than F6 (13% and <1%). On the other hand it was notable that the stramenopiles were more abundant at F6 than at B30 (41% and 5%). Of the stramenopiles-affiliated sequences, pelagomonadales represented up to 40% at F6, whereas at B30, the chrysophyceae was the dominant group (3.9%) (Table 2). Chlorophytes represented the third most abundant group in this study. They were almost exclusively composed of prasinophytes and were just over six-fold more abundant at B30 *vs* F6 (19% and 3% respectively). Prasinophyceae, Cryptophyceae and Bolidomonas were more abundant at B30, but the relative contributions of their phylotype OTUs were higher in F6 (Figures 2 and 3 and Table 2).

Phylogenetic relationships of picoeukaryotes based on maximum likelihood analysis of the V4 area of 18S rDNA gene sequences from stations F6 and B30 are shown in Supplementary Figure S2. The scale bar indicates 0.01 nucleotide changes per position. Yellow icons represent clones collected from the aquaculture area F6 and blue icons represent those at B30 (Supplementary Figure S2). Most of the OTUs obtained from F6 were represented by dinoflagellates, stramenopiles, prasinophytes, cryptophyta, haptophyta and cercozoa. As for B30, most OTUs were represented by dinoflagellates, stramenopiles, prasinophytes, cryptophyta, haptophyta and ciliates. The difference in the OTUs between the two stations were mainly associated with pelagophyceae, dinoflagellates, ciliates, prasinophytes and cercozoa (Table 2).

The contribution of the top 26 species to the average Bray–Curtis dissimilarity, which is 97.07%, in terms of occurrence and abundance for the samples from the Qinhuangdao aquaculture area and the contrasting station was analysed by SIMPER (Table 3). These OTUs were closely related to species belonging to pelagophyceae, dinophyceae and prasinophyceae, including *Aureococcus anophagefferens, Gymnodinium* sp., *Syndiniales* sp., *Micromonas* sp., etc. (Table 3).

Shannon and Simpson diversity indices were calculated for all samples to give an indication of species diversity. Non-parametric ACE and Chao 1 estimators and rarefaction curves were used to estimate the OTU richness in this study (Table 4). The Shannon index indicated higher diversity in the aquaculture area, as well as rarefaction curves reaching saturation (Figure 5).

Table 1. Sampling sites and their physical and environmental characteristics.

	F6	B30
Coordinates	119°27.849′E 39°41.564′N	121°59.871′E 38°11.923′N
Temperature(°C)	21.5	18.69
Salinity	33.3	31.179
Si (μmol l^{-1})	57.05	0.48
NO_3-N (μmol l^{-1})	2.01	0.002
NH_4-N (μmol l^{-1})	1.88	1.37
NO_2-N (μmol l^{-1})	0.08	0.02
PO_4-P (μmol l^{-1})	0.08	0.076

Si, silica; NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen; NO_2-N, nitrite nitrogen; PO_4-P, phosphate.

DISCUSSION

Our study sites, aquaculture waters (station F6) and open waters (station B30) showed distinct and dissimilar hydrographic features. In general, the main source of pollution from shellfish culture is the excreta of the shellfish, which can result in local anoxia of bottom sediments (Cao *et al.*, 2007). The seawater temperature, salinity and concentration of inorganic salt in the two environments were different. Notably, nitrate and silica concentrations were substantially elevated, and the primary productivity showed by the satellites map was significantly higher in the eutrophic aquaculture area compared with the contrasting station. In addition, Sun *et al.* (2014) found that the picoeukaryote count in the same aquaculture area in 2012 was about 3.23×10^5 cells mL^{-1} while in the same open sea area it was about 5.24×10^4. However, comparative study of the composition and genetic diversity of the picoeukaryote community in those two environments is still scant.

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Table 2. Sequence abundance and diversity of picoeukaryotic OTUs found in this study.

Phylogenetic groups		Sequences				OTUs			
Super group	Group	F6		B30		F6		B30	
		N	%	N	%	N	%	N	%
Alveolates									
	Cilliates	++	<1%	+++	13%	+	3%	++	10%
	Dinoflagellates	+++++	54%	++++	47%	+++	56%	++	48%
	Apicomonadea			+	<1%			+	<1%
	Perkinsidae	++	<1%	+	<1%	+	1%	+	<1%
	Total	+++++	54%	++++	60%	+++	60%	++	59%
Stramenopiles									
	Chrysophyceae	+++	<1%	++	4%	+	3%	+	7%
	Bacillariophytina	++	<1%	+	<1%	+	2%	+	1%
	Dictyochophyceae	++	<1%			+	<1%		
	Pelagomonadales	+++++	40%			+	1%		
	Bolidomonas	++	<1%	+	<1%	+	2%	+	<1%
	Labyrinthulomycetes	+	<1%	+	<1%	+	<1%	+	<1%
	MAST-1,2,4,8,9	++	<1%	++	<1%	+	<1%	+	<1%
	Total	+++++	41%	+++	5%	++	11%	++	11%
Chlorophytes									
	Prasinophytes	++++	3%	+++	19%	++	5%	+	5%
	Trebouxiophyceae	+	<1%			+	<1%		
	Ulvophyceae	+	<1%			+	<1%		
	Total	++++	3%	+++	19%	++	6%	+	5%
Hacrobia									
	Cryptophyta	+++	<1%	++	1%	++	7%	+	4%
	Haptophyta	+++	<1%	++	3%	+	4%	++	9%
	Picozoa	++	<1%	+	<1%	+	<1%	+	1%
	Centrohelida	+	<1%	+	<1%	+	<1%	+	<1%
	Total	+++	1%	+++	4%	++	12%	++	15%
Opisthokonta									
	Fungi	+++	<1%	+++	11%	++	5%	++	7%
	Choanoflagellida	+	<1%			+	<1%		
	Filasterea	++	<1%			+	<1%		
	Total	+++	<1%	+++	11%	+++	6%	++	7%
Rhizaria									
	Cercozoa	+++	<1%	+	<1%	+	3%	+	<1%
	Retaria	+	<1%			+	<1%		
	Total	+++	<1%	+	<1%	+	4%	+	<1%
Amoebozoa				+	<1%			+	<1%
Apusozoa		+	<1%			+	<1%		

N, number (+ = 0–10, ++ = 10–100, +++ = 100–1000, ++++ = 1000–10,000, +++++ = over 10,000).

The present results showed that there are also strong differences of community composition in the two sites. In previous studies, the diversity of picoeukaryotes in different seas has been investigated. Cheung *et al.* (2010) showed that coastal waters of Hong Kong were dominated by three super groups, the alveolates, stramenopiles and chlorophytes. Similar results were found in the north-eastern Red Sea coast (Acosta *et al.*, 2013), in the coast of the Gulf of Mexico (Rocke *et al.*, 2013) and in our two stations. But at the level of dominating groups, dinoflagellates, prasinophytes and ciliates, B30 is more similar to these previous studies, and was particularly dissimilar with F6. For example, dinoflagellates and ciliates were both abundant in B30, but in F6, ciliates account for only a little.

Dinoflagellates are important components of aquatic environments, where they play various functional roles (Taylor, 1984). Although photosynthetic dinoflagellates are important primary producers in marine ecosystems, some bloom-forming species produce toxins that can cause illness and even death in humans (Zingone & Enevoldsen, 2000). These HAB species are particularly prevalent in warm, stratified and nutrient-enriched coastal waters (Smayda & Reynolds, 2003). Documented HAB events have increased substantially during recent decades as a result of extensive coastal eutrophication and, possibly, global climate change (Chambouvet *et al.*, 2008). Based on the environmental monitoring of the red tide monitoring area in Qinhuangdao, and the red tide records of the China Oceanic Information Network in 2001–2011, it is clear that HAB dinoflagellates often cause 'red tides' in this area (Wang *et al.*, 2013). According to current research, uncultured *Gymnodinium* sp., *Woloszynskia* sp., *Gyrodinium* sp. and *Lessardia elongata* were in the top 26 OTUs, all representing photosynthetic dinoflagellates. Furthermore, alongside their 'red tide' roles, these are also important food organisms for shellfish.

Ciliated protozoa are one of the main components of the microbial community and they play an important role in the functioning of microbial food webs, especially in terms

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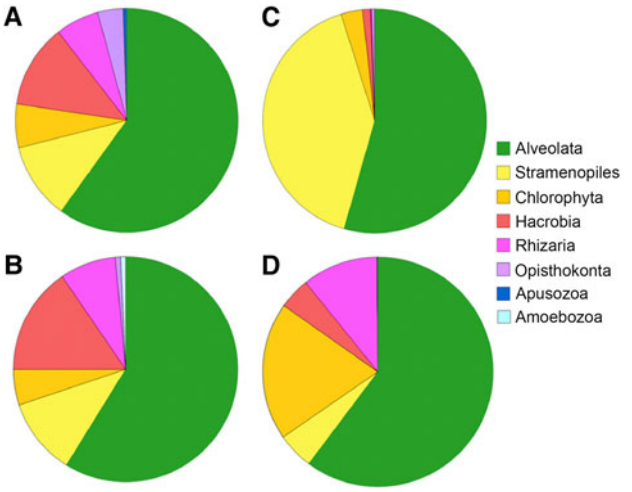

Fig. 2. Composition of picoeukaryote community with species number and relative abundance percentage of each super group assemblage. (A) species number percentage of F6; (B), species number percentage of B30; (C) relative abundance percentage of F6; (D) relative abundance percentage of B30.

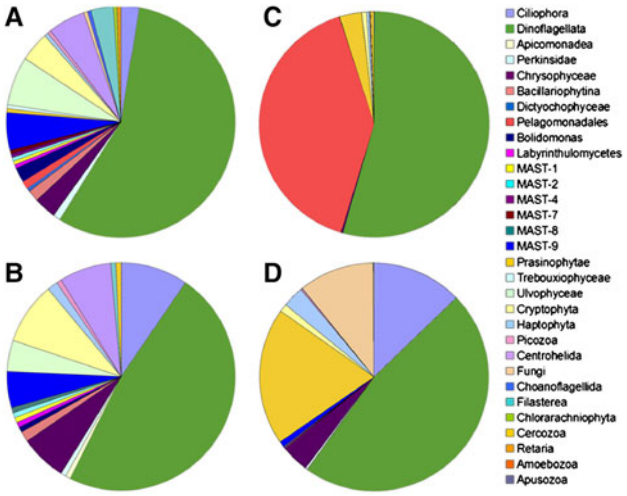

Fig. 3. Composition of picoeukaryote community with species number and relative abundance percentage of each group assemblage. (A) species number percentage of F6; (B) species number percentage of B30; (C) relative abundance percentage of F6; (D) relative abundance percentage of B30.

of energy flow and element cycling (Zöllner *et al.*, 2009). They act as primary producers in marine ecosystems and as consumers at different levels and thus play pivotal roles in the recovery and uptake of carbon nutrients and in their transfer to higher trophic levels (Kyewalyanga *et al.*, 2002). Similar results were found in a study by Doherty *et al.* (2007). In this study, free-living ciliates were more abundant in the open sea of B30, where they consisted mostly of pelagic species, such as species in the genus *Strombidium*. In contrast the eutrophic waters of F6 showed mostly periphytic species, such as *Aspidisca leptaspis*. These species locomote by relatively fast crawling on a substratum, and play an important role in water self-purification and wastewater-treatment processes. Some species of this type can be used as reliable indicators of water quality based on their higher tolerance to eutrophic or toxic environments than pelagic ones (Curds, 1992).

Table 3. Contribution of the top 26 species to the average Bray–Curtis dissimilarity (97.07%) in both occurrence and abundance between the samples of the Qinhuangdao aquaculture area and the contrasting station.

Species	Group F6 Av. Abund	Group B30 Av. Abund	Av. Diss	Contrib%	Cum.%	Closest relative
OTU287	30,223	N/A	39.09	40.27	40.27	*Aureococcus anophagefferens*
OTU355	9697	38	12.49	12.87	53.14	*Gymnodinium* clade sp. uncultured eukaryote
OTU310	3227	N/A	4.17	4.30	57.44	*Syndiniales* Group II sp. 1
OTU188	3049	6	3.94	4.05	61.49	*Syndiniales* Group II sp. 2 uncultured marine picoeukaryote
OTU13	3016	1	3.9	4.02	65.51	*Amoebophrya* sp. uncultured phytoplankton
OTU21	3212	197	3.9	4.02	69.53	*Syndiniales* Group II sp. 3 marine metagenome
OTU57	1881	20	2.41	2.48	72.00	*Woloszynskia* sp. uncultured alveolate
OTU154	1544	51	1.93	1.99	73.99	Alveolata OLI11255 sp. uncultured eukaryote
OTU127	1381	3	1.78	1.84	75.83	*Syndiniales* Group II sp. 4 uncultured marine eukaryote
OTU368	1162	12	1.49	1.53	77.36	*Micromonas pusilla*
OTU300	1191	83	1.43	1.48	78.84	Dinoflagellata sp. 1
OTU95	1071	28	1.35	1.39	80.23	Thecate dinoflagellate UDTSW0701
OTU311	982	N/A	1.27	1.31	81.54	*Syndiniales* Group II sp. 5 uncultured phytoplankton
OTU213	972	1	1.26	1.29	82.83	*Syndiniales* Group III sp. 6 uncultured marine eukaryote
OTU93	692	N/A	0.89	0.92	83.75	*Gyrodinium* sp.
OTU350	673	3	0.87	0.89	84.64	*Micromonas* sp. uncultured marine eukaryote
OTU338	571	N/A	0.74	0.76	85.41	*Syndiniales* Group II sp. 7
OTU190	554	N/A	0.72	0.74	86.14	*Amoebophrya* sp. ex *Prorocentrum minimum*
OTU172	559	12	0.71	0.73	86.87	*Syndiniales* Group I sp.
OTU359	481	1	0.62	0.64	87.51	*Amoebophrya* sp. 'Dinophysis'
OTU83	497	64	0.56	0.58	88.09	*Lessardia elongata*
OTU150	408	21	0.5	0.52	88.60	*Syndiniales* sp. 1
OTU307	340	N/A	0.44	0.45	89.06	*Amoebophrya* sp. 1 uncultured syndiniales
OTU208	151	461	0.4	0.41	89.47	*Bathycoccus* sp. uncultured eukaryote
OTU105	254	0	0.33	0.34	89.81	*Syndiniales* sp. 2
OTU373	246	0	0.32	0.33	90.14	*Amoebophrya* sp. 2 uncultured eukaryote

Av. Abund, average abundance; Av. Diss, average dissimilarity; Contrib%, percentage of contribution; Cum.%, cumulative percentage of contribution.

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Table 4. The species richness estimates of the two stations.

	ACE	Chao 1	Shannon	Simpson
B30	236	219	0.74	0.696
F6	217	217	2.71	0.188

Notably, *Myrionecta* sp., a cosmopolitan, estuarine and neritic photosynthetic marine planktonic ciliate appeared in eutrophic F6 water, which is also known to cause serious 'red-water' blooms (Herfort *et al.*, 2012).

Prasinophytes sequences recovered during this study included mainly uncultured *Bathycoccus, Micromonas* and *Ostreococcus* sp. within the order Mamiellales. These organisms are known to be more common in coastal areas than in open waters (Not *et al.*, 2005). We found that, although both samples shared comparable contributions of phylotype OTUs, prasinophytes were more abundant in open waters of B30 than in the eutrophic waters of F6. A similar result was found in a previous study using the cloning and 454 sequencing strategy (Cheung *et al.*, 2008). Viprey *et al.* (2008) reported a greater dominance of *Micromonas* and

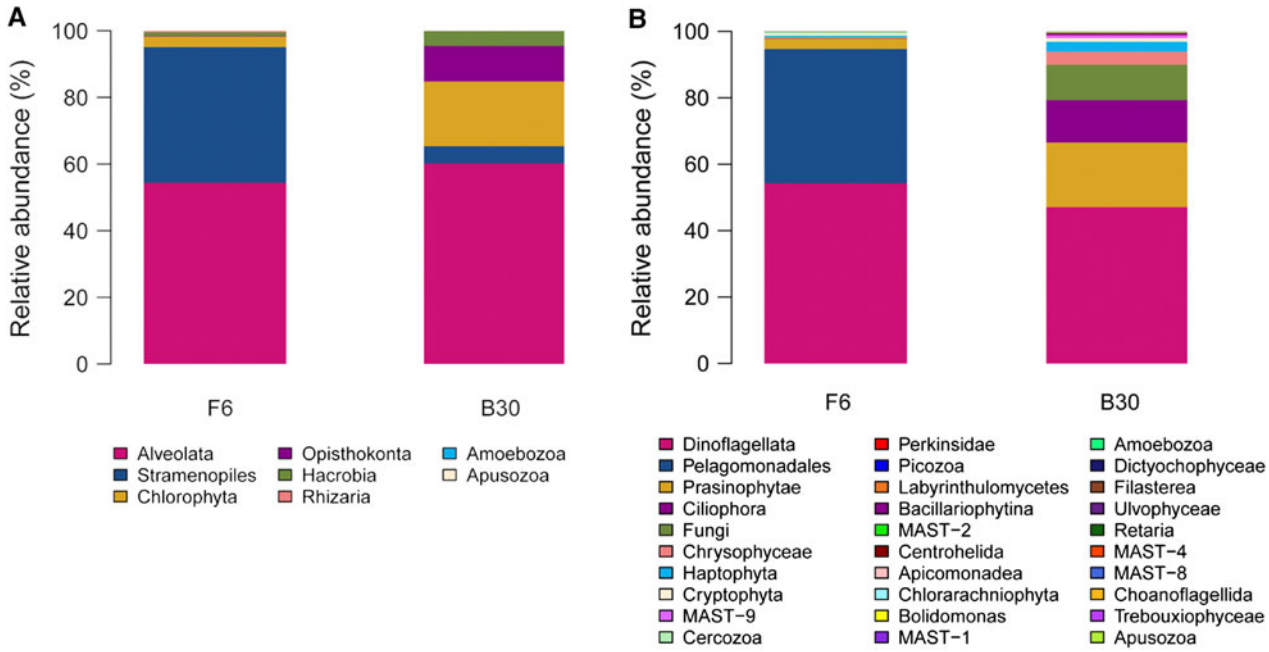

Fig. 4. Relative abundance of picoeukaryotes in super group (A) and group (B).

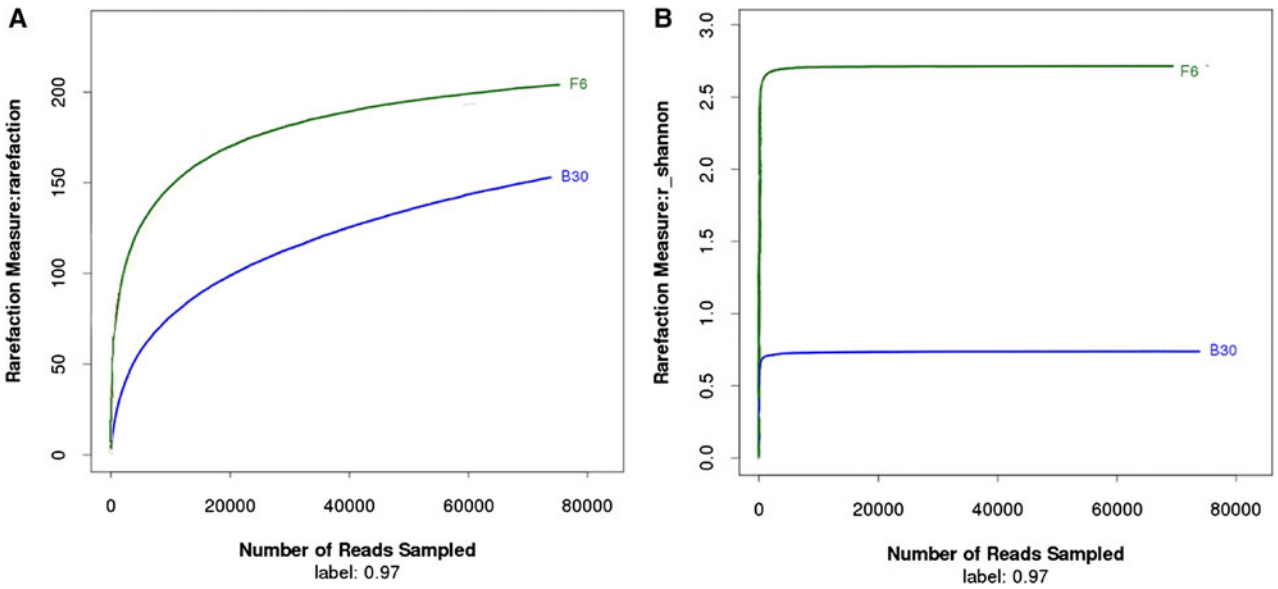

Fig. 5. Rarefaction curves and rarefaction Shannon curves of operational taxonomic units (OTUs). (A) rarefaction curves; (B) rarefaction Shannon curves.

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Ostreococcus environmental sequences in relatively mesotrophic waters compared with contrasting coastal waters. Taken together, these results suggest that these genera are well adapted to coastal waters of intermediate productivity, although the role of water temperature should not be neglected (Lovejoy *et al.*, 2007).

The top 26 OTUs were the main contributors to the average Bray–Curtis dissimilarity (97.07%) in both occurrence and abundance between the samples of the Qinhuangdao aquaculture area and the open area. The top one OTU was closely related to species belonging to pelagophyceae, and other OTUs belonged to dinophyceae and prasinophyceae.

Aureococcus anophagefferens (Pelagophyceae; DeYoe *et al.*, 1995) is a picoplanktonic (~2–3 μm) alga that periodically blooms, dramatically causing 'brown tide' in North America, Africa and Asia (Gobler & Sunda, 2012). Recently, large-scale brown tides have been reported in China, including present investigated waters of Qinhuangdao, northern China, where they have occurred in early summer for six consecutive years from 2009 to 2014 (Zhang *et al.*, 2012). Our result showed that *A. anophagefferens* was the most dissimilar OTU between the two stations; accounting for 40% of all the sequences in F6, but none in B30. While not recognized as a toxin producing alga, *A. anophagefferens* is classified as a harmful algal bloom (HAB) species as cell densities exceeding 1.7×10^6 mL^{-1} are sufficient to effectively attenuate light reaching seagrasses and other photosynthetic organisms, which has caused degradation of seaweed beds (Bricelj & Lonsdale, 1997). Then, this HAB has plagued many coastal ecosystems in the eastern USA and South Africa since its discovery in 1985 (Lu *et al.*, 2014). Indeed, the loss of *Zostera marine*, which is an important contributor to seaweed beds and preyed upon by scallop larvae, can lead to widespread shellfish mortality (Bricelj *et al.*, 2001). So, the abundance of *A. anophagefferens* found at the F6 station in our study appears to be the main reason for the large-scale brown tide in the Qinhuangdao aquaculture area, and needs special attention for monitoring in future.

It has been estimated that 7% of all known dinoflagellates are parasites of aquatic organisms, such as fish, crustaceans, annelids, appendicularians, radiolarians, ciliates, diatoms and other dinoflagellates (Drebes, 1984; Coats, 1999). Among dinoflagellates, the Syndiniales group represents the largest portion of dinoflagellate sequences from various marine ecosystems (especially the <2 μm picoplankton fraction), and this group also comprises the majority of sequences for marine environment clone libraries (López-García *et al.*, 2001; Moon-van der Staay *et al.*, 2001; Not *et al.*, 2007). Environmental sequences belonging to *Amoebophrya* have been detected in almost every marine ecosystem (Guillou *et al.*, 2008). The widespread existence of *Amoebophrya* sp. was 'rediscovered' by culture-independent methods and renamed as 'novel alveolate group II' (López-García *et al.*, 2001; Moon-van der Staay *et al.*, 2001; Díez *et al.*, 2001), but most of the reported clades are related solely to environmental sequence data. Most marine planktonic groups are potentially affected by these parasites, which like the viruses that control bacterial populations, play a top-down control role on their host populations. The distribution of the parasitic Amoebophryideae is closely related to the availability of nutrients. *Amoebophrya* sp. produce more dinospores under nutrient-replete conditions and they are more successful in infectivity than those produced by parasites grown at low nutrient concentrations (Yih & Coats, 2000). Siano *et al.* (2011) have also observed a strong positive correlation between dinospore populations and nitrate levels. In Qinhuangdao aquaculture area, the nitrate plus nitrite concentration was more than 100 times higher (>128 μg L^{-1}) than in the contrasting area. *Amoebophrya* sp. was an important contributor to the dissimilarity between the two stations. Toxic bloom-forming species of the dinoflagellate group are recognized as one of the most common hosts of *Amoebophrya* (Bai *et al.*, 2007). In this study, sequences affiliated with the host-specific *Amoebophrya* were widely related to several known species, for example *Amoebophrya* sp. ex *Gymnodinium* sp., *Amoebophrya* sp. ex *Prorocentrum minimum*, and *Amoebophrya* sp. ex *Gonyaulax* sp. (Johansson & Coats, 2002). The parasitic relationship between *Amoebophrya* sp. and bloom-forming species of dinoflagellates means that the red tide caused by those species might be restrained or possibly even degraded. Therefore, it might be speculated that the abundant *Amoebophrya* sp. could be one reason for the occurrence of 'brown tide' in this area. However, further study is needed to confirm their ecological roles in marine ecosystems.

This is the first study using the Illumina MiSeq platform to compare the picoeukaryotic diversity in surface waters of the Qinhuangdao scallop cultivation area and a contrasting offshore site. Water samples from two hydrographically different sites displayed different high-level taxonomic groups and phylotype OTUs for picoeukaryotes. The data suggest that aquaculture regions may develop very specific picoeukaryote communities which obtain higher diversity and more HAB species. With the rapid development of next-generation sequencing, the Illumina MiSeq platform sequencing technology has become an increasingly powerful tool for diversity surveys, aimed at a more comprehensive picture of marine picoeukaryotic diversity. Our research provides a basic dataset for the picoeukaryote community in an aquaculture eutrophication area, and will help in the understanding of brown tide issues.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0025315416000205

ACKNOWLEDGEMENTS

We thank the captain and crews of the RV 'Dong Fang Hong 2' for their support and the splendid atmosphere on board. We thank the chemistry group for chemical analyses.

FINANCIAL SUPPORT

Support for this work was provided by the National Natural Science Foundation of China (41076088, 31500339); Public Science and Technology Research Funds Projects of Ocean (201205031); China Postdoctoral Science Foundation (2015M570612); The Project Sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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https://doi.org/10.1017/S0025315416000205 Published online by Cambridge University Press