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Diurnal changes in bacterial communities in oxic surface and hypoxic middle seawater layers of the Changjiang River Estuary

Yan Huang[1], Lei Yuan[1], Yingping Fan[1], Habib U Rehman Jakhrani[1], Jianxin Wang[1]*

[1] Marine Microorganism Ecological & Application Laboratory, Zhejiang Ocean University, Zhoushan 316022, China

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Abstract

The Changjiang River Estuary (CRE) in the East China Sea suffers from seasonal hypoxia in summer. The vertical distributions and seasonal changes of microbial communities in the CRE were well documented. However, little is known about the diurnal changes of bacterial communities in the hypoxic zone of the CRE. Here, 16S rRNA gene analysis was used to explore the changes of bacterial communities in the oxic surface and hypoxic middle seawater layers during 24 h in the CRE. Significant differences between the hypoxic and oxic layers were observed: the phyla Cyanobacteria, Bacteroidetes and Acidimicrobiia were enriched in the oxic layer, whereas the phylum SAR406 and the class Deltaproteobacteria were more abundant in the hypoxic layer. In addition, some subtle diurnal variations of the bacterial relative abundance were found in both two layers. The relative abundance of *Synechococcus* increased at night, and this change was more obvious in the hypoxic layer. The similar trend was also found in some phototrophic and several heterotrophic bacteria, such as Rhodobacteraceae, OM60 and Flavobacteriaceae. Their relative abundances peaked at 16:00 in the oxic layer, while the relative abundances peaked at around 7:00 and decreased until 13:00 in the hypoxic layer. Together, the results of the present study suggest that some photosynthetic bacteria and several heterotrophic bacteria have similar diurnal variations implying the light and physicochemical heterogeneity in the course of a day are important for bacterial diurnal changes in the CRE.

Key words: bacterial communities, diurnal changes, hypoxic zone, the Changjiang River Estuary

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1 Introduction

Marine hypoxic zones are regions with dissolved oxygen (DO) concentration below 2 mg/L (Diaz, 2001; Rabalais and Turner, 2001; Chen et al., 2007; Chi et al., 2017). Hypoxia has become an environmental problem of public concern, because of its damage of the structure and function of ecosystems and its increasing number (Conley et al., 2011). For example, hypoxia usually destroys coastal ecosystems and affects fisheries through food web interactions (Zhu et al., 2011). In addition, hypoxia can change the natural redox conditions, then impacts the material cycles (Turner et al., 2008; Bianchi and Allison, 2009).

The Changjiang River Estuary (CRE) located offshore from the mouth of the Changjiang River is a region that has complex hydrological conditions (Yang et al., 2012). It is also strongly influenced by water masses and ocean currents. The CRE and its adjacent waters are attacked by the Changjiang Diluted Water (CDW), the Taiwan Warm Current (TWC), terrestrial riverine runoff and the Kuroshio branch in summer and suffer from eutrophication for decades (Zhang et al., 1999). Interestingly, seasonal hypoxia (up to 10 000 km^2) is easily formed in near-bottom water in the region off the CRE in summer (Li et al., 2002; Zhu et al., 2011), which may be caused by freshwater input, thermal warming and the decomposition of deposited organic matter from rivers mediated by microorganisms (Diaz and Rosenberg, 2008; Lohrenz et al., 2008; Grenz et al., 2010).

Low DO or hypoxia, can cause significant disturbances on the composition of microbial loop and changes in biogeochemical cycles (Naqvi et al., 2000), resulting from the indispensable role of oxygen in aerobic metabolism (Sato et al., 2016). How bacterial communities respond to hypoxia is vital for understanding the structure and function of estuarine ecosystems. Up to now, several studies on bacterial communities in the CRE hypoxic zones have been reported (Liu et al., 2012; Ye et al., 2016; Wu et al., 2019). Distinct microbial community differences were observed between hypoxic and oxic zones. For example, Alphaproteobacteria, SAR406 and Deltaproteobacteria were more abundant taxonomic groups in the hypoxic zones (Wu et al., 2019). Recently, a study explored the temporal distribution of bacterial communities of seawater in the CRE hypoxic area in June, August and October (Liu et al., 2012). They found that Gammaproteobacteria, Cytophaga–Flavobacteriia–Bacteroides (CFB), Deltaproteobacteria, Cyanobacteria and Firmicutes are the dominant groups, and seasonal environmental heterogeneity may be the reason for the shift of bacterial communities in the three

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*Corresponding author, E-mail: jxwang@zjou.edu.cn

months. However, knowledge of variations in bacterial communities during a short-term period (e.g., 1 d) in the CRE is still scarce. Only the diurnal variations of environmental factors and phytoplankton distributions have been studied in the CRE (Gao and Song, 2005; Gao et al., 2007; Yan et al., 2012; Lou and Hu, 2014).

There have been indications that bacteria are influenced by short-term fluctuation of growth conditions (e.g., light, temperature and nutrients) in marine waters (Suyama et al., 2002; Ruiz-González et al., 2012; García et al., 2018). Thus far, circadian rhythms of algal growth, bacterial gene transcription and gene expression have been well studied in open ocean sites (Vaulot et al., 1995; Gilbert et al., 2010; Ottesen et al., 2013, 2014), seafloor slopes (Fuhrman et al., 1985) and coastal waters (Gilbert et al., 2010; Ottesen et al., 2014). Like most phytoplankton, phototrophic *Cyanobacteria* was mostly studied with regards to diurnal changes in various aspects. For example, Lefort and Gasol (2014) found that *Synechococcus* grew during the light period and divided at night, whereas *Prochlorococcus* displayed the opposing pattern in winter in the coastal waters of the Northwest Mediterranean Sea. In addition, anaerobic photosynthetic bacteria, performing photosynthesis with sulfide, hydrogen or organic substrates, were reported to have higher viability of cells under light conditions than in dark conditions without nutrients (Suyama et al., 2002). Photosynthetic release and excretion by grazing activities are considered to be the main sources of dissolved organic matter (DOM) for most marine bacteria in oceanic environments (Suyama et al., 2002), and the generation of DOM is usually synchronized with the circadian cycle (Johan et al., 1990), which may lead to some heterotrophic groups showing corresponding variations.

In view of close associations within microbial loop (Porter, 1996), different preferences for organic substrates or phytoplankton species (Pinhassi et al., 2004; Alonso-Sáez and Gasol, 2007), and different sensitivities to sunlight or phototrophic abilities (Béjà et al., 2000; Kolber et al., 2000; Alonso-Sáez et al., 2006), this study assumes that bacterial groups are associated with the environmental conditions and might display different amplitudes and rhythms in their diurnal variations in the oxic and hypoxic layers in the CRE. In this study, the assumption by exploring the variation of bacterial community composition and diversity using 16S rRNA gene analysis was tried to be examine.

2 Materials and methods

2.1 *Sample collection*

During a summer cruise in July 2016, a total of 18 seawater samples were collected every three hours from 16:00 (July 22, 2016) to 16:00 (July 23, 2016) at the same geographical location (Station D3, 30°59.39′N, 122°46.26′E) in CRE (Fig. 1). Seawaters from the oxic surface layer (1.95–2.1 m in depth, abbreviated as S) and the hypoxic middle layer (9.95–10.0 m in depth, abbreviated as M) were respectively collected at each time node (Table 1). For each sample, 2 L seawater was collected using Niskin bottles mounted to an SBE32 CTD (Sea-Bird Electronics, USA). In order to remove large organisms and particles, each water sample was

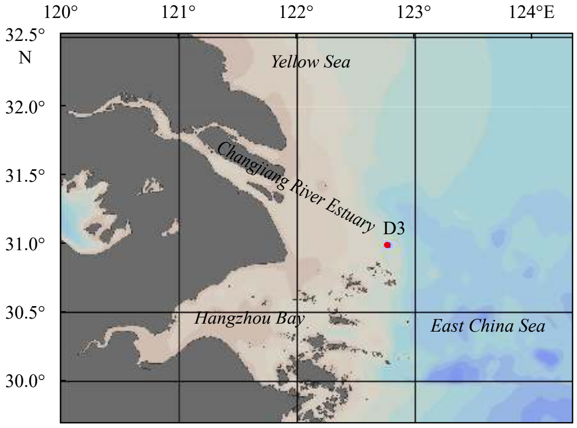

Fig. 1. The study area near the Changjiang River Estuary. The red dot named D3 is the sampling site.

Table 1. Environmental and physicochemical factors of the seawater from surface and middle layers of the 18 samples at Station D3 in the Changjiang River Estuary

Sample ID	Date (mm/dd/yy)	Time	Depth /m	S	T/°C	pH	DO /(mg·L^{-1})	PO$_4^{3-}$ /(μmol·L^{-1})	COD /(μmol·L^{-1})	NO$_2^-$ /(μmol·L^{-1})	NO$_3^-$ /(μmol·L^{-1})	SO$_4^{2-}$ /(μmol·L^{-1})
S16_1	7/22/16	16:00	2.005	21.70	24.78	7.78	3.67	0.87	0.45	0.74	47.28	56.55
S19	7/22/16	19:00	1.997	20.75	26.35	7.76	3.98	1.05	0.61	0.60	51.12	59.30
S22	7/22/16	22:00	1.982	21.43	25.94	7.97	5.52	0.46	0.79	0.98	44.38	59.41
S01	7/23/16	1:00	1.986	22.35	25.09	7.98	5.39	0.38	0.81	0.83	51.83	58.85
S04	7/23/16	4:00	1.962	24.10	23.88	7.74	3.32	0.79	0.42	0.64	42.10	56.04
S07	7/23/16	7:00	1.973	21.17	24.02	7.63	2.04	0.90	0.31	0.68	35.25	50.43
S10	7/23/16	10:00	1.987	23.05	24.97	7.77	3.66	0.80	0.78	0.94	39.75	61.32
S13	7/23/16	13:00	1.975	21.61	26.20	7.78	4.11	0.76	0.57	0.80	42.85	60.08
S16_2	7/23/16	16:00	1.951	21.95	25.63	7.78	3.77	0.83	0.42	0.81	42.31	55.03
M16_1	7/22/16	16:00	9.934	30.04	21.52	7.69	2.08	0.85	0.20	0.52	31.24	38.41
M19	7/22/16	19:00	9.944	31.56	20.86	7.68	1.44	0.83	0.33	0.43	23.80	35.49
M22	7/22/16	22:00	9.896	32.68	20.51	7.68	1.31	1.02	0.10	0.36	20.52	33.02
M01	7/23/16	1:00	9.936	32.48	20.55	7.68	1.36	0.84	0.10	0.26	21.61	32.79
M04	7/23/16	4:00	9.922	31.08	21.02	7.65	1.64	0.93	0.13	0.36	26.95	38.41
M07	7/23/16	7:00	9.901	31.98	20.76	7.63	1.41	0.86	0.22	0.40	25.84	34.70
M10	7/23/16	10:00	9.969	30.82	21.16	7.63	1.39	0.78	0.12	0.75	35.11	49.19
M13	7/23/16	13:00	9.926	30.51	21.25	7.64	1.63	0.94	0.07	0.35	25.67	28.64
M16_2	7/23/16	16:00	9.941	31.06	21.14	7.67	1.68	0.96	0.10	0.54	26.35	39.31

Note: S and M in the sample IDs represent surface and middle layers, respectively; DO, dissolved oxygen; COD, chemical oxygen demand; S, salinity; T, temperature.

pre-filtered through a filter with 3 μm pore size (with a gentle vacuum pressure less than 33.3 kPa) and then filtered through a 0.22 μm filter (Millipore Corporation, USA) to collect the free-living microbial cells. Samples were temporarily stored at –20°C on board, then were transferred to –80°C in the laboratory until the extraction of DNA. The samples were grouped according to the sampling time into two groups: Day (6:00–18:00, abbreviated as D) and Night (18:00–24:00 and 0:00–6:00, abbreviated as N) (Ottesen et al., 2014; García et al., 2018). Each sample was named using the abbreviation of the layer, followed by the sampling time number. For example, the S19 sample was taken at 19:00 from the oxic surface seawater. The S16_1 represents the surface sample collected at 16:00 on the first day.

2.2 *Physicochemical measurements of seawater*

For each sample, the physicochemical parameters of seawater, including depth, salinity and temperature, were documented using the CTD *in situ*. Other parameters of seawater, including the concentrations of nitrate (NO_3^-), nitrite (NO_2^-), chemical oxygen demand (COD), silicate (SiO_3^{2-}), phosphate (PO_4^{3-}) and DO, were measured according to the General Administration of Quality Supervision Inspection and Quarantine and Standardization Administration of China (2007) prior to chemical parameter analysis (Wu et al., 2019). NO_3^- was determined using the copper-cadmium column reduction method. NO_2^- was determined using the diazo coupling method. COD was determined using the alkaline potassium permanganate method. SiO_3^{2-} was determined using the silicon molybdenum blue method. PO_4^{3-} was determined using the phosphorus molybdenum blue method. The DO value was determined using the iodometric method. According to the definition of hypoxia, the middle layer samples could be defined as hypoxic (DO$\leqslant$2 mg/L), whereas the surface samples could be defined as oxic (DO>2 mg/L).

2.3 *Microbial sampling and DNA extraction*

The DNA of all the samples was extracted using a FastDNA spin kit for soil (MP Biomedicals, USA), according to the manufacturer's instruction. The DNA extracts were quantified using a NanoDrop ND2000 (Thermo Fisher Scientific, USA) and were subsequently submitted to Biozeron Co. (China) for 16S rRNA gene amplification (V4–V5 region) using dual-indexed bacterial barcoded primer pairs 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) (Stubner, 2002). Before performing PCR, the barcode sequences were linked to the primers during primer synthesis. The PCR was performed under the following program: denaturation at 95°C for 3 min, followed by 35 cycles of amplification (95°C for 30 s, 55°C for 30 s and 72°C for 30 s) and a final extension of 10 min at 72°C. Finally, purified PCR products were sequenced on the Illumina HiSeq platform. The raw sequence data in this study were deposited in GenBank under BioProject PRJNA632685 (BioSample Accessions: SRR11787864–SRR11787881).

2.4 *16S rRNA gene data processing and analysis*

The raw reads FASTQ files were processed and analyzed using the QIIME v1.8.0 bioinformatics program (Caporaso et al., 2010). The original paired reads were joined with fast length adjustment of short reads with the default settings (Magoč and Salzberg, 2011). Reads were assigned to each sample based on their unique barcodes. Chimeric sequences were detected using the UCHIME module of the USEARCH program (Edgar et al., 2011). The full dataset (n=18) contained 655 588 clean reads. After filtering the chimeric reads, the sequences with 97% similarity were clustered into operational taxonomic units (OTUs) using the pick_open_reference_otus.py script in QIIME (Caporaso et al., 2010). The taxonomy composition of OTUs was determined according to the Greengenes database (13_8_release) (DeSantis et al., 2006). Some taxa annotated as "Chloroplast" "Eukaryota" "Archaea" and "Mitochondria" were filtered from the OTU table using *filter_taxa_from_otu_table.py* script in QIIME (Caporaso et al., 2010).

For the diversity calculations, the OTU table was subsampled (rarefied) by randomly removing pseudo-random sequences using the *single_rarefaction.py* script in QIIME (Caporaso et al., 2010). The sequence number after subsampling was 20 653 in each sample. Then the subsampled OTU table was used to calculate the alpha diversity of each sample using the *alpha_diversity.py* script in QIIME (Caporaso et al., 2010). The diversity and richness indexes, including observed OTUs, Shannon and Chao1 (Lee and Chao, 1994), are shown in Table A1. To compare the diversities and richness of bacterial communities in different groups (DS, NS, DM and NM) named by the sampling time (day or night) and water layers (surface or middle), alpha diversity indexes were analyzed using the function ggsignif in the ggplot2 package (Wickham and Chang, 2009). The beta diversity was explored with the Bray-Curtis dissimilarity index using the function vegdist of the vegan package in R (Bray and Curtis, 1957; Oksanen et al., 2010).

2.5 *Statistical analysis*

The statistical analyses were performed in the R statistical environment (Oksanen et al., 2010). A heatmap was plotted to visualize the Spearman correlations among the environmental factors using the function ggcorplot in the ggplot2 package (Wickham and Chang, 2009). The alpha diversity indexes were presented as the means ± standard deviation (SD), and the Wilcoxon test was used to make pairwise comparisons (nonparametric data). To estimate the similarity among the samples, the data of the bacterial OTUs was processed using non-metric multidimensional scaling. Detrended correspondence analysis was used to select a linear multivariate redundancy analysis (RDA) or a unimodal ordination method (Canonical Correspondence Analysis) (ter Braak and Smilauer, 2002). Given the length of the first axis of the detrended correspondence analysis (1.058 SD<4 SD), RDA combined with a Monte Carlo permutation test (999 permutations) was probably the most suitable method to analyze the influence of environmental variations on microbial communities. Most of the collinear factors were eliminated, leaving six factors: depth, NO_3^-, NO_2^-, COD, PO_4^{3-} and pH. Hellinger was used to standardize the OTU data, and the dissimilarity in environmental factors was log-transformed. Analysis of similarity (ANOSIM) was used to test the dissimilarity of environmental factors, which was calculated based on the Bray-Curtis distance. Spearman indexes were used to investigate the correlation among environmental factors or between the environmental factors and bacterial community composition at the family level.

3 Results

3.1 *Environmental variations in seawater*

The environmental factors that described the geographical location and the water properties of the studied site were summarized in Table 1. Briefly, the salinity of the oxic samples was distinctly lower than that of the hypoxic ones (p<0.001). Inversely, the concentrations of NO_3^- and DO in the surface seawater were distinctly higher than those in the middle water (p<0.001). Notably, the temperature, and the NO_3^-, NH_4^+ and PO_4^{3-} concentrations in Sample M22 were the lowest among the hypoxic samples. In the hypoxic layer, only the concentrations of

NO_3^- (p<0.05) and temperature (p<0.05) were significantly different between day and night, while there was no distinct difference (p>0.05) in the oxic samples. To evaluate the relationships among these variables, correlation coefficients (Spearman) and significances were determined (Fig. A1). NO_3^- was positively correlated to temperature (r=0.91, p<0.05) and DO (r=0.91, p<0.05). Meanwhile, salinity was distinctly negatively related to NO_3^- (r=–0.87, p<0.05) and temperature (r=–0.94, p<0.05).

3.2 *Alpha and beta diversity of bacterial communities*

In total, 655 588 bacterial sequences with high quality were obtained from all the samples. Briefly, the observed OTUs index of the bacteria ranged from 323 to 468; the Chao1 index of the bacteria ranged from 438.11 to 645.75; the Shannon index of the bacteria ranged from 5.05 to 5.75 (Table A1). Chao1 index indicates the extent of the community richness. Here, Chao1 index for DS was significantly lower than DM (p<0.01, Wilcoxon test; Fig. 2a), which indicated in the daytime bacterial community had lower richness in the oxic layer than that in the hypoxic layer. The Shannon index represented the degree of community diversity. There was no significant difference in the Shannon index between each two groups (p>0.05, Wilcoxon test; Fig. 2b), except between DS and NM (p=0.016, Fig. 2b). Overall, the bacterial richness and diversity in the two layers were quite different, whereas there were no obvious differences on the bacterial richness and diversity of each water layer during 24 h. Beta diversity was measured to capture changes in community composition across different environments. Patterns in microbial community structure among samples were collectively examined using ANOSIM and non-metric multidimensional scaling based on the Bray-Curtis distance ordinations of the OTUs (Singh et al., 2015). As shown in Fig. 3a, the oxic and hypoxic groups were separated by the first axis. ANOSIM also portrayed the significant difference in the bacterial community structure between the depth groups (r=0.410 5, p=0.004). However, there was no significant difference observed according to the time (r=–0.012 7, p=0.462;

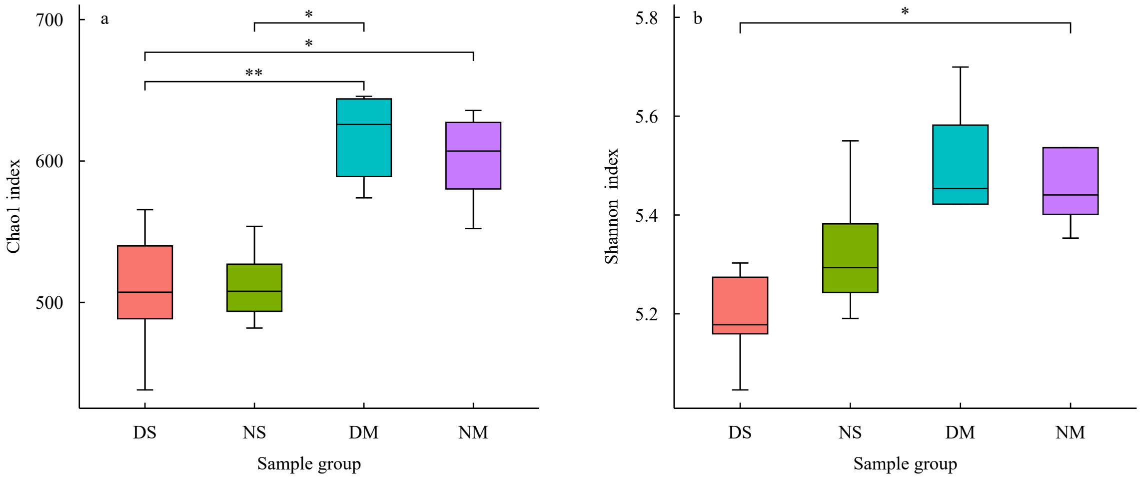

Fig. 2. The alpha diversity measured using the Chao1 and Shannon indexes was plotted for different groups (DS, NS, DM and NM). Groups were named according to their sampling time (day or night, abbreviated as D and N) and sampling location (oxic surface or hypoxic middle layers, abbreviated as S and M). The line inside the box represented the median, while the whiskers represented the lowest and highest values within the 1.5 interquartile range. The significance of the difference between each pair of sample groups was shown at the top. *** p<0.001; ** 0.001⩽p<0.01; * 0.01⩽p<0.1.

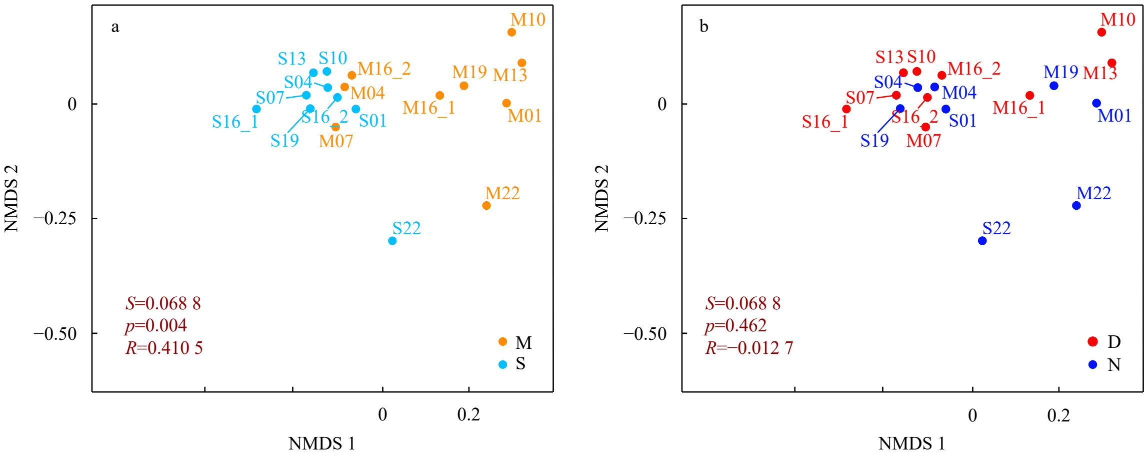

Fig. 3. The non-metric multidimensional scaling (NMDS) results showing the relationship among all the samples based on the bacterial community composition using operational taxonomic units (S=0.068 8, S represents stress). Samples were color coded according to the depth (orange for hypoxic seawater of middle layer and light blue for oxic seawater of surface layer, abbreviated as M and S, respectively) and time (red for day and dark blue for night, abbreviated as D and N, respectively).

Fig. 3b). These results indicated that there were more significant variations in community structure across depths than between day and night.

3.3 *Composition of bacterial communities*

In all samples, the dominant phyla were Proteobacteria (52.9%–75.2%), Bacteroidetes (9.7%–26.2%), Actinobacteria (5.1%–13.1%) and SAR406 (2.0%–9.3%) (Figs 4a and c). The percentages of Bacteroidetes ($p<0.05$), Actinobacteria ($p<0.05$) and Cyanobacteria ($p=0.113$) in the surface samples were slightly higher than those in the seawater samples of middle layer. However, the percentage of SAR406 ($p=0.006$) in the oxic samples was noticeably lower than in the hypoxic ones. It was also found that the relative amount of Cyanobacteria ($p=0.122$) in the daytime group was higher than that in the night group. In contrast, the relative amounts of SAR406 ($p=0.181\,2$), ZB3 ($p<0.05$) and Verrucomicrobia ($p<0.1$) displayed the opposite trend. Notably, compared with each individual sample, Cyanobacteria (4.9%), ZB3 (3.1%) and Chloroflexi (1.4%) showed the highest relative abundances in S16_1, S22 and M22 samples, respectively.

At the class level, Alphaproteobacteria (17.7%–42.7%), Gammaproteobacteria (17.3%–37.5%), Flavobacteriia (9.0%–24.1%) and Acidimicrobiia (4.9%–20.9%) were the most abundant clades (Figs 4b and d). More pronounced fluctuations in bacterial community composition were observed at the class taxonomic level. In particular, for AB16 ($p<0.01$) and Deltaproteobacteria ($p<0.01$), their percentages in the oxic layer were distinctly lower than those in the hypoxic layer. In addition, Flavobacteriia ($p<0.05$), Acidimicrobiia ($p<0.05$), Betaproteobacteria ($p=0.27$) and Synechococcophycideae ($p=0.1$) were more abundant in the oxic samples. The percentage of AB16 ($p=0.17$) was slightly higher in the night samples than that in the daytime ones. In contrast, higher relative abundances of Alphaproteobacteria ($p=0.05$), Betaproteobacteria ($p=0.25$), Flavobacteriia ($p<0.1$) and Synechococcophycideae ($p=0.13$) were observed in daytime samples. As mentioned above, Synechococcophycideae, which is affiliated to Cyanobacteria, was the most abundant class in S16_1. Notably, compared with other samples, S22 and M22 contained relatively high percentage of BS119, which belongs to the ZB3 clade.

A heatmap that exhibited the relative abundance of the major classified families (the top 30 families) was summarized in Fig. 5. It shows the relative abundance of each family in each sample and highlights the differences in the two seawater layers and different time nodes. The relative abundances of Pelagibacteraceae ($p=0.38$), Rhodospirillaceae ($p=0.01$) and A714017 ($p<0.01$) were relatively high in hypoxic samples, whereas OCS115 ($p<0.05$), Flavobacteriaceae ($p<0.1$), Cryomorphaceae

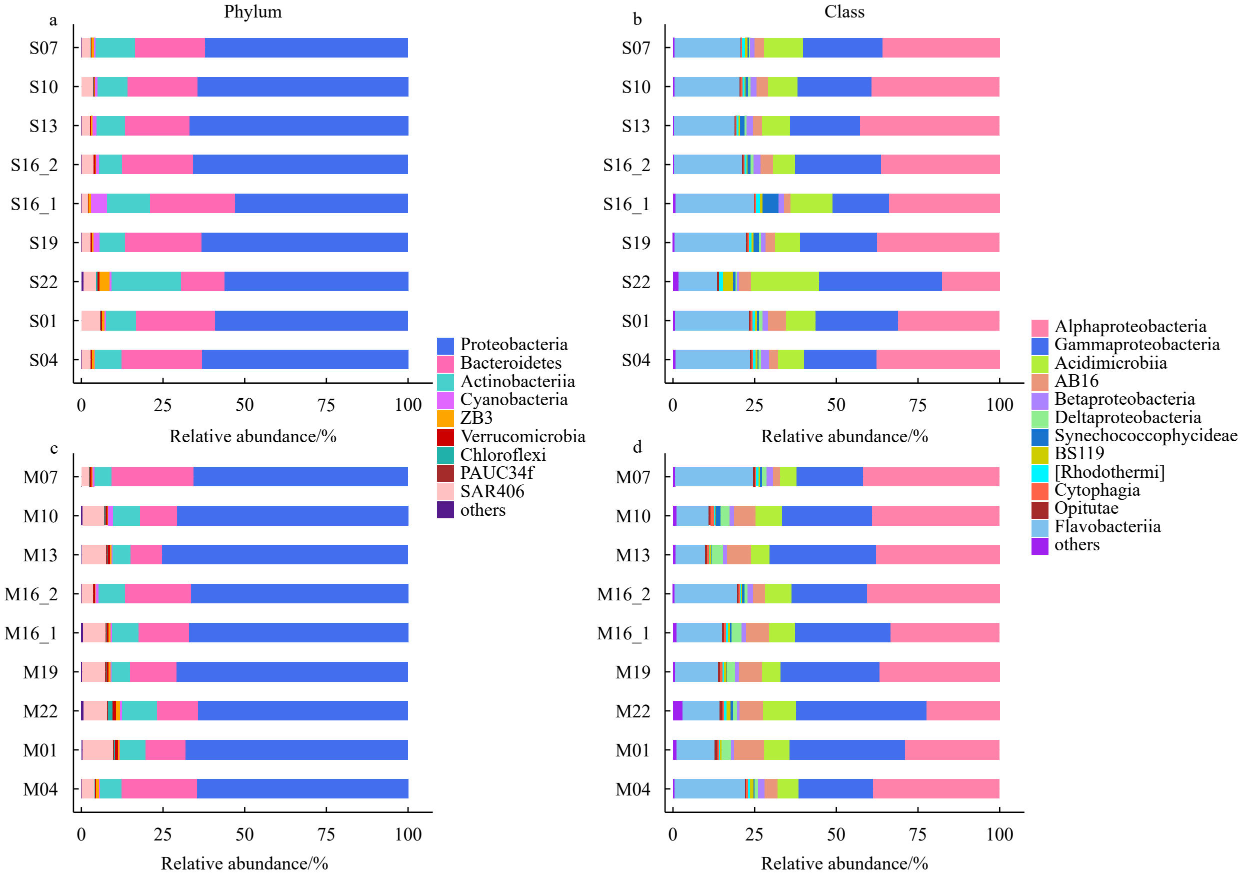

Fig. 4. The bacterial community composition at different taxonomic levels in each seawater sample. a. The relative abundance of the dominant bacterial phyla in oxic samples of surface layer (sample ID: S); b. the relative abundance of the dominant bacterial classes in oxic samples of surface layer (sample ID: S); c. the relative abundance of the dominant bacterial phyla in hypoxic samples of middle layer (sample ID: M); d. the relative abundance of the dominant bacterial classes in hypoxic samples of middle layer (sample ID: M). In a and c, “others” referred to the phyla with a total relative abundance of less than 0.08%. In b and d, “others” referred to classes with a total relative abundance of less than 0.2%. The number referred to the time when the samples were collected, e.g., “16_1” indicated that the sampling time was 16:00 on the first day.

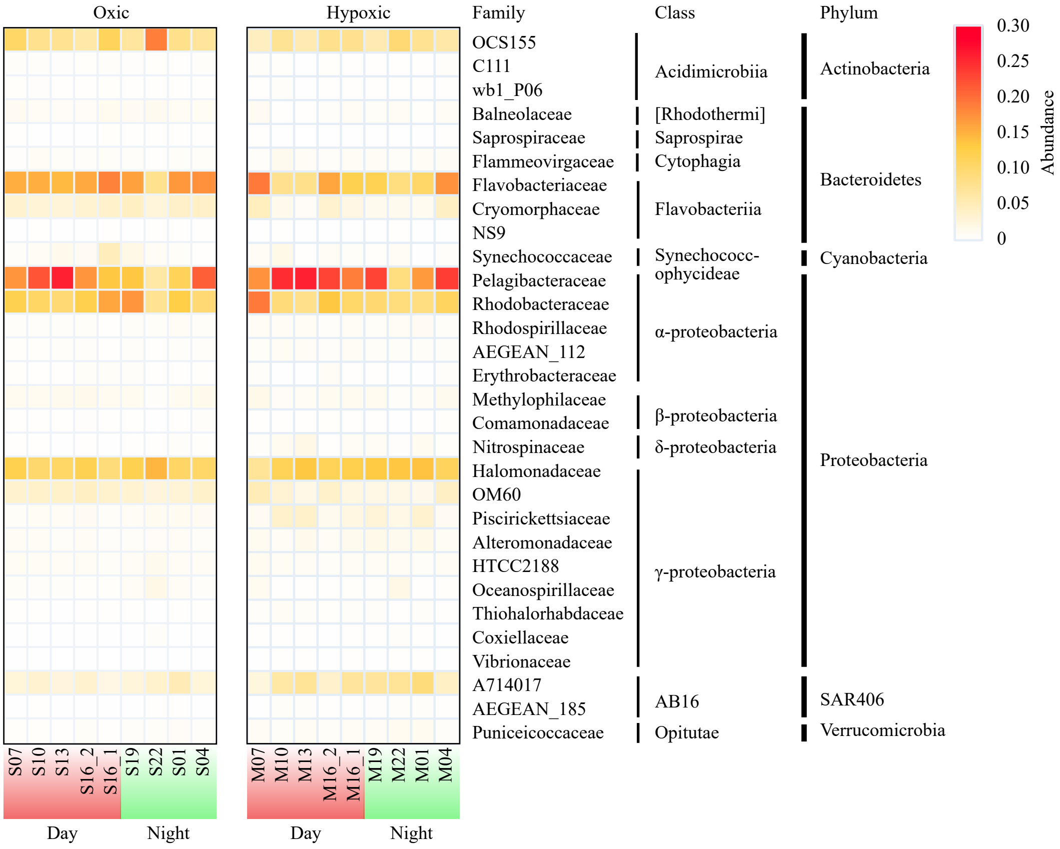

Fig. 5. A heatmap showing the abundance of different bacteria within the community at the family level (top 30) of all the samples. The samples were named according to a letter that represents the seawater layer (S for surface and M for middle) and a number that referred to the time at which the sample was collected, e.g., “16_1” indicated that the sampling time was 16:00 on the first day.

($p<0.05$) and OM60 ($p=0.05$) showed the reverse trend. In both layers, Pelagibacteraceae were more abundant in the daytime, whereas the abundances of Flavobacteriaceae and Rhodobacteraceae were relatively high from 16:00 to 19:00. *Synechococcus* and *Prochlorococcus*, which are affiliated with the Cyanobacteria group, exhibited low ratios in all samples (Fig. 6). The relative abundance of *Prochlorococcus* was unexpectedly low. The changes in the relative abundances of *Synechococcus* and *Prochlorococcus* in the oxic layer were not correlated ($r=0.009\,2$, $p>0.1$). In contrast, the trends in relative abundances of the two genera were significantly correlated in the hypoxic samples ($r=0.52$, $p<0.05$). *Synechococcus* species were more abundant in the oxic samples than those in the hypoxic samples. In both oxic and hypoxic layers, the percentage of *Synechococcus* was relatively low at 1:00. *Synechococcus* presented some differences over the course of the 24 h period in the two layers. In the oxic water,

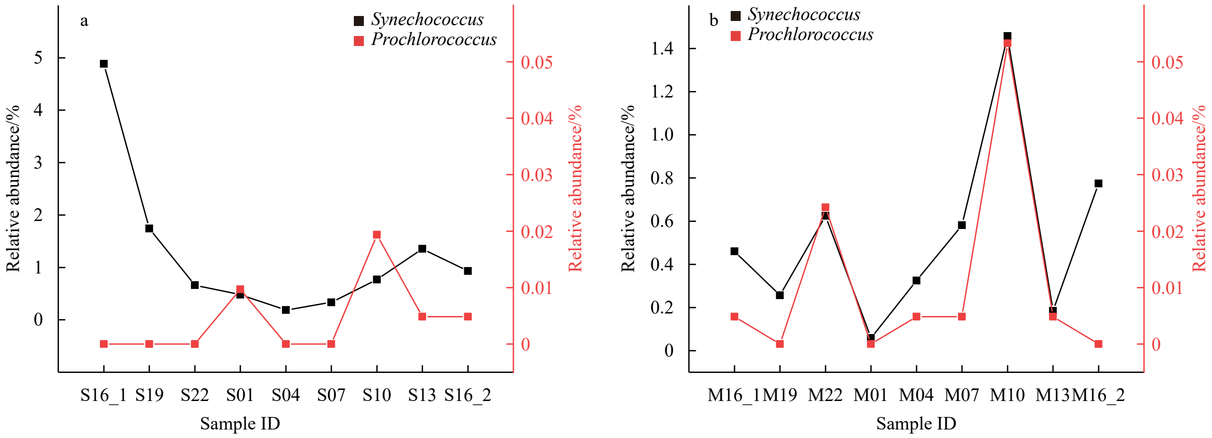

Fig. 6. The abundance of *Synechococcus* and *Prochlorococcus* over time in the oxic (a) and hypoxic (b) samples. The samples were named according to a letter that represented the seawater layer (S for surface and M for middle) and a number that referred to the time at which the sample was collected, e.g., “16_1” indicated that the sampling time was 16:00 on the first day.

the concentration of *Synechococcus* at 16:00 (4.89%) was highest, then decreased until 4:00 (1.89%). Reversely, the percentage of *Synechococcus* peaked at M10 (10:00; 14.57%) in the hypoxic samples. The diel changes of the genus *Synechococcus* and the families Flavobacteriaceae, Rhodobacteraceae and OM60 were displayed in Fig. 7. In the oxic samples, Rhodobacteraceae correlated with *Synechococcus* and Flavobacteriaceae ($r>0.5$, $p<0.1$). In the hypoxic samples, Rhodobacteraceae and OM60 displayed similar trends to *Synechococcus* with respect to the diel cycle ($r\geqslant 0.45$), except for Flavobacteriaceae. The diel changes in the hypoxic zone were more stable than those in the oxic zone. It was found that the relative abundances of these three families (Flavobacteriaceae, Rhodobacteraceae and OM60) decreased in the evening (16:00–1:00), increased to maximum values at 7:00, then decreased to minimum values at 13:00, before increasing again. In view of close associations within microbial loop, correlation coefficients of the relative abundance between *Synechococcus* and the top 29 families (except Synechococcaceae) were summarized in Table A2. In the oxic layer, *Synechococcus* was correlated with HTCC2188 ($r<-0.6$, $p<0.1$), Nitrospinaceae ($r<-0.7$, $p<0.05$), and Rhodobacteraceae ($r>0.5$, $p<0.1$). In the hypoxic layer, *Synechococcus* was correlated with OM60 ($r>0.45$, $p=0.18$), wb1_P06 ($r>0.6$, $p<0.1$), A714017 ($r<-0.6$, $p<0.1$) and C111 ($r>0.6$, $p=0.05$).

3.4 *The correlation between bacterial communities and environmental variations*

RDA was used to analyze variation of the community structure at the OTU level as a function of environmental factors including NO_3^-, NO_2^-, COD and PO_4^{3-} concentrations, depth and pH. Collectively, these data explained 40% of the variation in community structure (Fig. 8). On the horizontal axis (RDA1, 30%

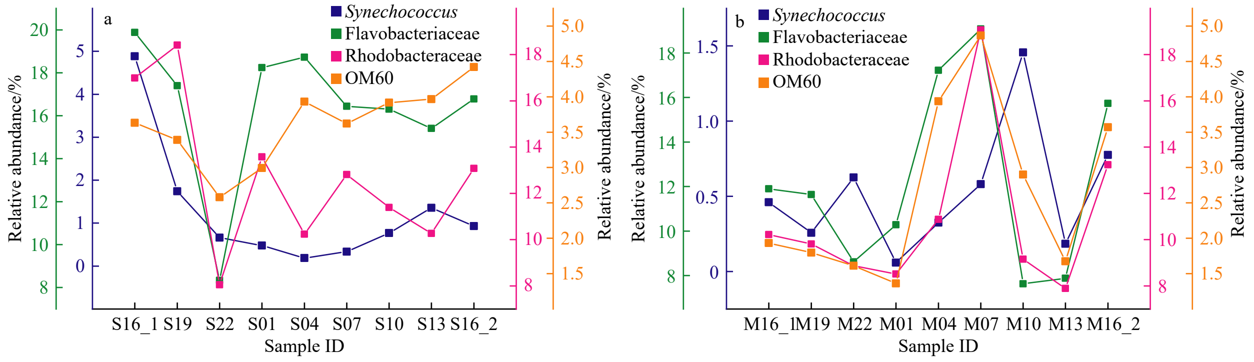

Fig. 7. The abundance of *Synechococcus*, Flavobacteriaceae, Rhodobacteraceae and OM60 over time in the oxic (a) and hypoxic (b) samples. The samples were named according to a letter that represented the seawater layer (S for surface and M for middle) and a number that referred to the time at which the sample was collected, e.g., “16_1” indicated that the sampling time was 16:00 on the first day.

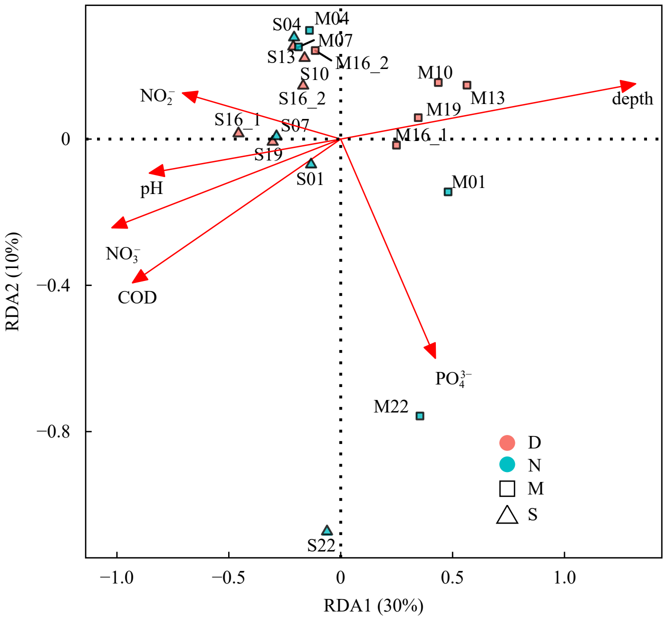

Fig. 8. The redundancy analysis (RDA) results showing the ordination diagram of the bacterial community composition (operational taxonomic units) associated with environmental factors, except collinear ones. Environmental factors were indicated as arrows. DO, dissolved oxygen; COD, chemical oxygen demand. D and N represent day and night groups, respectively; M and S represent middle and surface layer groups, respectively.

of constrained variability), the most influential constraining variable was depth (biplot score, 0.88) followed by NO_3^- (biplot score, –0.68). On the vertical axis (RDA2, 10% of constrained variability), the most influential constraining variable is PO_4^{3-} (biplot score, –0.40), followed by COD (biplot score, –0.26). The permutest function results showed that six factors (depth, NO_2^-, NO_3^-, pH, COD and PO_4^{3-}) were significantly related to the variation in bacterial community composition (p<0.05). However, this result did not represent each factor's contribution. The function envfit was used to measure each factor onto an ordination. The depth, salinity, temperature and DO were significantly related to the bacterial community structure (r>0.45, p<0.05). However, PO_4^{3-} and NO_2^- had little effect on the bacterial community (r<0.2, p>0.1). In addition, the RDA1 axis clearly separated the oxic and hypoxic samples.

To further identify the relationship between environmental factors and the bacterial community structure (the top 30 families), a heatmap was constructed based on agglomerative hierarchical clustering with complete linkage to provide an overview of the identified connections among the studied samples (Fig. 9). Depth, salinity, temperature, DO and NO_3^- were significantly correlated with some families. With respect to the bacterial communities, AEGEAN_112, Nitrospinaceae, Piscirickettsiaceae, A714017 and AEGEAN_185 were positively correlated with depth and salinity (r>0.52, p<0.05), but were negatively related to temperature and NO_3^- (r<–0.59, p<0.05). Flavobacteriaceae and Cryomorphaceae displayed the same results: the relative abundances were negatively related to depth and salinity (r<–0.49, p<0.05), but positively correlated with temperature and NO_3^- (r>0.46, p<0.05). Several families of the class Gammaproteobacteria were significantly related to most environmental factors. These families, including Piscirickettsiaceae, Alteromonadaceae, Thiohalorhabdaceae and Vibrionaceae, were negatively related to COD, NO_2^-, DO, temperature and NO_3^- (r<–0.47, p<0.05). The families Methylophilaceae and OM60 clades were positively associated with DO and pH (r>0.60, p<0.01). A number of families were also found to be associated with depth, salinity and temperature. Combining the RDA with a Monte Carlo permutation test (Fig. 8), depth was linearly related to some factors (such as salinity, temperature and pH), and was a proxy for a number of other factors that potentially have more direct effects on the microorganisms. Light, pressure and other physical factors can all vary with depth, and the interaction of these factors can influence the ecological communities.

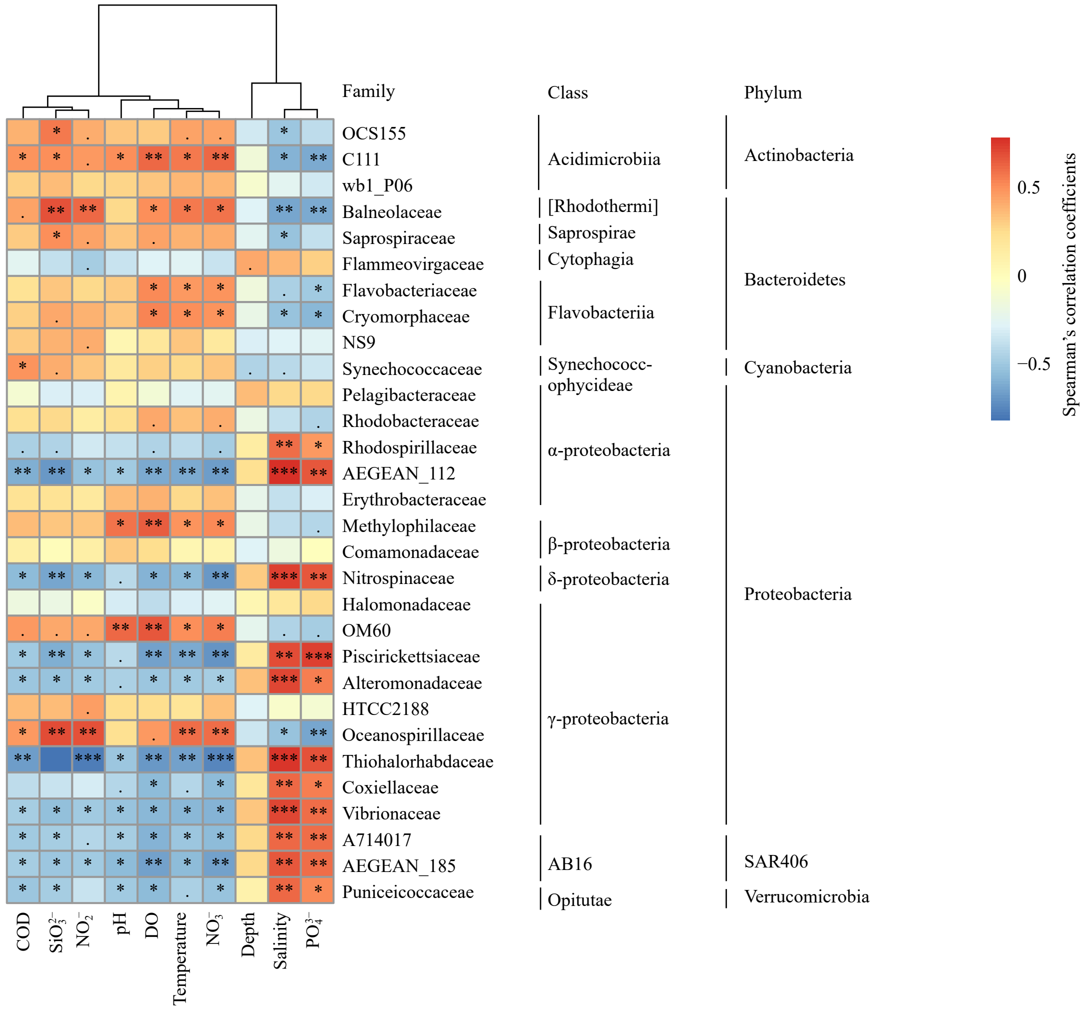

Fig. 9. A heatmap showing the environmental factors associated with the bacterial community compositions at the family level of all the samples. The Spearman's correlation coefficients, ranging from –1 to 1, were displayed as colors ranging from blue to red. The significant differences (p values) were shown in each rectangle. *** p<0.001; ** 0.001⩽p<0.01; * 0.01⩽p<0.05; · 0.05⩽p<0.1; DO, dissolved oxygen; COD, chemical oxygen demand.

4 Discussion

4.1 *Comparison of bacterial communities between oxic and hypoxic layers*

In our study, relatively distinct bacterial communities were observed between the oxic and hypoxic layers. In addition, the richness and diversity of bacteria in the hypoxic layers were greater than the oxic ones, as found in the northern Gulf of Mexico (Campbell et al., 2019) and northwestern Mediterranean Sea (Pommier et al., 2010). The relative abundances of some phyla, like Cyanobacteria, Bacteroidetes and Acidimicrobiia, were greater in the oxic layer (p<0.05), whereas the phylum SAR406 and the class Deltaproteobacteria were more abundant in hypoxic conditions (p<0.05), which were also observed abundant in the low oxygen (2 mg/L<DO<3 mg/L) water layers in the previous study of the CRE in July 2016 (Wu et al., 2019).

As expected, the groups enriched in the oxic layer were mostly phototrophic (Cyanobacteria) or aerobic bacteria (Acidimicrobiia and Flavobacteriia). Bacteroidetes, in particular the class Flavobacteriia, are specialized in the degradation of polysaccharides, which are the constituents of marine algae (for example, xylan in red algae, and laminarin in brown algae and diatoms) (de Jesus Raposo et al., 2013; Synytsya et al., 2015). Previous studies have found that diatoms are the dominant species of phytoplankton in summer in the CRE (Lin et al., 2008; Ye et al., 2016; Fan et al., 2019). Ye et al. (2016) ever found that the diatoms bloomed in August around 31°N, 123°E where is quite close to the sampling site of this study, indicating diatoms grow in this area. As the primary colonizers of marine phytoplankton, Flavobacteriia has been shown to be more abundant during phytoplankton blooms, especially in the coastal surface waters (Alonso et al., 2007; Teeling and Amann, 2012). A high proportion of Flavobacteriia share the characteristic of gliding motility, which allows them to grow on some algal cells and utilize phytoplankton-derived polysaccharides (Mann et al., 2013; McBride et al., 2009; Qin et al., 2010; Tang et al., 2017). The family Cryomorphaceae has been shown to exhibit great similarity to Flavobacteriaceae according to phenotype and genotype (Bernardet et al., 1996; Bowman, 2014). In addition, Cryomorphaceae is also associated with phytoplankton blooms (Pinhassi et al., 2004). Marine algae converts a substantial fraction of fixed carbon dioxide into various polysaccharides, which suggested that more marine carbon cycling may occur in the oxic layers (Cottrell and Kirchman, 2000; Isao et al., 1990; Richardson and Jackson, 2007).

SAR406 (Marine Group A), also named as Marinimicrobia, is ubiquitous in many deep oceans (Rinke et al., 2013; Gordon and Giovannoni, 1996; Wright et al., 2012). The SAR406 clade is also particularly abundant in oxygen minimum zones (Gordon and Giovannoni, 1996; Schattenhofer et al., 2009; Hu et al., 2016), and permanently or seasonally stratified anoxic basins (Fuchs et al., 2005; Stevens and Ulloa, 2008; Martha et al., 2010). The relative abundance of SAR406 was higher in hypoxic samples (p=0.006). Additionally, the clade was significantly negatively associated with DO and positively associated with depth and salinity. Wright et al. (2014) suggested that the SAR406 clade has the ability to reduce, or possibly oxidize sulfur compounds by analyzing partial genome data of SAR406. Subsequently, Thrash et al. (2017) identified the metabolic potential of SAR406 using a coupled shotgun metagenomic and metatranscriptomic method and found complete pathways for sulfur reduction and nitrate reduction. Otherwise, SAR406 has also been shown to degrade complex carbohydrates via aerobic respiration (Rinke et al., 2013; Thrash et al., 2017). This result might explain why the SAR406 clade was also found in the oxic layer in the present study.

Gammaproteobacteria is also slightly more abundant in the hypoxic samples, and has been proven to be an adaptive group under hypoxic conditions (Devereux et al., 2015; Jessen et al., 2017). When compared with the oxic water layers (5–16 m) in the similar depth of our middle layer in the CRE, the class Gammaproteobacteria also existed in oxic layers (Dong et al., 2014). It indicated that some subgroups of Gammaproteobacteria might be anaerobe or tolerant to the hypoxic environments (He et al., 2019). Actually, Gammaproteobacteria was found to be the dominant group in some other anoxic environments, such as the Blue Hole (He et al., 2019) and the Baltic Sea (Broman et al., 2017). Another abundant group in the hypoxic layer was Deltaproteobacteria, which was frequently found in anaerobic conditions and associated with sulfate reduction (Jørgensen and Bak, 1991; Coleman et al., 1993; Füssel et al., 2017). These findings suggest that more sulfate reduction may occur in hypoxic zones. Similar results were also found in the Zhujiang River Estuary (Liu et al., 2015) and the Blue Hole (He et al., 2019).

4.2 *The diel changes in bacterial communities in the oxic and hypoxic layers*

The photic zone of aquatic habitats is subjected to strong physicochemical gradients (Haro-Moreno et al., 2018). Episodic forcing over short-time scales was deemed to cause changes in phytoplankton community structures (Guadayol et al., 2009). Changes in temperature, light, UV and nutrient availability are known to moderate the growth of photosynthetic organisms, and light has often been considered as the most important factor determining diel variability (Gao et al., 2019). Interestingly, in this study, the composition of bacterial communities at 22:00 was significantly different from other times (Figs 3 and 4). From the tide graph (Fig. A2), we found that 22:00 was the low tide period. A previous study suggested that high DOM concentrations and increased prey activities could occur at low tide (Chauhan et al., 2009), which will alter the bacterial abundance in seawater. In addition, some other uncontrollable factors, e.g., viral lysis and physicochemical conditions, are also likely to contribute to shifts in the bacterial composition (Rappé et al., 2000; Bouvier and Del Giorgio, 2007). This study also found the bacterial communities were different at 16:00 in both layers, which may be caused by some environmental factors. Similar conditions occurred in other studies (Pernthaler and Pernthaler, 2005; Wang et al., 2013). Environmental microbial samples are greatly influenced by a variety of uncontrolled factors, so it is normal that different bacterial composition occurs in different days at the same time if it weakly affects the overall trend of the community structure. Here, the relative abundances of some phototrophic bacteria and several heterotrophic bacteria had similar fluctuations in the diurnal changes of the studied site.

Cyanobacteria, which can photosynthesize, is common in the aquatic systems (Bryant, 1995). Since the livelihood of Cyanobacteria is directly dependent upon light, a comprehensive understanding of the metabolism of these organisms is required to account for the effects of day-night transitions and circadian regulation. In the present study, the circadian rhythms of *Synechococcus* and *Prochlorococcus* were not too apparent. In the surface layer, the relative abundance of *Synechococcus* had a steady increase from 4:00 to 13:00, but a drop at 16:00. Dolan and Šimek (1999) found a similar phenomenon: the *Synechococcus* cell count peaked in the late afternoon and early evening hours in the bay of Villefranche (northwestern Mediterranean). Nevertheless, in the middle water layer of our study, a general increase was observed from 1:00 to 10:00, and a slight decrease was observed from 10:00 to 13:00. This phenomenon was also found in several previous studies, like the open Mediterranean Sea (Llabrés et al., 2011) and the northern South China Sea (Liu et al., 2016). Jacquet et al. (1998) suggested that the *Synechococcus* cell cycle was carried out in stages, with the daily photoperiod possibly being genetically controlled by a “clock”. Owing to different environmental conditions and nutrient in different layers, the daily change of *Synechococcus* was not entirely consistent. In the surface layer, *Synechococcus* is possibly affected by light, wind, ultraviolet ray and other uncontrolled physical factors (Gao et al., 2019), while in the middle layer, nutrients and grazing may be the domain factors (Suyama et al., 2002; García et al., 2018).

Little *Prochlorococcus* was found at the studied site by 16S rRNA gene Illumina HiSeq technology. There are several reasons that may explain why little *Prochlorococcus* presented in the samples. The distribution of *Prochlorococcus* could be sensitive to multiple factors (Jiao et al., 2002), including temperature, which is a crucial environmental factor (Olson et al., 1990; Partensky et al., 1999). *Prochlorococcus* was proposed to be limited to the minimum temperature (26°C) in summer in Chinese seas (Jiao et al., 2002), which may explain the lack of *Prochlorococcus* in our samples where the temperature was lower than 26.4°C. Additionally, physical conditions such as mixing and stratification may also work (Olson et al., 1990; Partensky et al., 1996).

Tight coupling between phytoplankton and bacteria could cause some bacteria to follow similar circadian cycles (Kirchman, 2008). Here, the families Rhodobacteraceae and Flavobacteriaceae displayed similar trends to *Synechococcus* in the diel cycle. More Rhodobacteraceae was found in the oxic samples, and positively related to DO concentration. Studies have shown that Rhodobacteraceae comprises aerobic phototrophs, which can utilize light to live (Pujalte et al., 2014) and some Rhodobacteraceae have been found in algae-associated biofilms (Elifantz et al., 2013). A previous study found that Rhodobacteraceae is the dominant family associated with diatom *Leptocylindrus* (Ajani et al., 2018). Ye et al. (2016) found that diatoms can sink to the deep layer and then release organic materials. Here, Rhodobacteraceae also existed in the hypoxic layer, especially during the night. A slight increase of relative abundance occurred at night, which may be caused by the sinking dead diatoms. Several researchers have also found similar results that some Rhodobacteraceae are likely to thrive under hypoxic conditions (Drews, 1981). The relative abundance of the family Flavobacteriaceae is also associated with that of *Synechococcus*. Zheng et al. (2017) suggested that Flavobacteriaceae tends to aggregate or attach to the *Synechococcus* cells, and could degrade complex organic matter by directly attaching to and attacking algal cells using exoenzymes (Kirchman, 2002; Gómez-Pereira et al., 2012; Teeling and Amann, 2012). A previous study found the abundance of *Cytophaga-Flavobacterium* had diurnal fluctuations in Monterey Bay (USA), and reached the highest during the high tide over a tide cycle (Olapade, 2012). In this study, there was a similar rhythm between the tide variation and the relative abundance change of Flavobacteriaceae in the hypoxic layer. Cottrell and Kirchman (2000) purposed that their high abundance might be associated with DOM which directly inflowed during the high wind mixing.

The OM60 clade is the marine gammaproteobacterial branch of aerobic anoxygenic phototrophic bacteria and has been reported to coincide with high chlorophyll concentrations (Fuchs et al., 2007; Yan et al., 2009). Here, the relative abundance of OM60 was steady and high in the daytime in the oxic layer. On the contrary, in the hypoxic layer, the relative abundance of OM60 was low and fluctuated. Furthermore, the OM60 clade was positively correlated with DO, indicating that the clade may prefer habitats with sufficient oxygen concentrations. From night to early morning, the percentage of OM60 decreased, then rose. This phenomenon may due to the tide changes or the lack of light at night, meaning that individuals of the clade could not grow well. Nevertheless, the respiration of the phytoplankton may have produced sufficient dissolved oxygen during the night that OM60 could thrive (Fuchs et al., 2007; Yan et al., 2009).

In addition to the above-mentioned families, there are other families associated with *Synechococcus* such as HTCC2188, C111, wb1_P06, Nitrospinaceae and A714017. However, little is known about the ecological functions about these families. More efforts are needed to focus on these families to explain the correlation of different communities in marine environments. Together, the results suggest that some photosynthetic bacteria and several heterotrophic bacteria may have similar circadian rhythms to *Synechococcus*. Considering close associations within microbial loop (Porter, 1996), different preferences for organic substrates or phytoplankton species (Pinhassi et al., 2004; Alonso-Sáez and Gasol, 2007), some bacteria may have diurnal circadian rhythms associated with phytoplankton. The trend was not distinct in the upper layer, which may due to the influence of various uncontrolled physical factors.

5 Conclusions

In this study, we detected the bacterial communities in the oxic and hypoxic layers in the CRE during July 2016. Distinct community structure was observed between oxic and hypoxic layers. In addition, we found that some photosynthetic autotrophic bacteria and several heterotrophic bacteria have similar diurnal variations, hinting that there are some potential ecological associations between them. Our results may contribute to further understanding of the factors that affect the microbial communities in the estuary. More efforts are needed to verify the above-mentioned findings in other coastal areas, and more efforts are needed to explore the diurnal changes in bacterial, archaeal or eukaryotic plankton in oceanic hypoxic zones and potential influences on microbial loop.

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References

Ajani P A, Kahlke T, Siboni N, et al. 2018. The microbiome of the cosmopolitan diatom *Leptocylindrus* reveals significant spatial and temporal variability. Frontiers in Microbiology, 9: 2758, doi: 10.3389/fmicb.2018.02758

Alonso C, Warnecke F, Amann R, et al. 2007. High local and global diversity of *Flavobacteria* in marine plankton. Environmental Microbiology, 9(5): 1253–1266, doi: 10.1111/j.1462-2920.2007.01244.x

Alonso-Sáez L, Gasol J M. 2007. Seasonal variations in the contributions of different bacterial groups to the uptake of low-molecular-weight compounds in Northwestern Mediterranean coastal waters. Applied and Environmental Microbiology, 73(11): 3528–3535, doi: 10.1128/AEM.02627-06

Alonso-Sáez L, Gasol J M, Lefort T, et al. 2006. Effect of natural sunlight on bacterial activity and differential sensitivity of natural bacterioplankton groups in northwestern Mediterranean coastal waters. Applied and Environmental Microbiology, 72(9): 5806–5813, doi: 10.1128/AEM.00597-06

Béjà O, Aravind L, Koonin E V, et al. 2000. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Science, 289(5486): 1902–1906, doi: 10.1126/science.289.5486.1902

Bernardet J F, Segers P, Vancanneyt M, et al. 1996. Cutting a Gordian knot: emended classification and description of the genus Flavobacterium, emended description of the family Flavobacteriaceae, and proposal of *Flavobacterium hydatis* nom. nov. (Basonym, *Cytophaga aquatilis* Strohl and Tait 1978). International Journal of Systematic Bacteriology, 46(1): 128–148, doi: 10.1099/00207713-46-1-128

Bianchi T S, Allison M A. 2009. Large-river delta-front estuaries as natural “recorders” of global environmental change. Proceedings of the National Academy of Sciences of the United States of America, 106(20): 8085–8092, doi: 10.1073/pnas.0812878106

Bouvier T, Del Giorgio P A. 2007. Key role of selective viral-induced mortality in determining marine bacterial community composition. Environmental Microbiology, 9(2): 287–297, doi: 10.1111/j.1462-2920.2006.01137.x

Bowman J P. 2014. The family Cryomorphaceae. In: Rosenberg E, DeLong E F, Lory S, et al., eds. The Prokaryotes: Other Major Lineages of Bacteria and the Archaea. Berlin, Heidelberg: Springer-Verlag, 539–550

Bray J R, Curtis J T. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs, 27(4): 325–349, doi: 10.2307/1942268

Broman E, Sachpazidou V, Pinhassi J, et al. 2017. Oxygenation of hypoxic coastal Baltic Sea sediments impacts on chemistry, microbial community composition, and metabolism. Frontiers in Microbiology, 8: 2453, doi: 10.3389/fmicb.2017.02453

Bryant D A. 1995. The molecular biology of cyanobacteria: Edited by Donald A. Bryant. 1994. 881 pp. Kluwer Academic Publishers, Dordrecht, The Netherlands. ISBN 0-7923-3222-9. Photosynthesis Research, 45(2): 177–179, doi: 10.1007/BF00032589

Campbell L G, Thrash J C, Rabalais N N, et al. 2019. Extent of the annual Gulf of Mexico hypoxic zone influences microbial community structure. PLoS One, 14(4): e0209055, doi: 10.1371/journal.pone.0209055

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

Chauhan A, Cherrier J, Williams H N. 2009. Impact of sideways and bottom-up control factors on bacterial community succession over a tidal cycle. Proceedings of the National Academy of Sciences of the United States of America, 106(11): 4301–4306, doi: 10.1073/pnas.0809671106

Chen Chung-Chi, Gong Gwo-Ching, Shiah F K. 2007. Hypoxia in the East China Sea: one of the largest coastal low-oxygen areas in the world. Marine Environmental Research, 64(4): 399–408, doi: 10.1016/j.marenvres.2007.01.007

Chi Lianbao, Song Xiuxian, Yuan Yongquan, et al. 2017. Distribution and key influential factors of dissolved oxygen off the Changjiang River Estuary (CRE) and its adjacent waters in China. Marine Pollution Bulletin, 125(1–2): 440–450

Coleman M L, Hedrick D B, Lovley D R, et al. 1993. Reduction of Fe(III) in sediments by sulphate-reducing bacteria. Nature, 361(6411): 436–438, doi: 10.1038/361436a0

Conley D J, Carstensen J, Aigars J, et al. 2011. Hypoxia is increasing in the coastal zone of the Baltic Sea. Environmental Science & Technology, 45(16): 6777–6783

Cottrell M T, Kirchman D L. 2000. Natural assemblages of marine Proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Applied and Environmental Microbiology, 66(4): 1692–1697, doi: 10.1128/AEM.66.4.1692-1697.2000

de Jesus Raposo M F, de Morais A M M B, de Morais R M S C. 2013. Bioactivity and applications of polysaccharides from marine microalgae. In: Ramawat K G, Mérillon J M, eds. Polysaccharides: Bioactivity and Biotechnology. Cham: Springer International Publishing, 233–252

DeSantis T Z, Hugenholtz P, Larsen N, et al. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology, 72(7): 5069–5072, doi: 10.1128/AEM.03006-05

Devereux R, Mosher J J, Vishnivetskaya T A, et al. 2015. Changes in northern Gulf of Mexico sediment bacterial and archaeal communities exposed to hypoxia. Geobiology, 13(5): 478–493, doi: 10.1111/gbi.12142

Diaz R J. 2001. Overview of hypoxia around the world. Journal of Environmental Quality, 30(2): 275–281, doi: 10.2134/jeq2001.302275x

Diaz R J, Rosenberg R. 2008. Spreading dead zones and consequences for marine ecosystems. Science, 321(5891): 926–929, doi: 10.1126/science.1156401

Dolan J R, Šimek K. 1999. Diel periodicity in *Synechococcus* populations and grazing by heterotrophic nanoflagellates: analysis of food vacuole contents. Limnology and Oceanography, 44(6): 1565–1570, doi: 10.4319/lo.1999.44.6.1565

Dong Yi, Zhao Yuan, Zhang Wenyan, et al. 2014. Bacterial diversity and community structure in the East China Sea by 454 sequencing of the 16S rRNA gene. Chinese Journal of Oceanology and Limnology, 32(3): 527–541, doi: 10.1007/s00343-014-3215-2

Drews G. 1981. *Rhodospirillum salexigens*, spec. nov., an obligatory halophilic phototrophic bacterium. Archives of Microbiology, 130(4): 325–327, doi: 10.1007/BF00425949

Edgar R C, Haas B J, Clemente J C, et al. 2011. Uchime improves sensitivity and speed of chimera detection. Bioinformatics, 27(16): 2194–2200, doi: 10.1093/bioinformatics/btr381

Elifantz H, Horn G, Ayon M, et al. 2013. Rhodobacteraceae are the key members of the microbial community of the initial biofilm formed in Eastern Mediterranean coastal seawater. FEMS Microbiology Ecology, 85(2): 348–357, doi: 10.1111/1574-6941.12122

Fan Xin, Cheng Fangjin, Yu Zhiming, et al. 2019. The environmental implication of diatom fossils in the surface sediment of the Changjiang River Estuary (CRE) and its adjacent area. Journal of Oceanology and Limnology, 37(2): 552–567, doi: 10.1007/s00343-019-8037-9

Fuchs B M, Spring S, Teeling H, et al. 2007. Characterization of a marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis. Proceedings of the National Academy of Sciences of the United States of America, 104(8): 2891–2896, doi: 10.1073/pnas.0608046104

Fuchs B M, Woebken D, Zubkov M V, et al. 2005. Molecular identification of picoplankton populations in contrasting waters of the Arabian Sea. Aquatic Microbial Ecology, 39(2): 145–157

Fuhrman J A, Eppley R W, Hagström Å, et al. 1985. Diel variations in bacterioplankton, phytoplankton, and related parameters in the Southern California Bight. Marine Ecology Progress Series, 27: 9–20, doi: 10.3354/meps027009

Füssel J, Lücker S, Yilmaz P, et al. 2017. Adaptability as the key to success for the ubiquitous marine nitrite oxidizer *Nitrococcus*. Science Advances, 3(11): e1700807, doi: 10.1126/sciadv.1700807

Gao Kunshan, Beardall J, Häder D P, et al. 2019. Effects of ocean acidification on marine photosynthetic organisms under the concurrent influences of warming, UV radiation, and deoxygenation. Frontiers in Marine Science, 6: 322, doi: 10.3389/fmars.2019.00322

Gao Xuelu, Song Jinming. 2005. Phytoplankton distributions and their relationship with the environment in the Changjiang Estuary, China. Marine Pollution Bulletin, 50(3): 327–335, doi: 10.1016/j.marpolbul.2004.11.004

Gao Xuelu, Song Jinming, Li Ning, et al. 2007. Spatial distribution and diurnal variation of chemical oxygen demand at the beginning of the rainy season in the Changjiang (Yangtze) River Estuary. Chinese Journal of Oceanology and Limnology, 25(3): 254–260, doi: 10.1007/s00343-007-0254-y

García F C, Calleja M L, Al-Otaibi N, et al. 2018. Diel dynamics and coupling of heterotrophic prokaryotes and dissolved organic matter in epipelagic and mesopelagic waters of the central Red Sea. Environmental Microbiology, 20(8): 2990–3000, doi: 10.1111/1462-2920.14336

General Administration of Quality Supervision Inspection and Quarantine, Standardization Administration of China. 2007. GB17378.5—2007. The specification for marine monitoring—Part 5: Sediment analysis (in Chinese). Beijing: Standards Press of China.

Gilbert J A, Field D, Swift P, et al. 2010. The taxonomic and functional diversity of microbes at a temperate coastal site: a “multi-omic” study of seasonal and diel temporal variation. PLoS One, 5(11): e15545, doi: 10.1371/journal.pone.0015545

Gómez-Pereira P R, Schüler M, Fuchs B M, et al. 2012. Genomic content of uncultured Bacteroidetes from contrasting oceanic provinces in the North Atlantic Ocean. Environmental Microbiology, 14(1): 52–66, doi: 10.1111/j.1462-2920.2011.02555.x

Gordon D A, Giovannoni S J. 1996. Detection of stratified microbial populations related to *Chlorobium* and *Fibrobacter* species in the Atlantic and Pacific Oceans. Applied and Environmental Microbiology, 62(4): 1171–1177, doi: 10.1128/AEM.62.4.1171-1177.1996

Grenz C, Denis L, Pringault O, et al. 2010. Spatial and seasonal variability of sediment oxygen consumption and nutrient fluxes at the sediment water interface in a sub-tropical lagoon (New Caledonia). Marine Pollution Bulletin, 61(7–12): 399–412

Guadayol Ò, Peters F, Marrasé C, et al. 2009. Episodic meteorological and nutrient-load events as drivers of coastal planktonic ecosystem dynamics: a time-series analysis. Marine Ecology Progress Series, 381: 139–155, doi: 10.3354/meps07939

Haro-Moreno J M, López-Pérez M, de la Torre J R, et al. 2018. Fine metagenomic profile of the Mediterranean stratified and mixed water columns revealed by assembly and recruitment. Microbiome, 6(1): 128, doi: 10.1186/s40168-018-0513-5

He Hui, Fu Lulu, Liu Qian, et al. 2019. Community structure, abundance and potential functions of bacteria and archaea in the Sansha Yongle Blue Hole, Xisha, South China Sea. Frontiers in Microbiology, 10: 2404, doi: 10.3389/fmicb.2019.02404

Hu Ping, Tom L, Singh A, et al. 2016. Genome-resolved metagenomic analysis reveals roles for candidate phyla and other microbial community members in biogeochemical transformations in oil reservoirs. mBio, 7(1): e01669–15

Isao K, Hara S, Terauchi K, et al. 1990. Role of sub-micrometre particles in the ocean. Nature, 345(6272): 242–244, doi: 10.1038/345242a0

Jacquet S, Lennon J F, Marie D, et al. 1998. Picoplankton population dynamics in coastal waters of the northwestern Mediterranean Sea. Limnology and Oceanography, 43(8): 1916–1931, doi: 10.4319/lo.1998.43.8.1916

Jessen G L, Lichtschlag A, Ramette A, et al. 2017. Hypoxia causes preservation of labile organic matter and changes seafloor microbial community composition (Black Sea). Science Advances, 3(2): e1601897, doi: 10.1126/sciadv.1601897

Jiao Nianzhi, Yang Yanhui, Koshikawa H, et al. 2002. Responses of picoplankton to nutrient perturbation in the South China Sea, with special reference to the coast-wards distribution of *Prochlorococcus*. Acta Botanica Sinica, 44(6): 731–739

Johan W, Rassoulzadegan F, Hagström Å. 1990. Periodic bacterivore activity balances bacterial growth in the marine environment. Limnology and Oceanography, 35(2): 313–324, doi: 10.4319/lo.1990.35.2.0313

Jørgensen B B, Bak F. 1991. Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat, Denmark). Applied and Environmental Microbiology, 57(3): 847–856, doi: 10.1128/AEM.57.3.847-856.1991

Kirchman D L. 2002. The ecology of *Cytophaga–Flavobacteria* in aquatic environments. FEMS Microbiology Ecology, 39(2): 91–100

Kirchman D L. 2008. Microbial Ecology of the Oceans. 2nd ed. Hoboken: John Wiley and Sons, 187–190

Kolber Z S, van Dover C L, Niederman R A, et al. 2000. Bacterial photosynthesis in surface waters of the open ocean. Nature, 407(6801): 177–179, doi: 10.1038/35025044

Lee S M, Chao Anne. 1994. Estimating population size via sample coverage for closed capture-recapture models. Biometrics, 50(1): 88–97, doi: 10.2307/2533199

Lefort T, Gasol J M. 2014. Short-time scale coupling of picoplankton community structure and single-cell heterotrophic activity in winter in coastal NW Mediterranean Sea waters. Journal of Plankton Research, 36(1): 243–258, doi: 10.1093/plankt/fbt073

Li Daoji, Zhang Jing, Huang Daji, et al. 2002. Oxygen depletion off the Changjiang (Yangtze River) estuary. Science in China Series D: Earth Sciences, 45(12): 1137–1146, doi: 10.1360/02yd9110

Lin Fengzhu, Wu Yulin, Yu Haicheng, et al. 2008. Phytoplankton community structure in the Changjiang Estuary and its adjacent waters in 2004. Oceanologia et Limnologia Sinica (in Chinese), 39(4): 401–410

Liu Jiwen, Fu Bingbing, Yang Hongmei, et al. 2015. Phylogenetic shifts of bacterioplankton community composition along the Pearl Estuary: the potential impact of hypoxia and nutrients. Frontiers in Microbiology, 6: 64

Liu Min, Xiao Tian, Wu Ying, et al. 2012. Temporal distribution of bacterial community structure in the Changjiang Estuary hypoxia area and the adjacent East China Sea. Environmental Research Letters, 7(2): 025001, doi: 10.1088/1748-9326/7/2/025001

Liu Haijiao, Xue Bing, Feng Yuanyuan, et al. 2016. Size-fractionated chlorophyll *a* biomass in the northern South China Sea in summer 2014. Chinese Journal of Oceanology and Limnology, 34(4): 672–682, doi: 10.1007/s00343-016-5017-1

Llabrés M, Agustí S, Herndl G J. 2011. Diel in situ picophytoplankton cell death cycles coupled with cell division. Journal of Phycology, 47(6): 1247–1257, doi: 10.1111/j.1529-8817.2011.01072.x

Lohrenz S E, Redalje D G, Cai Weijun, et al. 2008. A retrospective analysis of nutrients and phytoplankton productivity in the Mississippi River plume. Continental Shelf Research, 28(12): 1466–1475, doi: 10.1016/j.csr.2007.06.019

Lou Xiulin, Hu Chuanmin. 2014. Diurnal changes of a harmful algal bloom in the East China Sea: observations from GOCI. Remote Sensing of Environment, 140: 562–572, doi: 10.1016/j.rse.2013.09.031

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

Mann A J, Hahnke R L, Huang Sixing, et al. 2013. The genome of the alga-associated marine Flavobacterium *Formosa agariphila* KMM 3901^T reveals a broad potential for degradation of algal polysaccharides. Applied and Environmental Microbiology, 79(21): 6813–6822, doi: 10.1128/AEM.01937-13

Martha S, Fuchs B M, Rudolf A, et al. 2010. 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

McBride M, Xie G, Martens E C, et al. 2009. Novel features of the polysaccharide-digesting gliding bacterium *Flavobacterium johnsoniae* as revealed by genome sequence analysis. Applied and Environmental Microbiology, 75(21): 6864–6875, doi: 10.1128/AEM.01495-09

Naqvi S W A, Jayakumar D A, Narvekar P V, et al. 2000. Increased marine production of N_2O due to intensifying anoxia on the Indian continental shelf. Nature, 408(6810): 346–349, doi: 10.1038/35042551

Oksanen J, Blanchet F G, Friendly M, et al. 2010. Vegan: community ecology package. R package version 2.5-7. http://CRAN.R-project.org/package=vegan [2020-11-28]

Olapade O A. 2012. Diel fluctuations in the abundance and community diversity of coastal bacterioplankton assemblages over a tidal cycle. Microbial Ecology, 63(1): 96–102, doi: 10.1007/s00248-011-9940-6

Olson R J, Chisholm S W, Zettler E R, et al. 1990. Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep Sea Research Part A: Oceanographic Research Papers, 37(6): 1033–1051, doi: 10.1016/0198-0149(90)90109-9

Ottesen E A, Young C R, Eppley J M, et al. 2013. Pattern and synchrony of gene expression among sympatric marine microbial populations. Proceedings of the National Academy of Sciences of the United States of America, 110(6): E488–E497, doi: 10.1073/pnas.1222099110

Ottesen E A, Young C R, Gifford S M, et al. 2014. Multispecies diel transcriptional oscillations in open ocean heterotrophic bacterial assemblages. Science, 345(6193): 207–212, doi: 10.1126/science.1252476

Partensky F, Blanchot J, Lantoine F, et al. 1996. Vertical structure of picophytoplankton at different trophic sites of the tropical northeastern Atlantic Ocean. Deep Sea Research Part I: Oceanographic Research Papers, 43(8): 1191–1213, doi: 10.1016/0967-0637(96)00056-8

Partensky F, Hess W R, Vaulot D. 1999. *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiology and Molecular Biology Reviews, 63(1): 106–127, doi: 10.1128/MMBR.63.1.106-127.1999

Pernthaler A, Pernthaler J. 2005. Diurnal variation of cell proliferation in three bacterial taxa from coastal North Sea waters. Applied and Environmental Microbiology, 71(8): 4638–4644, doi: 10.1128/AEM.71.8.4638-4644.2005

Pinhassi J, Sala M M, Havskum H, et al. 2004. Changes in bacterioplankton composition under different phytoplankton regimens. Applied and Environmental Microbiology, 70(11): 6753–6766, doi: 10.1128/AEM.70.11.6753-6766.2004

Pommier T, Neal P R, Gasol J M, et al. 2010. Spatial patterns of bacterial richness and evenness in the NW Mediterranean Sea explored by pyrosequencing of the 16S rRNA. Aquatic Microbial Ecology, 61(3): 221–233, doi: 10.3354/ame01484

Porter K G. 1996. Integrating the microbial loop and the classic food chain into a realistic planktonic food web. In: Polis G A, Winemiller K O, eds. Food Webs. Boston: Springer, 51–59

Pujalte M J, Lucena T, Ruvira M A, et al. 2014. The family Rhodobacteraceae. In: Rosenberg E, DeLong E F, Lory S, et al., eds. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Berlin, Heidelberg: Springer, 439–512

Qin Qilong, Zhang Xiying, Wang Xumin, et al. 2010. The complete genome of *Zunongwangia profunda* SM-A87 reveals its adaptation to the deep-sea environment and ecological role in sedimentary organic nitrogen degradation. BMC Genomics, 11(1): 247, doi: 10.1186/1471-2164-11-247

Rabalais N N, Turner R E. 2001. Hypoxia in the northern Gulf of Mexico: description, causes and change. In: Rabalais N N, Turner R E, eds. Coastal Hypoxia: Consequences for Living Resources and Ecosystems. Washington: American Geophysical Union, 1–36

Rappé M S, Vergin K, Giovannoni S J. 2000. Phylogenetic comparisons of a coastal bacterioplankton community with its counterparts in open ocean and freshwater systems. FEMS Microbiology Ecology, 33(3): 219–232, doi: 10.1111/j.1574-6941.2000.tb00744.x

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

Rinke C, Schwientek P, Sczyrba A, et al. 2013. Insights into the phylogeny and coding potential of microbial dark matter. Nature, 499(7459): 431–437, doi: 10.1038/nature12352

Ruiz-González C, Lefort T, Massana R, et al. 2012. Diel changes in bulk and single-cell bacterial heterotrophic activity in winter surface waters of the northwestern Mediterranean Sea. Limnology and Oceanography, 57(1): 29–42, doi: 10.4319/lo.2012.57.1.0029

Sato M, Horne J K, Parker-Stetter S L, et al. 2016. Hypoxia impacts on food web linkages in a pelagic ecosystem. https://ui.adsabs.harvard.edu/abs/2016AGUOSME24E0756S/abstract [2018-7-30]

Schattenhofer M, Fuchs B M, Amann R, 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

Singh P, Teal T K, Marsh T L, et al. 2015. Intestinal microbial communities associated with acute enteric infections and disease recovery. Microbiome, 3(1): 45, doi: 10.1186/s40168-015-0109-2

Stevens H, Ulloa O. 2008. Bacterial diversity in the oxygen minimum zone of the eastern tropical South Pacific. Environmental Microbiology, 10(5): 1244–1259, doi: 10.1111/j.1462-2920.2007.01539.x

Stubner S. 2002. Enumeration of 16S rDNA of *Desulfotomaculum* lineage 1 in rice field soil by real-time PCR with SybrGreen^{TM} detection. Journal of Microbiological Methods, 50(2): 155–164, doi: 10.1016/S0167-7012(02)00024-6

Suyama T, Shigematsu T, Suzuki T, et al. 2002. Photosynthetic apparatus in *Roseateles depolymerans* 61A is transcriptionally induced by carbon limitation. Applied and Environmental Microbiology, 68(4): 1665–1673, doi: 10.1128/AEM.68.4.1665-1673.2002

Synytsya A, Čopíková J, Kim W J, et al. 2015. Cell wall polysaccharides of marine algae. In: Kim S K, ed. Springer Handbook of Marine Biotechnology. Berlin: Springer, 543–590

Tang Kai, Lin Yingfan, Han Yu, et al. 2017. Characterization of potential polysaccharide utilization systems in the marine *Bacteroidetes gramella flava* JLT2011 using a multi-omics approach. Frontiers in Microbiology, 8: 220

Teeling H, Amann R. 2012. Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science; 336(6081): 608–611, doi: 10.1126/science.1218344

ter Braak C J F, Smilauer P. 2002. Canoco reference manual and canodraw for windows user’s guide: software for canonical community ordination (version 4.5). Ithaca NY, USA: Microcomputer Power

Thrash J C, Seitz K W, Baker B J, et al. 2017. Metabolic roles of uncultivated bacterioplankton lineages in the northern Gulf of Mexico “dead zone”. mBio, 8(5): e01017–17

Turner R E, Rabalais N N, Justic D. 2008. Gulf of Mexico hypoxia: alternate states and a legacy. Environmental Science & Technology, 42(7): 2323–2327

Vaulot D, Marie D, Olson R J, et al. 1995. Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the equatorial Pacific Ocean. Science, 268(5216): 1480–1482, doi: 10.1126/science.268.5216.1480

Wang Lei, Zhong Chao, Liu Xin, et al. 2013. The comparative study on the diurnal variations of phytoplankton community between the northeastern South China Sea and the East China Sea in summer. Haiyang Xuebao (in Chinese), 35(6): 170–177

Wickham H, Chang W. 2009. ggplot2: an implementation of the grammar of graphics. R package version 0.7. https://www.researchgate.net/publication/245585126_ggplot2_An_Implementation_of_the_Grammar_of_Graphics [2018-6-30]

Wright J J, Konwar K M, Hallam S J. 2012. Microbial ecology of expanding oxygen minimum zones. Nature Reviews Microbiology, 10(6): 381–394, doi: 10.1038/nrmicro2778

Wright J J, Mewis K, Hanson N W, et al. 2014. Genomic properties of marine group a bacteria indicate a role in the marine sulfur cycle. The ISME Journal, 8(2): 455–468, doi: 10.1038/ismej.2013.152

Wu Dongmei, Dai Qiuping, Liu Xuezhu, et al. 2019. Comparison of bacterial community structure and potential functions in hypoxic and non-hypoxic zones of the Changjiang Estuary. PLoS One, 14(6): e0217431, doi: 10.1371/journal.pone.0217431

Yan Shi, Fuchs B M, Lenk S, et al. 2009. Biogeography and phylogeny of the NOR5/OM60 clade of Gammaproteobacteria. Systematic and Applied Microbiology, 32(2): 124–139, doi: 10.1016/j.syapm.2008.12.001

Yan Weijin, Yang Libiao, Wang Fang, et al. 2012. Riverine N_2O concentrations, exports to estuary and emissions to atmosphere from the Changjiang River in response to increasing nitrogen loads. Global Biogeochemical Cycles, 26(4): GB4006

Yang Dezhou, Yin Baoshu, Liu Zhiliang, et al. 2012. Numerical study on the pattern and origins of Kuroshio branches in the bottom water of southern East China Sea in summer. Journal of Geophysical Research, 117(C2): C02014

Ye Qi, Wu Ying, Zhu Zhuoyi, et al. 2016. Bacterial diversity in the surface sediments of the hypoxic zone near the Changjiang Estuary and in the East China Sea. MicrobiologyOpen, 5(2): 323–339, doi: 10.1002/mbo3.330

Zhang J, Zhang Z F, Liu S M, et al. 1999. Human impacts on the large world rivers: would the Changjiang (Yangtze River) be an illustration?. Global Biogeochemical Cycles, 13(4): 1099–1105, doi: 10.1029/1999GB900044

Zheng Qiang, Wang Yu, Xie Rui, et al. 2017. Dynamics of heterotrophic bacterial assemblages within *Synechococcus* cultures. Applied and Environmental Microbiology, 84(3): e01517–17

Zhu Zhuoyi, Zhang Jing, Wu Ying, et al. 2011. Hypoxia off the Changjiang (Yangtze River) Estuary: oxygen depletion and organic matter decomposition. Marine Chemistry, 125(1–4): 108–116

Appendix:

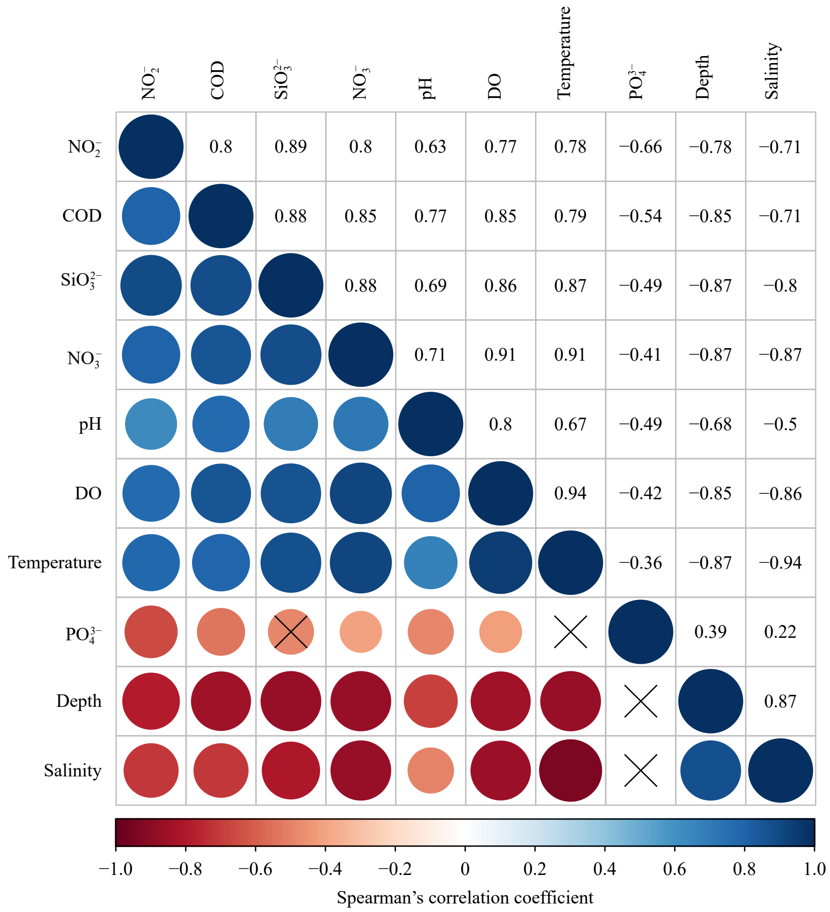

Fig. A1. Relationships among physicochemical and environmental factors. A correlation matrix showed the Spearman’s correlation coefficients, which ranged from –1 to 1, among the environmental factors. The correlation coefficients were shown in the corresponding circles. Blue and red indicated positive and negative values, respectively. The black crossed on some cells indicated an insignificant correlation (p>0.05). DO, dissolved oxygen; COD, chemical oxygen demand.

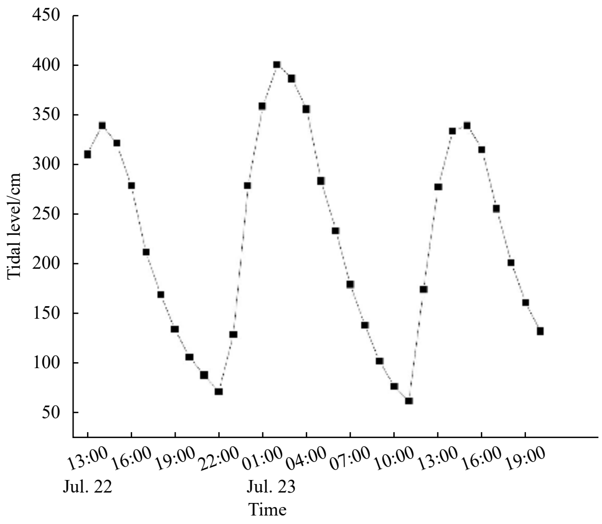

Fig. A2. The tidal graph showing the tide changes from July 22 13:00 to July 23 19:00 in 2016 in Wusong (31°23′N, 121°29′E), Shanghai. The original data were downloaded from https://www.cnss.com.cn/tide/. The tidal observation point is the closest one to the sampling site of this study and is used for reference.

Table A1. Alpha diversity indexes for each sample, including observed OTUs, Chao1, Shannon indexes

Sample ID	Bacterial sequences reads	Normalized observed OTUs index	Normalized Chao1 index	Normalized Shannon index
S16_1	40 471	323	438.111	5.160
S19	39 810	338	497.638	5.191
S22	20 653	401	518.109	5.261
S01	40 648	374	481.859	5.550
S04	35 956	392	553.803	5.326
S07	39 104	336	539.947	5.178
S10	41 686	359	507.222	5.274
S13	37 159	336	488.442	5.046
S16_2	34 361	367	565.571	5.303
M16_1	39 930	466	645.754	5.582
M19	38 884	454	589.584	5.417
M22	31 041	458	552.182	5.753
M01	33 070	447	635.750	5.464
M04	41 446	401	624.500	5.353
M07	38 264	421	625.915	5.454
M10	39 243	468	589.016	5.699
M13	35 169	468	643.918	5.422
M16_2	28 693	377	573.938	5.168

Note: The seawater samples were taken from surface and middle layers (Abbreviation in ID number: S and M). The numbers in the sample ID indicate at what time the sample was taken over the 24 h period. For example, the sample S16_1 was the surface sample taken at 16:00 on the first day of sampling. OTU, operational taxonomic unit.

Table A2. The correlation coefficients and significances between the top 29 families and *Synechococcus* in oxic and hypoxic layers

Family	Oxic layer		Hypoxic layer	
	r	p	r	p
OCS155	−0.117	0.765	0.367	0.332
C111	−0.025	0.949	0.667	0.05
wb1_P06	−0.326	0.391	0.636	0.066
Balneolaceae	0.075	0.847	0.217	0.576
Saprospiraceae	−0.117	0.765	0.517	0.154
Flammeovirgaceae	−0.067	0.865	−0.167	0.668
Flavobacteriaceae	0.367	0.185	0.386	0.213
Cryomorphaceae	−0.05	0.898	0.55	0.125
NS9	0.033	0.932	0.226	0.558
Pelagibacteraceae	−0.067	0.865	−0.033	0.932
Rhodobacteraceae	0.55	0.087	0.45	0.124
Rhodospirillaceae	−0.427	0.17	−0.5	0.17
AEGEAN_112	−0.417	0.265	−0.017	0.966
Erythrobacteraceae	0.417	0.265	0.075	0.847
Methylophilaceae	0.017	0.966	−0.033	0.932
Comamonadaceae	0.159	0.683	0.301	0.431
Nitrospinaceae	−0.767	0.016	−0.5	0.17
Halomonadaceae	−0.267	0.488	−0.483	0.187
OM60	0.45	0.13	0.483	0.187
Piscirickettsiaceae	−0.467	0.205	−0.317	0.406
Alteromonadaceae	−0.333	0.381	−0.417	0.103
HTCC2188	−0.653	0.057	0.301	0.431
Oceanospirillaceae	−0.226	0.559	0.117	0.764
Thiohalorhabdaceae	−0.134	0.731	−0.1	0.798
Coxiellaceae	−0.282	0.462	−0.2	0.606
Vibrionaceae	−0.236	0.54	−0.133	0.732
A714017	−0.567	0.112	−0.633	0.067
AEGEAN_185	−0.218	0.572	−0.167	0.668
Puniceicoccaceae	−0.367	0.332	−0.433	0.244