Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 49, No. 1, March 2020
pages (23-33)

ISSN 1730-413X
eISSN 1897-3191

Diversity and biogeography of picoplankton communities from the Straits of Malacca to the South China Sea

by

Zhao-Yu Jiang[1,2,*],
Fu-Lin Sun[2]

DOI: **10.1515/ohs-2020-0003**
Category: **Original research paper**
Received: **April 25, 2019**
Accepted: **August 26, 2019**

[1]*College of Life Sciences, Linyi University, Linyi 276000, China*

[2]*State Key Laboratory of Tropical Oceanography, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China*

* Corresponding author: *jiangzhaoyu@lyu.edu.cn*

Abstract

Marine picoplankton, including prokaryotic and eukaryotic picoplankton, drive many biogeochemical processes, such as carbon, nitrogen and sulfur cycles, making them crucial to the marine ecosystem. Despite the fact that picoplankton is prevalent, its diversity and spatial distribution from the Straits of Malacca (SM) to the South China Sea (SCS) remain poorly investigated. This work explores the phylogenetic diversity and community structure of picoplankton in relation to environmental factors from the SM to the SCS. To this end, the Illumina MiSeq sequencing technique was applied to 16S and 18S rRNA genes. The results showed significant differences in the dynamics of picoplankton between the open sea and the strait region. *Proteobacteria* and *Cyanobacteria* constituted a larger part of the prokaryotic group. Within *Cyanobacteria*, the abundance of *Prochlorococcus* in the open sea was significantly higher than that of *Synechococcus*, while the opposite trend was observed in the strait. *Dinoflagellata*, *Cnidaria*, *Retaria*, *Tunicata*, and *Arthropoda* dominated among the eukaryotic taxa. High-throughput sequencing data indicated that salinity, temperature and NO_2-N were the key factors determining the prokaryotic community structure, while temperature and dissolved oxygen determined the eukaryotic community structure in the studied region. The network analysis demonstrated that the cooperation and competition were also important factors affecting the picoplankton community.

Key words: Picoplankton, Illumina MiSeq sequencing, 16S rRNA gene, 18S rRNA gene, Straits of Malacca, South China Sea

DE GRUYTER

The *Oceanological and Hydrobiological Studies* is online at www.oandhs.pl

Introduction

Picoplankton between 0.2 and 3 µm in diameter, including eukaryotic picophytoplankton, picozooplankton and heterotrophic bacteria, are essential components of plankton communities in all aquatic ecosystems (Flombaum et al. 2013; Mena et al. 2016). Picophytoeukaryotes, *Prochlorococcus* and *Synechococcus*, constitute most of the picoplankton, which drive the biogeochemical cycles of biogenic elements in the marine ecosystem, such as carbon and nitrogen cycling. These tiny primary producers significantly contribute to both primary production and biomass in open ocean regions, especially in oligotrophic waters, where they can reach their highest abundance up to 10^6 cells ml^{-1} (Zhang et al. 2008; Buitenhuis et al. 2012; Tamm et al. 2018) and account for 50–80% of the total carbon production and up to 50% of the biomass (Richardson & Jackson 2007). Different ambient conditions may lead to varying phylogenetic composition of picoplankton, which plays multiple ecological functions in biogeochemical cycles in the ocean (De Vargas et al. 2015; del Campo et al. 2016; Xenopoulos et al. 2017). Therefore, the identification of potential environmental factors affecting the abundance and community composition of picoplankton is currently one of the major challenges in marine microbial ecology.

The South China Sea (SCS), one of the largest marginal seas in the world, has rich biological resources (Jiang et al. 2014). The SCS is located in the subtropical and tropical northwest Pacific Ocean and the southern SCS is connected with the Andaman Sea and the Indian Ocean through the Straits of Malacca (SM), which are among the busiest international sea routes (Jiang et al. 2014; Wang et al. 2007). The northern SCS is characterized by significant environmental gradients owing to the discharge of the Pearl River and is affected by many types of physical forcing, such as monsoons, perennial cold cyclonic eddies, Taiwan Shoals, the Kuroshio Current and others (Morton & Blackmore 2001; Chen et al. 2006; Lin et al. 2011; Ning et al. 2004; Jiang et al. 2014). Waters of the SCS and the SM are significantly affected by the Asian monsoon.

Despite the large number of ecological researches on picoplankton in multifarious waters of the Pacific Ocean (Moon-van der Staay et al. 2000; Mackinson et al. 2015; Rii et al. 2016; Liang et al. 2017), the Atlantic Ocean (Schattenhofer et al. 2009; 2011; Zubkov et al. 2000), the Mediterranean Sea (Meador et al. 2010; Man-Aharonovich et al. 2010; Cerino et al. 2012) and the Arabian Sea (Fuchs et al. 1998), few studies have focused on the community structure of both eukaryotic and prokaryotic picoplankton in different marine regimes from the SM to the SCS along environmental gradients using the high-throughput sequencing technique.

Materials and methods

Sample collection

The study area extends from the Straits of Malacca to the South China Sea (1°N–21°N, 96°E–114°E). Surface seawater samples were collected from 12 locations during a multidisciplinary cruise carried out in the Eastern Indian Ocean by R/V Shiyan 3, the South China Sea Institute of Oceanology, Chinese Academy of Sciences, from 14 to 19 April 2017 (Fig. 1). The surface water for phylogenetic analysis (2 l) was filtered through a polycarbonate filter with a pore size of 0.22 µm (Millipore Isopore, Bedford, USA) after pre-filtration through a polycarbonate filter with a pore size of 3 µm (Millipore Durapore, Bedford, USA). The filters (0.22 µm pore size) were snap frozen in liquid nitrogen and then stored at –80°C in the laboratory until DNA extraction. Seawater for nutrient analysis was filtered through polycarbonate filters with a pore size of 0.45 µm, collected in acid-washed polyethylene containers and immediately frozen (–20°C) until analysis.

Physical and chemical analysis

Temperature, salinity and dissolved oxygen (DO) were measured at the sampling locations with a Sea-Bird SBE9 Conductivity-Temperature-Depth (CTD) profiler (USA). Chemical characteristics, including concentrations of nitrate (NO_3-N), nitrite (NO_2-N), ammonium (NH_4-N), silicate (SiO_3-Si) and phosphorus (PO_4-P) were analyzed according to Jiang et al. (2014, 2015).

Total community DNA extraction and sequencing analysis

Total genomic DNA was extracted from the filters using the PowerWater DNA Isolation Kit (MoBio, Carlsbad, CA, United States) according to the manufacturer's protocol. The DNA was stored at –80°C for subsequent analyses. The V4-V5 region of the 16S rRNA gene with the 515f/907r primer set (Xiong et al. 2014) and the V9 region of the 18S rRNA gene with the 1380f/1510r primer set (Amaral-Zettler et al. 2009) were selected for targeting amplicons. Next generation sequencing library preparations and Illumina MiSeq

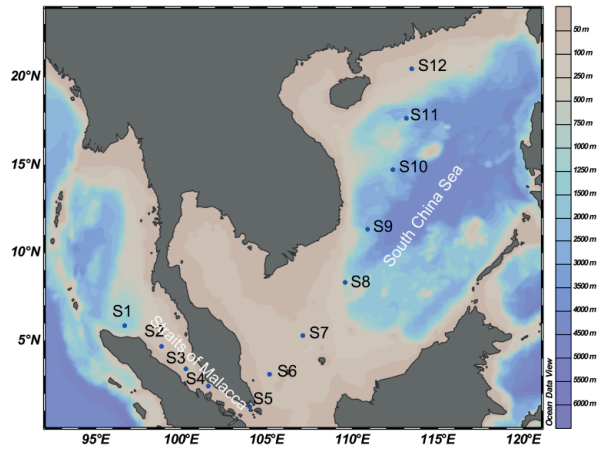

Figure 1

Location of the sampling sites

PE250 sequencing were conducted at the Novogene Company (Beijing, China). All sequences obtained in this study were deposited in the NCBI Sequence Read Archive (SRA) with accession numbers PRJNA492365 for the 16S rRNA gene and PRJNA492462 for the 18S rRNA gene.

Statistical analysis

The software package QIIME 1.9.1 (Caporaso et al. 2010) was used for 16S and 18S rRNA gene data analysis. The forward and reverse reads were derived from the original DNA fragments, which were merged by using FLASH (Magoč & Salzberg 2011) and assigned to a sample based on a barcode and truncated by cutting off the barcode and primer sequence. Sequences were grouped into operational taxonomic units (OTUs) using the clustering program VSEARCH 1.9.6 (Edgar 2010) at 97% sequence identity. Rarefaction analysis was conducted using the original detected OTUs. The taxonomic assignment was performed by the RDP classifier at a confidence threshold of 0.8 (Wang et al. 2007).

The Shannon index, the Chao1 index and Good's coverage of samples were determined as described by Schloss et al. 2009. Weighted and unweighted UniFrac, Bray–Curtis and principal coordinate analysis (PCoA) were performed by QIIME. The unweighted pair group method with arithmetic mean (UPGMA) clustering was conducted by unweighted and weighted UniFrac based on the protocol published by Caporaso et al. 2010.

Heatmap and redundancy analysis (RDA) was conducted using the R statistical package to determine the correlation between community composition and environmental parameters (Zhang et al. 2014).

Network analysis

Network analysis was performed according to Qin et al. (2010). Forty most abundant OTUs of picoplankton communities were selected and Spearman correlations were calculated. In order to reduce the network complexity, correlations r below −0.60 or above 0.60 (n = 162 and n = 128 for 16S and 18S rRNA gene data, respectively) were visualized in a graph using Cytoscape 2.8.2 (Shannon et al. 2003).

Results

Community composition

After quality filtering of the raw reads, a total of 901 199 16S rRNA gene sequences (on average 75 100 reads per sample) and 1 238 847 18S rRNA gene sequences (on average 103 237 reads per sample) from 12 samples were obtained and clustered into 1914 OTUs and 3931 OTUs for 16S and 18S rRNA genes (97% cutoff), respectively. The Shannon index indicated that eukaryotic picoplankton α-diversity was significantly higher (independent t-test and paired t-test; $p < 0.05$) than that of the prokaryotic picoplankton (Table 1).

In total, 34 prokaryotic phyla and 55 eukaryotic phyla were detected. The top five bacterial phyla accounted for more than 97.7% of the total abundance.

Table 1

Sequencing information and diversity index analyses

Site	OTU		Chao1		Shannon		Coverage	
	16S	18S	16S	18S	16S	18S	16S	18S
S1	1000	2341	977.50	2641.87	5.20	8.87	0.997	0.993
S2	1092	1920	1045.07	2233.97	6.38	7.91	0.997	0.994
S3	891	2069	1050.14	2496.52	4.32	7.78	0.996	0.993
S4	995	1593	957.65	1909.62	6.26	7.50	0.998	0.995
S5	762	1791	738.70	2226.80	4.20	7.60	0.997	0.993
S6	746	1872	781.84	2334.47	4.50	6.94	0.997	0.993
S7	991	2174	969.97	2615.01	5.30	7.94	0.997	0.993
S8	972	2388	936.86	2728.43	5.17	8.45	0.997	0.993
S9	878	1803	875.60	2270.83	4.90	5.98	0.997	0.993
S10	822	2146	811.78	2405.77	4.96	8.71	0.997	0.995
S11	693	1978	672.34	2301.63	4.31	7.20	0.998	0.994
S12	753	2111	746.50	2448.65	4.48	7.22	0.997	0.994

Proteobacteria (40.4%; α – 31.8%, δ – 4.8%) and *Cyanobacteria* (39.2%; *Prochlorococcus* – 23.1%, *Synechococcus* – 15.5%) were the most abundant prokaryotic group, followed by *Actinobacteria* (8.1%), *Bacteroidetes* (6.8%), *Firmicutes* (1.9%), SAR406 (1.4%; Fig. 2a). At sampling sites S3 and S5, *Cyanobacteria* reached the highest relative abundance, while the relative abundance of *Proteobacteria* was the lowest among the twelve sampling sites. At sampling sites S3 and S5, *Cyanobacteria* reached a higher relative abundance, while *Proteobacteria* were relatively less abundant among the twelve sampling sites. The top 10 eukaryotic phyla were: *Dinoflagellata* (8.9%), *Cnidaria* (8.2%), *Retaria* (8.1%), *Tunicata* (8.1%), *Arthropoda* (7.8%), *Protalveolata* (4.1%), *Ciliophora* (3.9%), *Ochrophyta* (2.4%), *Prymnesiophyceae* (1.5%) and MAST-3 (1.3%). *Cnidaria* and *Retaria* were the least abundant at site S3, whereas *Tunicata* reached the highest abundance at site S9.

OTU analyses and multi-sample comparisons

The results of the principal coordinate analysis (PCoA) based on weighted UniFrac distances revealed that most differences could be attributed to geographical location (Fig. 3). The first two PCoA axes explained more variance in the abundant prokaryotic communities (84.43%; Fig. 3a) than in the eukaryotic communities (42.36%; Fig. 3b). Samples from the SCS and site S1 were grouped together (upper side and right side of the PCoA plots, respectively), while samples from four other sampling sites of the SM were separated.

Based on the composition of the top 40 OTUs, samples were clustered into two groups (Fig. 4). The top 40 OTUs of the 16S RNA gene at sampling sites S3, S4 and S5 were clustered together into a subgroup and the remaining OTUs were included in another group (Fig. 4a). *Cyanobacteria*, especially *Synechococcus* (OTU4, OTU25, OTU59, OTU899 and OTU1805) were abundant in the SM. Different OTUs, even those associated with the same classification, showed different distribution patterns. Within *Proteobacteria*, the relative abundance of OTU2 and OTU1494 was higher in the SCS, but the relative abundance of OTU19, OTU28 and OTU29 was high at site S2 located in the SM. Sampling sites S4 and S5 were in the same cluster, other sampling sites were in the same group based on the top 40 OTUs of the 18S RNA gene (Fig. 4b). Each site featured several highly abundant OTUs. The relative abundance of OTU7, OTU11, OTU12, OTU13, OTU15 and OTU19 was higher in the SM than in the SCS. The relative abundance of OTU22, OTU25 and OTU35 was higher at site S7.

Relationships between picoplankton community structure and environmental variables

Redundancy analysis (RDA) was performed to identify possible correlations between environmental factors and picoplankton distribution (Fig. 5). Each environmental variable in the RDA biplot was represented by an arrow and the length of an individual arrow indicated how much variance was explained by that variable. The RDA analysis based on the prokaryotic picoplankton community composition was consistent with PCoA (Fig. 3a). Sampling sites S2, S3, S4 and S5 are located on the right-hand side of the RDA plot. However, the RDA models for eukaryotic picoplankton assemblages indicated that temperature

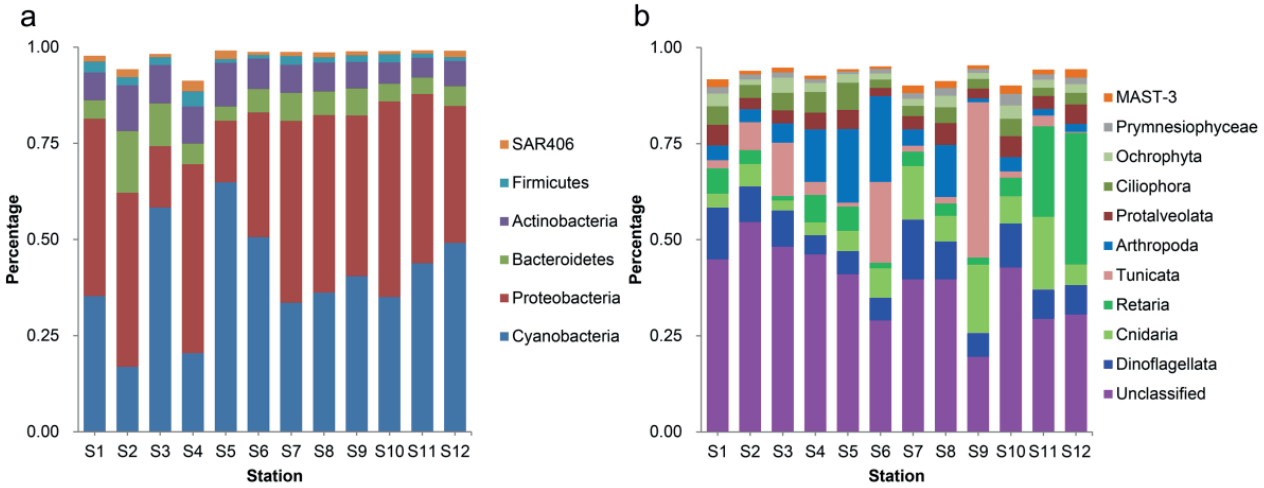

Figure 2

Picoplankton community composition at the phylum level: (a) prokaryotic phyla and (b) eukaryotic phyla

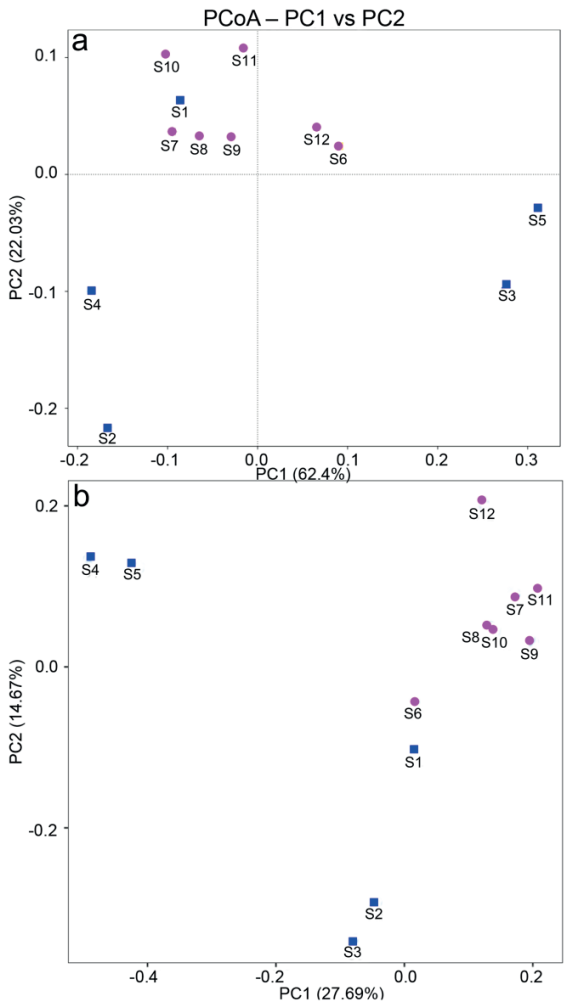

Figure 3

Weighted UniFrac principal coordinates analysis (PCoA) of picoplankton communities from the Straits of Malacca to the South China Sea; (a) prokaryotes and (b) eukaryotes

and DO (statistically significant environmental factors; $p < 0.05$) only partly accounted for the diversification of communities from the SM to the SCS (Fig. 3b). In the two RDA models of prokaryotic picoplankton and eukaryotic picoplankton, the environmental factors explained about 96.6 and 61.6% of the total variance in the community composition, respectively.

The RDA analysis revealed that salinity ($p < 0.01$), temperature ($p < 0.1$) and NO_2-N ($p < 0.1$) were statistically the most significant variables, explaining the pattern of the prokaryotic picoplankton community composition (Fig. 5a). However, temperature and DO concentrations were significant factors determining the composition of the eukaryotic picoplankton community ($p < 0.05$) (Fig. 5b).

Network analysis of the picoplankton community

As the whole networks of the total OTUs are too complicated to display, only a very limited number of key OTUs with higher abundance were considered. The top 40 OTUs were examined (Fig. 6). All curves of the network connectivity fitted to power-law model ($r < -0.60$ or $r > 0.60$). There are similarities in terms of network size and structure between 16S and 18S rRNA-based sequences. Prokaryotic taxa displayed closer interactions than the eukaryotic community (162 and 128 connections for 16S and 18S rRNA-based sequences, respectively). Positive connections (97/162 and 72/128 for 16S and 18S rRNA-based sequences, respectively) prevail in both prokaryotic and eukaryotic communities.

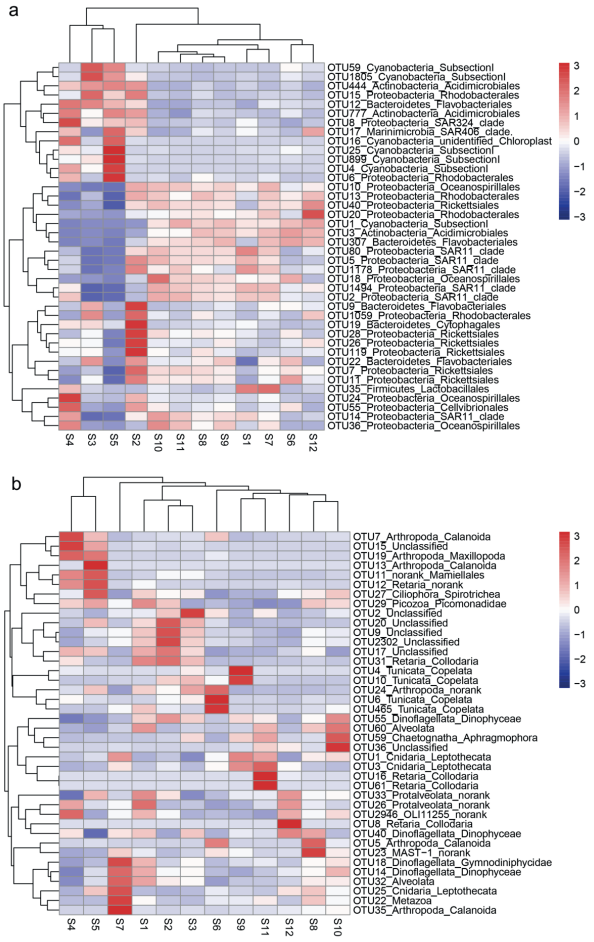

Figure 4

Heatmap of the 40 most abundant operational taxonomic units (OTUs) of picoplankton communities from the Straits of Malacca to the South China Sea; (a) prokaryotes and (b) eukaryotes

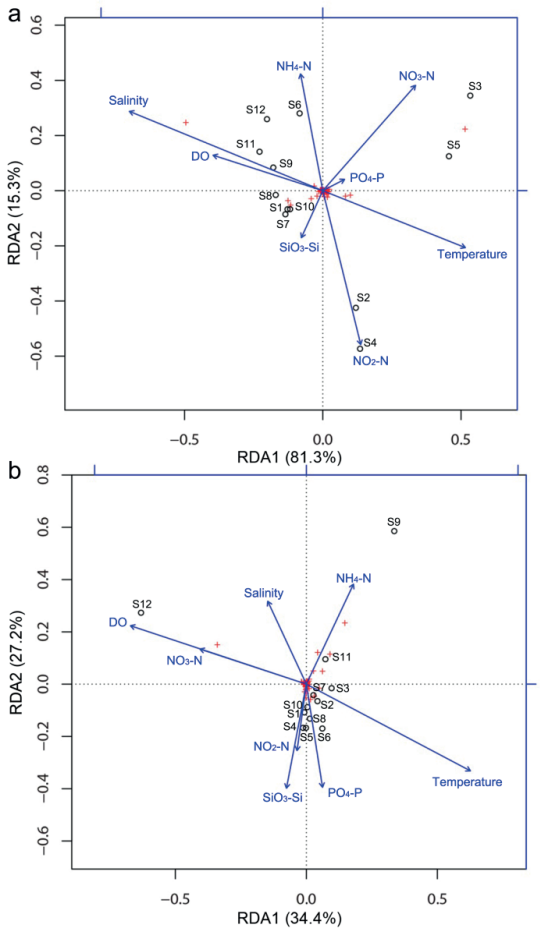

Figure 5

Redundancy analysis (RDA) ordination plot of environmental parameters and picoplankton communities; (a) prokaryotes and (b) eukaryotes

Discussion

Diversity and biogeography of picoplankton communities

In the present study, the Illumina MiSeq sequencing technology was employed to describe the diversity and biogeography of picoplankton (including prokaryote and eukaryote) communities from the SM to the SCS. The comparison of the community structure based on OTU percentages indicated a divergent distribution pattern of individual picoplanktic taxa.

The results showed that *Proteobacteria* and *Cyanobacteria* were the dominant phyla of prokaryotic picoplankton and *Alphaproteobacteria* accounted for more than 75% of *Proteobacteria* in the study area. According to previous studies and based on phylogenetic and phenotypic properties as well as strong adaptability to various marine environments (Sunagawa et al. 2015; Shi et al. 2018), *Proteobacteria* were identified as the most abundant bacterial phylum in all marine water masses (Suh et al. 2014; Sunagawa et al. 2015; Sun et al. 2015; Li et al. 2017; Shi et al. 2018).

Being important autotrophic picoprokaryotes, *Cyanobacteria* not only regulate the global primary productivity, but also drive carbon and nitrogen cycles in the marine ecosystem (Xia et al. 2015). *Synechococcus* and *Prochlorococcus* are the most abundant groups of picophytoplankton in the world's oceans and they have different ecological niches (Zwirglmaier et al. 2008; Xia et al. 2015). *Prochlorococcus* is the dominant

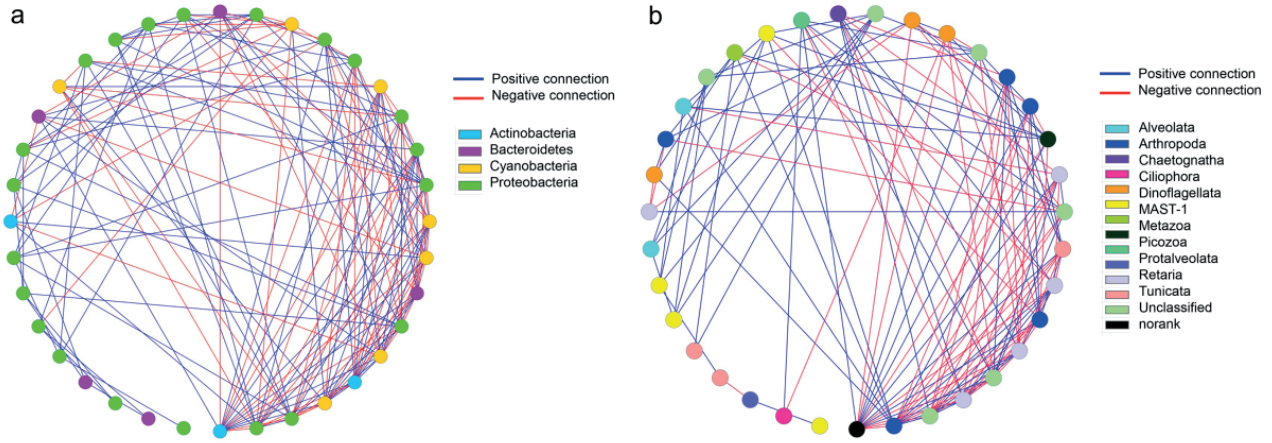

Figure 6

Network interactions of the top 40 OTUs (degree sorted circle layout); (a) prokaryotic picoplankton and (b) eukaryotic picoplankton

genus in oligotrophic areas, while *Synechococcus* inhabits mainly coastal mesotrophic and eutrophic environments, such as coastal waters and upwelling regions (Zwirglmaier et al. 2008; Xia et al. 2015). This study revealed that within *Cyanobacteria*, the abundance of *Prochlorococcus* in the open sea was significantly higher than that of *Synechococcus* ($p < 0.05$), while in the strait area the abundance of *Prochlorococcus* was significantly lower than that of *Synechococcus* ($p < 0.05$). Chen et al. (2011) mentioned that picophytoplankton might be restricted by high concentrations of nutrients and (or) heavy metals in the estuary and coastal areas of the northern SCS. In general, the open sea is usually characterized by oligotrophic conditions, while the strait area is highly eutrophicated by nutrients from terrestrial input and transportation. Therefore, the distribution of *Synechococcus* and *Prochlorococcus* showed varying characteristics from the SM to the SCS.

Five phyla – *Dinoflagellata, Cnidaria*, *Retaria*, *Tunicata* and *Arthropoda* – dominated in eukaryotic picoplankton from the SM to the SCS. We distinguished ten most abundant eukaryotic phyla and the results showed that only three of them (*Dinoflagellata*, *Ochrophyta* and *Prymnesiophyceae*) belonged to phytoplankton, while the remaining seven phyla were affiliated with zooplankton. Dinoflagellates are the major group of unicellular protists inhabiting both marine and freshwater environments (Price & Bhattacharya 2017). These aquatic protists account for the main part of the marine plankton and play an important role in the marine primary productivity, while being represented by some toxic species (Price & Bhattacharya 2017). They form the basis of food chains and are a large component of the food web in the marine ecosystem (Taylor et al. 2008; Sze et al. 2018). High diversity of dinoflagellates was detected by DNA metabarcoding using high-throughput sequencing in Singapore's waters (Sze et al. 2018). Annett et al. (2010) reported that both heterotrophic and autotrophic dinoflagellates contributed as much as 27% to the total phytoplankton biomass in Ryder Bay. The phylum *Cnidaria*, containing over 10 000 species of animals, is usually classified into five classes: *Anthozoa*, *Cubozoa*, *Hydrozoa*, *Scyphozoa* and *Staurozoa* (Kayal et al. 2013). They occur only in aquatic environments, mostly in marine ecosystems, and are ecologically important marine invertebrates (Kayal et al. 2013). *Cnidarians* are found from deep waters near hydrothermal vents to the polar seabed and tropical reefs (Rocha et al. 2015). The SCS features an extraordinary richness of reef corals (571 known species; Huang et al. 2015), due to the large number of Anthozoa (*Cnidaria*).

Environmental factors affecting the picoplankton community

In the present study, the weighted UniFrac PCoA indicated that picoplankton communities of prokaryotes and eukaryotes from the SM and the SCS belong to different groups. Samples from the SCS and site S1 were grouped together and placed on the opposite side of the four other sampling sites in the PCoA plots. Jiang et al. (2015) mentioned that geographical proximity is an important factor affecting the structure of phytoplankton communities from the Pearl River estuary to the SCS. Although site S1 was located in the SM mouth facing the Indian Ocean, the picoplankton communities exhibited characteristics of the open sea, such as the SCS in this study. It has been reported that sampling sites located near islands had a similar bacterial community (Ling et al. 2012). The distance between land and a sampling location may play an important role in the distribution of eukaryotic ultraplankton (Jiang et al. 2014). Therefore, the geographical location could influence the structure of picoplankton communities from the SM to the SCS.

RDA was conducted to further explore the relationships between the environmental factors and the community structure of prokaryotic and eukaryotic picoplankton. With regard to prokaryotes, the distribution of the sampling sites in the UniFrac PCoA analysis was consistent with the RDA results. Prokaryotic samples were clustered into two groups: the strait area (sampling sites S2–S5) and open waters (sampling sites S1, S6–S12). Temperature ($p < 0.01$) as well as NO_2-N ($p < 0.1$) and NO_3-N concentrations could affect the prokaryotic community structure in the SM, while salinity significantly affected it in the coastal and open waters. The relationship between the prokaryotic plankton community and environmental parameters varies in different waters (Sun et al. 2015; Suh et al. 2014; Shi et al. 2017; Halsey et al. 2017; Cram et al. 2015; Xia et al. 2015). Previous studies showed that the concentration of NO_3-N is not necessarily a limiting factor for the growth of bacteria in estuaries and coastal waters, but in oligotrophic waters it could be a major limiting factor affecting the bacterioplankton community due to its low concentration (Xia et al. 2015). In this study, sampling sites S2, S3, S4 and S5 located in the MS maintained a relatively higher temperature than other sampling sites due to anthropogenic influence and received a large amount of inorganic and organic nutrients originating from anthropogenic activities, whereas salinity was the key factor shaping the prokaryotic picoplankton communities at other sampling sites located in the open sea.

In terms of eukaryotic picoplankton, there was a discrepancy between UniFrac PCoA and RDA. This indicated that other variables, in addition to the eight selected environmental factors, may also contribute to the community cluster patterns, such as grazing. Further research is needed to identify specific factors. Environmental factors (temperature, DO, salinity, grazing and nutrient concentrations) can significantly affect the eukaryotic plankton community in the natural environment (Bernardi Aubry et al. 2013; Jiang et al. 2014; Suikkanen et al. 2007; Suzuki et al. 1997; Wu et al. 2014; De Vargas et al. 2015). Wu et al. (2014) reported that temperature and irradiance affected the picoeukaryotic distribution at the surface and 60 m sampling depth in the SCS ($p < 0.001$). Temperature, irradiance, nutrient concentrations and salinity were mainly correlated with changes in the main phytoplankton groups and the phytoplankton community composition (Xiao et al. 2018). Jiang et al. (2014) demonstrated that temperature, salinity, phosphorus and silicate had a significant impact on the community structure of eukaryotic ultraplankton of the northern SCS. This study indicated that temperature and DO played an important role in shaping the eukaryotic picoplankton community composition from the SM to the SCS ($p < 0.05$). Picophytoplankton (including *Synechococcus*, *Prochlorococcus* and picoeukaryotes) are usually dominant in waters characterized by high temperature and low content of nutrients (Agawin et al. 2000; Li 2002; Finkel et al. 2010; Moran et al. 2010; Chen et al. 2014). Temperature and DO affect the growth rate of picoplankton (Chen et al. 2014; Wu et al. 2014). Therefore, both prokaryotic and eukaryotic picoplankton communities were significantly influenced by temperature ($p < 0.01$ or $p < 0.05$).

Cooperation dominates in the interactions within prokaryotic and eukaryotic picoplankton communities

Complicated networks were constructed by a variety of species interacting with each other in the complex marine ecosystem (Montoya et al. 2006). The marine ecosystem performs the system-level functions (such as biogeochemical cycling, ecosystem stability) owing to these complicated network interactions, and the functions could not be fulfilled by individual populations (Zhou et al. 2010). Community network models were built to demonstrate the interactions between species. The determined competition or cooperation relationships between species were based on nutrients, space, material and information (Zhang et al. 2014). Although ecological networks are very important to the ecosystem, there are few researches on networks of the picoplankton community. Based on Fig. 6, we found that the network size and structure of the prokaryotic community was significantly different from that of the eukaryotic community. In general, the interaction of bacterioplankton was relatively close compared to eukaryotic picoplankton. The results also showed that the cooperation dominated the relationships of both prokaryotic and eukaryotic communities. This is in line with the previous study (Zhang et al. 2014), which has reported that positive connections dominated the interactions between taxa in the DNA-based networks of the total bacterioplankton in the SCS.

Conclusions

In the current study, we focused on the diversity and biogeographic patterns of picoplankton communities, including both prokaryotic and eukaryotic groups from the Straits of Malacca (SM) to the South China Sea (SCS). The results suggested that *Proteobacteria* and *Cyanobacteria* dominated in the prokaryotic group and *Dinoflagellata*, *Cnidaria*, *Retaria*, *Tunicata*, and *Arthropoda* dominated among the prokaryotic taxa. Within *Cyanobacteria*, the richness of *Prochlorococcus* was significantly higher compared to *Synechococcus* in the open sea, while the opposite relationship was observed in the strait area. Geographical location could affect the structure of picoplankton communities. Salinity, temperature and NO_2-N determined the community structure of prokaryotic picoplankton, whereas temperature and DO determined the community structure of eukaryotic picoplankton. The community network models further indicated that both prokaryotic and eukaryotic communities from the MS to the SCS experience high cooperation between each other to maintain high abundance in a long-term process of evolution. This study excluded grazing, therefore further experiments are required to determine the effect of grazing on the picoplankton.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 41506127) and the Project of Guangdong Science and Technology Department (No. 2017A020216008).

References

Amaral-Zettler, L.A., McCliment, E.A., Ducklow, H.W. & Huse, S.M. (2009). A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PloS one* 4(7): e6372. DOI: 10.1371/journal.pone.0006372.

Annett, A.L., Carson, D.S., Crosta, X., Clarke, A. & Ganeshram, R.S. (2010). Seasonal progression of diatom assemblages in surface waters of Ryder Bay, Antarctica. *Polar Biology* 33(1): 13–29. DOI: 10.1007/s00300-009-0681-7.

Buitenhuis, E.T., Li, W.K., Vaulot, D., Lomas, M.W., Landry, M.R. (2012). Picophytoplankton biomass distribution in the global ocean. *Earth System Science Data* 4(1): 37–46. DOI: 10.5194/essd-4-37-2012.

Campbell, B.J., Yu, L., Heidelberg, J.F., & Kirchman, D.L. (2011). Activity of abundant and rare bacteria in a coastal ocean. *Proceedings of the National Academy of Sciences* 108(31): 12776–12781. DOI: 10.1073/pnas.1101405108.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D. et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* 7(5): 335–336. DOI: 10.1038/nmeth.f.303.

Cerino, F., Aubry, F.B., Coppola, J., La Ferla, R., Maimone, G. et al. (2012). Spatial and temporal variability of pico-, nano- and microphytoplankton in the offshore waters of the southern Adriatic Sea (Mediterranean Sea). *Continental Shelf Research* 44: 94–105. DOI: 10.1016/j.csr.2011.06.006.

Chen, B., Liu, H., Huang, B. & Wang, J. (2014). Temperature effects on the growth rate of marine picoplankton. *Marine Ecology Progress Series* 505: 37–47. DOI: 10.3354/meps10773.

Chen, B., Wang, L., Song, S., Huang, B., Sun, J. et al. (2011). Comparisons of picophytoplankton abundance, size, and fluorescence between summer and winter in northern South China Sea. *Continental Shelf Research* 31(14): 1527–1540. DOI: 10.1016/j.csr.2011.06.018.

Chen, C.C., Shiah, F.K., Chung, S.W., & Liu, K.K. (2006). Winter phytoplankton blooms in the shallow mixed layer of the South China Sea enhanced by upwelling. *Journal of Marine Systems* 59(1–2): 97–110. DOI: 10.1016/j.jmarsys.2005.09.002.

Cram, J.A., Chow, C.E.T., Sachdeva, R., Needham, D.M., Parada, A.E. et al. (2015). Seasonal and interannual variability of the marine bacterioplankton community throughout the water column over ten years. *The ISME Journal* 9(3): 563–580. DOI: 10.1038/ismej.2014.153.

De Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F. et al. (2015). Eukaryotic plankton diversity in the sunlit ocean. *Science* 348(6237): 1261605. DOI: 10.1126/science.1261605.

del Campo, J., Guillou, L., Hehenberger, E., Logares, R., López-García, P. et al. (2016). Ecological and evolutionary significance of novel protist lineages. *European Journal of Protistology* 55: 4–11. DOI: 10.1016/j.ejop.2016.02.002.

Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19): 2460–2461. DOI: 10.1093/bioinformatics/btq461.

Flombaum, P., Gallegos, J.L., Gordillo, R.A., Rincón, J., Zabala, L.L. et al. (2013). Present and future global distributions of the marine Cyanobacteria Prochlorococcus and Synechococcus. *Proceedings of the National Academy of Sciences* 110(24): 9824–9829. DOI: 10.1073/pnas.1307701110.

Fuchs, B.M., Woebken, D., Zubkov, M.V., Burkill, P., & Amann, R. (2005). Molecular identification of picoplankton populationsin contrasting waters of the Arabian Sea. *Aquatic Microbial Ecology* 39(2): 145–157. DOI: 10.3354/ame039145.

Halsey, K.H., Giovannoni, S.J., Graus, M., Zhao, Y., Landry, Z. et al. (2017). Biological cycling of volatile organic carbon by phytoplankton and bacterioplankton. *Limnology and Oceanography* 62(6): 2650–2661. DOI: 10.1002/lno.10596.

Huang, D., Licuanan, W.Y., Hoeksema, B.W., Chen, C.A., Ang, P.O. et al. (2015). Extraordinary diversity of reef corals in the South China Sea. *Marine Biodiversity* 45(2): 157–168. DOI: 10.1007/s12526-014-0236-1.

Jiang, Z.Y., Wang, Y.S., Cheng, H., Sun, C.C., & Wu, M.L. (2015). Variation of phytoplankton community structure from the Pearl River estuary to South China Sea. *Ecotoxicology* 24(7–8): 1442–1449. DOI: 10.1007/s10646-015-1494-9.

Jiang, Z.Y., Wang, Y.S., & Sun, F.L. (2014). Spatial structure of eukaryotic ultraplankton community in the northern South China Sea. *Biologia* 69(5): 557–565. DOI: 10.2478/s11756-014-0361-0.

Kayal, E., Roure, B., Philippe, H., Collins, A.G. & Lavrov, D.V. (2013). Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology* 13(1): 5. DOI: 10.1186/1471-2148-13-5.

Li, J., Jiang, X., Li, G., Jing, Z., Zhou, L. et al. (2017). Distribution of picoplankton in the northeastern South China Sea with special reference to the effects of the Kuroshio intrusion and the associated mesoscale eddies. *Science of the Total Environment* 589: 1–10. DOI: 10.1016/j.scitotenv.2017.02.208.

Liang, Y., Zhang, Y., Zhang, Y., Luo, T., Rivkin, R.B. et al. (2017). Distributions and relationships of virio-and picoplankton in the epi-, meso- and bathypelagic zones of the Western Pacific Ocean. *FEMS Microbiology Ecology* 93(2): fiw238. DOI: 10.1093/femsec/fiw238.

Lin, L., Wang, Y.S., Sun, C.C., Li, N., Wang, H. et al. (2011). Demonstration of a new indicator for studying upwelling in the northern South China Sea. *Oceanologia* 53(2): 605–622. DOI: 10.5697/oc.53-2.605.

Ling, J., Dong, J.D., Wang, Y.S., Zhang, Y.Y., Deng, C. et al. (2012). Spatial variation of bacterial community structure of the Northern South China Sea in relation to water chemistry. *Ecotoxicology* 21(6): 1669–1679. DOI: 10.1007/s10646-012-0941-0.

Mackinson, B.L., Moran, S.B., Lomas, M.W., Stewart, G.M., & Kelly, R.P. (2015). Estimates of micro-, nano-, and picoplankton contributions to particle export in the northeast Pacific. *Biogeosciences* 12(11): 3429–3446. DOI: 10.5194/bg-12-3429-2015.

Magoč, T. & Salzberg, S.L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27(21): 2957–2963. DOI: 10.1093/bioinformatics/btr507.

Man-Aharonovich, D., Philosof, A., Kirkup, B.C., Le Gall, F., Yogev, T. et al. (2010). Diversity of active marine picoeukaryotes in the Eastern Mediterranean Sea unveiled using photosystem-II psbA transcripts. *The ISME Journal* 4(8): 1044–1052. DOI: 10.1038/ismej.2010.25.

Meador, T.B., Gogou, A., Spyres, G., Herndl, G.J., Krasakopoulou, E. et al. (2010). Biogeochemical relationships between ultrafiltered dissolved organic matter and picoplankton activity in the Eastern Mediterranean Sea. *Deep Sea Research Part II: Topical Studies in Oceanography* 57(16): 1460–1477. DOI: 10.1016/j.dsr2.2010.02.015.

Mena, C., Reglero, P., Ferriol, P., Torres, A.P., Aparicio-González, A. et al. (2016). Prokaryotic picoplankton spatial distribution during summer in a haline front in the Balearic Sea, Western Mediterranean. *Hydrobiologia* 779(1): 243–257. DOI: 10.1007/s10750-016-2825-4.

Montoya, J.M., Pimm, S.L., & Solé, R.V. (2006). Ecological networks and their fragility. *Nature* 442(7100): 259–264. DOI: 10.1038/nature04927.

Moon-van der Staay, S.Y., De Wachter, R. & Vaulot, D. (2001). Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. *Nature* 409(6820): 607–610. DOI: 10.1038/35054541.

Morton, B. & Blackmore, G. (2001). South China Sea. *Marine Pollution Bulletin* 42(12): 1236–1263. DOI: 10.1016/S0025-326X(01)00240-5.

Ning, X.R., Chai, F., Xue, H., Cai, Y., Liu, C. et al. (2004). Physical-biological oceanographic coupling influencing phytoplankton and primary production in the South China Sea. *Journal of Geophysical Research: Oceans* 109(C10): C1005. DOI: 10.1029/2004JC002365.

Price, D.C. & Bhattacharya, D. (2017). Robust Dinoflagellata phylogeny inferred from public transcriptome databases. *Journal of Phycology* 53(3): 725–729. DOI: 10.1111/jpy.12529.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S. et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464(7285): 59–65. DOI: 10.1038/nature08821.

Richardson, T.L., & Jackson, G.A. (2007). Small phytoplankton and carbon export from the surface ocean. *Science* 315(5813): 838–840. DOI: 10.1126/science.1133471.

Rii, Y.M., Duhamel, S., Bidigare, R.R., Karl, D.M., Repeta, D.J. et al. (2016). Diversity and productivity of photosynthetic picoeukaryotes in biogeochemically distinct regions of the South East Pacific Ocean. *Limnology and Oceanography* 61(3): 806–824. DOI: 10.1002/lno.10255.

Rocha, J., Calado, R. & Leal, M. (2015). Marine Bioactive Compounds from Cnidarians. In: S.K. Kim (Ed.) *Springer Handbook of Marine Biotechnology* (pp. 823–849). Springer, Berlin, Heidelberg.

Schattenhofer, M., Fuchs, B.M., Amann, R., Zubkov, M.V., Tarran, G.A. et al. (2009). Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean. *Environmental Microbiology* 11(8): 2078–2093. DOI: 10.1111/j.1462-2920.2009.01929.x.

Schattenhofer, M., Wulf, J., Kostadinov, I., Glöckner, F.O., Zubkov, M.V. et al. (2011). Phylogenetic characterisation of picoplanktonic populations with high and low nucleic acid content in the North Atlantic Ocean. *Systematic and Applied Microbiology* 34(6): 470–475. DOI: 10.1016/j.syapm.2011.01.008.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M. et al. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75(23): 7537–7541. DOI: 10.1128/AEM.01541-09.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T. et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research* 13(11): 2498–2504. DOI: 10.1101/gr.1239303.

Shi, R., Li, J., Qi, Z., Zhang, Z., Liu, H. et al. (2018) Abundance and community composition of bacterioplankton in the Northern South China Sea during winter: geographic position and water layer influences. *Biologia* 73(2): 197–206. DOI: 10.2478/s11756-018-0023-8.

Suh, S.S., Park, M., Hwang, J., Lee, S., Chung, Y. et al. (2014). Distinct patterns of marine bacterial communities in the South and North Pacific Oceans. *Journal of Microbiology* 52(10): 834–841. DOI: 10.1007/s12275-014-4287-6.

Sun, F.L., Wang, Y.S., Wu, M.L., Sun, C.C. & Cheng, H. (2015). Spatial and vertical distribution of bacterial community in the northern South China Sea. *Ecotoxicology* 24(7–8): 1478–1485. DOI: 10.1007/s10646-015-1472-2.

Sunagawa, S., Coelho, L.P., Chaffron, S., Kultima, J.R., Labadie, K. et al. (2015). Structure and function of the global ocean microbiome. *Science* 348(6237): 1261359. DOI: 10.1126/science.1261359.

Sze, Y., Miranda, L.N., Sin, T.M., & Huang, D. (2018). Characterising planktonic dinoflagellate diversity in Singapore using DNA metabarcoding. *Metabarcoding and Metagenomics* 2: e25136. DOI: 10.3897/mbmg.2.25136.

Tamm, M., Laas, P., Freiberg, R., Nõges, P. & Nõges, T. (2018). Parallel assessment of marine autotrophic picoplankton using flow cytometry and chemotaxonomy. *Science of the Total Environment* 625: 185–193. DOI: 10.1016/j.scitotenv.2017.12.234.

Taylor, F.J.R., Hoppenrath, M., & Saldarriaga, J.F. (2008).

Dinoflagellate diversity and distribution. *Biodiversity and Conservation* 17(2): 407–418. DOI: 10.1007/s10531-007-9258-3.

Wang, G., Chen, D. & Su, J. (2008). Winter eddy genesis in the eastern South China Sea due to orographic wind jets. *Journal of Physical Oceanography* 38(3): 726–732. DOI: 10.1175/2007JPO3868.1.

Wang, Q., Garrity, G.M., Tiedje, J.M., & Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* 73(16): 5261–5267. DOI: 10.1128/AEM.00062-07.

Wu, W., Huang, B., Liao, Y. & Sun, P. (2014). Picoeukaryotic diversity and distribution in the subtropical–tropical South China Sea. *FEMS Microbiology Ecology* 89(3): 563–579. DOI: 10.1111/1574-6941.12357.

Xenopoulos, M.A., Downing, J.A., Kumar, M.D., Menden-Deuer, S. & Voss, M. (2017). Headwaters to oceans: Ecological and biogeochemical contrasts across the aquatic continuum. *Limnology and Oceanography* 62(S1): S3–S14. DOI: 10.1002/lno.10721.

Xia, X., Guo, W. & Liu, H. (2015). Dynamics of the bacterial and archaeal communities in the Northern South China Sea revealed by 454 pyrosequencing of the 16S rRNA gene. *Deep Sea Research Part II: Topical Studies in Oceanography* 117: 97–107. DOI: 10.1016/j.dsr2.2015.05.016.

Xiao, W., Wang, L., Laws, E., Xie, Y., Chen, J. et al. (2018). Realized niches explain spatial gradients in seasonal abundance of phytoplankton groups in the South China Sea. *Progress in Oceanography* 162: 223–239. DOI: 10.1016/j.pocean.2018.03.008.

Xiong, J., Ye, X., Wang, K., Chen, H., Hu, C. et al. (2014). Biogeography of the sediment bacterial community responds to a nitrogen pollution gradient in the East China Sea. *Applied and Environmental Microbiology* 80(6): 1919–1925. DOI: 10.1128/AEM.03731-13.

Zhang, Y., Jiao, N. & Hong, N. (2008). Comparative study of picoplankton biomass and community structure in different provinces from subarctic to subtropical oceans. *Deep Sea Research Part II: Topical Studies in Oceanography* 55(14–15): 1605–1614. DOI: 10.1016/j.dsr2.2008.04.014.

Zhang, Y., Zhao, Z., Dai, M., Jiao, N. & Herndl, G.J. (2014). Drivers shaping the diversity and biogeography of total and active bacterial communities in the South China Sea. *Molecular Ecology* 23(9): 2260–2274. DOI: 10.1111/mec.12739.

Zhou, J., Deng, Y., Luo, F., He, Z., Tu, Q. et al. (2010). Functional molecular ecological networks. *MBio* 1(4): e00169-10. DOI: 10.1128/mBio.00169-10.

Zubkov, M.V., Sleigh, M.A., & Burkill, P.H. (2000). Assaying picoplankton distribution by flow cytometry of underway samples collected along a meridional transect across the Atlantic Ocean. *Aquatic Microbial Ecology* 21(1): 13–20. DOI: 10.3354/ame021013.

Zwirglmaier, K., Jardillier, L., Ostrowski, M., Mazard, S., Garczarek, L. et al. (2008). Global phylogeography of marine Synechococcus and Prochlorococcus reveals a distinct partitioning of lineages among oceanic biomes. *Environmental Microbiology* 10(1): 147–161.