The effect of southwest monsoon on the meso-scale biogeographic patterns of the bacteria in the northeast of South China Sea

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Abstract: Complicated physical systems and strong seasonal monsoons are features of the north-east South China Sea (SCS). To understand how monsoon-driven changes affect the bacterial community structure and distribution in the surface water from shelf to slope in the northeast SCS (NESCS), we collected water samples during the intermonsoon (Spring, 2021) and Southwest monsoon (May, 2021) respectively. In our research, we found that α diversity of bacteria did not differ significantly during intermonsoon and monsoon periods, nor did it differ significantly between shelf and slope. However, bacterial community were well differentiated between groups (Shelf-I, Slope-I, Shelf-M, and Slope-M). In both seasons, there was a significant geographical distance decay relationship, but the monsoon did not change the biogeographic pattern of bacteria. Finally, all environmental factors in both seasons, except salinity, have a significant impact on bacteria, and the correlation is enhanced during the monsoon.

1 INTRODUCTION

The South China Sea (SCS) is the biggest marginal sea in the northwest Pacific Ocean. There is a vast continental shelf in the northern South China Sea. The South China Sea is located in the tropical and subtropical zone, where the northeast monsoon prevails in winter and the southwest monsoon prevails in summer[18], while spring and autumn are the intermonsoon periods. Influenced by the monsoon, the Eastern Guangdong coastal current flows to the northeast in summer, and is also affected by the runoff from the the Pearl River[18]. As a key environmental variable affecting the whole South China Sea, the monsoon mainly controls the phytoplankton ecosystem in the north of the South China Sea by changing the depth of the mixed layer and triggering local upwelling, thus affecting the nutrient supply of the upper layer[8], consequently higher trophic level of biological community changed during the monsoon[16].

Bacteria are an important composition of the microbial food web. Autotrophic picophytoplankton is also widely consumed by many juvenile zooplankton, affecting their growth[1]. Therefore, it is of high importance to study the bacterial community. However, the composition and distribution of bacteria may differ between different areas and seasons. For examples, Gong et al. reported the variation of bacterial community composition from the shelf to slope area influenced by upwelling in the northern SCS (NSCS)[5]. The abundance of *Synechococcus* and *Protochlorococcus* had opposite trend from onshore to offshore[19]. And several studies about the seasonal changes. were conducted in the SCS[9]. The bacterial community disturbance driven by monsoon had been reported mainly in Eastern tropical Indian Ocean[4] , which was a less mentioned topic in SCS.

Community Similarity usually decreases as distance increases, a phenomenon known as distance decay relationship, which is used as a quantitative technique to describe biographical patterns[13]. Geographical distance is one of the reasons for the decline of biological similarity. So far, It has shown that the spatial scale may affect the biogeographic patterns in SCS[12]. However, few concerns were given for the potential biogeographic pattern change by different season in SCS.

In our research, we used the method of DNA metabarcoding to explore the spatiotemporal distribution pattern of bacteria in the oligotrophic surface waters of the north-east South China Sea (NESCS) during intermonsoon (spring) and monsoon (summer) period. We

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hypothesize that the monsoon could distinguish the bacterial community composition and change the biogeographic pattern.

2 METHOD AND MATERIALS

2.1 Sampling Sites and Collection

During the intermonsoon (May 18-22, 2021) and the southwest monsoon period (August 16-20, 2021) on the shelf (depth < 200m) and slope (depth > 200m) of the northeast South China Sea respectively, two surveys were conducted on the R.V. Yanping 2 ship (Figure 1). During the two cruises, 11 samples were collected during the intermoon period, and 13 samples were collected during the monsoon period along the latitudinal gradient transect. The bacteria sampling method is as follows: taking 4L seawater from the surface with a bucket, and using 200 μM bolting-silk pre-filtration to remove debris, large and medium-sized zooplankton, then 0.22 μM pore diameter (diameter 47 mm polycarbonate, Millipore, Massachusetts, USA) for filtration. All samples were immediately stored in liquid nitrogen on site, and then transferred to the laboratory − 80 ° C refrigerator for storage until DNA was extracted.

Use RBR concerto3 CTD (RBR Ltd, Canada) to record temperature, salinity, chlorophyll a concentration and DO_2 (dissolved oxygen concentration). To ensure the validity of the data, the equipment has been pre-calibrated in the laboratory

2.2 DNA Extraction, PCR Amplication And Illumina Miseq Sequencing

The DNA of bacteria was extracted using the Dnasy PowerSoil Kit (QIAGEN, California, USA), following to the steps provided by the manufacturer. The concentration of total DNA, as well as purity were detected with NanoDrop ND-100 spectrophotometer (Thermo Fisher Scientific, Delaware, USA). The bacteria were amplified in 16S V3-V4 regions (480bp) using primers, Forward: 341F (5 '- CCTAYGGGRBGCASCAG-3') and Reverse: 806R (5 '- GACTACNNGGTTATCTAAT-3')[11].

The PCR components contained 0.25 μl of high-fidelity DNA Polymerase (5U/μl), 5 μl of Reaction buffer (5×), 5 μl of High GC buffer (5×) 2 μl (10 mM) of dNTPs, 2 μl of DNA Template, 1 μl (10 uM) of each Forward and Reverse primer respectively, and 8.75 μl of ddH_2O. Thermal circulation includes initial denaturation at 98℃ for 5 min, and then 25 cycles consisted of denaturation at 98℃ for 30 s, being annealled at 53℃ for 30 s, and extension at 72℃ for 45 s, with a final extension of 5 min at 72℃. PCR products

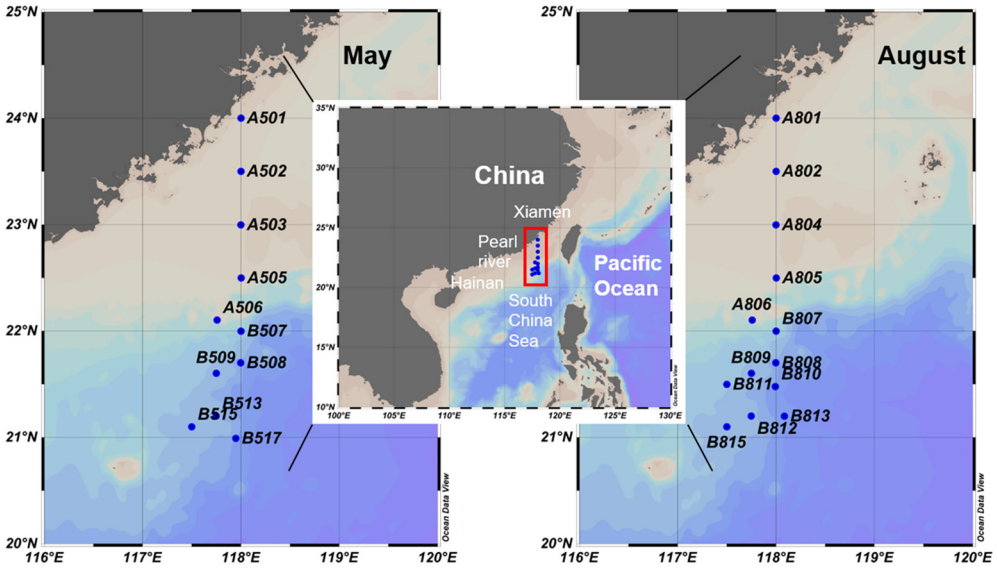

Figure 1: Sampling locations in the northeast of South China Sea during May (intermonsoon) and August (Southwest monsoon). Station labels initialize "A" meaning that the bottom depth of the station is less than 200 m for shelf area and "B" meaning that is more than 200 m for slope area.

were conducted on a 2% agarose gel, and fragments from the gel were purified using Agencourt AMPure Beads (Beckman Coulter). After purifying on the gel, production of PCR were amplified using the PicoGreen dsDNA Assay Kit (Invitrogen). The PCR amplification products were sequenced at the Illumina MiSeq PE250 platform from the Shanghai Personal Biotechnology Co., Ltd (Degnan & Ochman, 2012). Using Illumina's TruSeq Nano DNA LT Library Prep Kit to prepare libraries. The PCR amplification products were pooled to form a library for sequencing.

2.3 Sequence Data Processing

The raw sequence data were amplified using QIIME2. The DEMUX plugin is used to multiplex the raw sequence data, and then the CutAdapt plugin is used to cut the primer[10]. Then use the DADA2 plugin to perform quality filtering, denoising, merging and chimera removal on the sequence. Non-monomorphic amplified sequence variants (ASV)

was compared by MAFFT. For each amplicon sequence variant (ASV), using UCLUST[2] with 100% identity sequence to assign high-quality representative sequences. ASV is also used to construct the phylogenetic tree of Fasttree2[14]. Taxonomic classification was conducted using Silva 16S rRNA gene database Release 132[15]. All chloroplasts, mitochondria, archaea, eukaryotes, unknown sequences and ASVs containing single sequences were removed from the data set. Finally, to reduce potential mistakes caused by unequal sequences in every samples, we randomly resampled all the ASV to make sure the same number of sequences per sample (39254) on MOTHUR v 1.33.3[17].

2.4 Statistical Analyses

Using ggplot2 package to visualize and using t.test in "ggpubr" package to test the significance difference of environment variables.

The composition of bacteria communities were plotted at the top10 Class levels. Alpha diversity indices (Chao1 and Shannon–Wiener indices) was calculated using package "vegan" in R and were visualized using the "ggplot2" package. Using wilcox tests to evaluate differences between groups (Shelf-M vs Slope-M, Shelf-I vs Slope-I, Shelf-I vs Shelf-M, Slope-I vs Slope-M).

Before following analysis, the community data were Hellinger-transformed to improve normality and homoscedasticity. To realize the visualization of the overall change of community composition of bacteria, non-metric multidimensional scaling (NMDS) based on a Bray–Curtis metric was done. A measure of goodness of fit of the ordination was given by a stress value, being set at < 0.20 to minimize misinterpretation. An analysis of similarity (ANOSIM) was applied to detect for significant differences between groups (pairwise comparison: Shelf-M vs Slope-M, Shelf-I vs Slope-I, Shelf-I vs Shelf-M, Slope-I vs Slope-M. The same below). The global R in ANOSIM has a range of 0 to 1, meaning the degree of difference between groups; R = 0 indicating no difference, whereas R = 1 suggesting complete difference. Moreover, the variations in community compositions between groups were done respectively for bacteria through a Permutational multivariate analysis of variance (PERMANOVA).

To detect distance-decay relationship, we fitted linearly community similarity (1-Bray-curtis dissimilarity) with geographical distance(km) and the relationship were tested using Spearman's rank correlation. The significant difference of the slope between bacteria between seasons were detected using package "simba" in R.

To dectect the effect environmental factors, Mantel tests using Pearson method were conducted.

3 RESULTS

During the intermonsoon period, the sea surface temperature ranged from 26.21 to 31.07°C, salinity ranged from 31.89 to 34.16 psu, and chlorophyll *a* ranged from 0.02 to 0.78 µg/L, dissolved oxygen in 185.39 - 206.27 µg/L. In the monsoon period, the temperature ranged from 25.88 - 30.06°C, the salinity ranged from 32.87 - 34.10 psu, and the chlorophyll *a* ranged from 0 - 1.25 µg/L, dissolved oxygen between 190.80 and 204.99 µg/L. Between the shelf and the slope (Figure 2), significant differences in temperature between the two seasons (intermonsoon period: t = -3.34, $p < 0.05$; monsoon period: t = -3.71, p<0.05) were detected, chlorophyll a is significantly different in monsoon period (t = 3.68, $p < 0.05$), and dissolved oxygen is significantly different during intermonsoon period (t = 2.40, $p < 0.05$) and monsoon period (t = 2.85, $p < 0.05$). During the intermonsoon and monsoon periods (Figure 2), significant differences in temperature (t = 2.84, $p < 0.05$) and salinity (t = 2.5971, $p < 0.05$) were observed in the slope area, and significant differences of chlorophyll *a* (t = 4.855, $p < 0.001$) were observed in the shelf area.

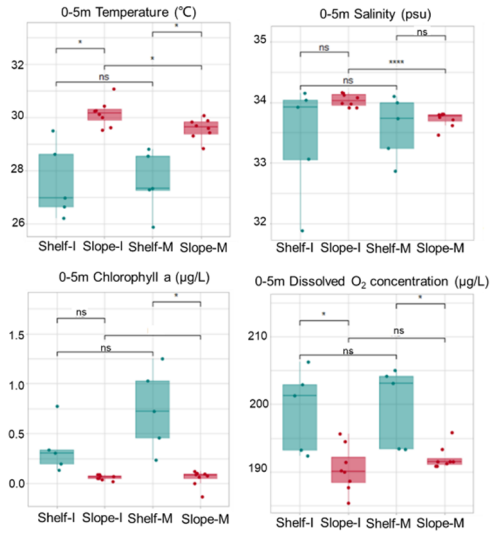

Figure 2: Boxplots of environmental variabilities, comparing the differences between slope and shelf, and between intermonsoon (May, green line box) and monsoon (August, red line box). a: surface temperature (surface 0-5 m); b: 0-5 m surface salinity; c: chlorophyll *a* concentration in 0-5 m water column; d, dissolved oxygen concentration in 0-5 m water column. Significance by t-test: ns ($p \geq 0.05$); * ($0.01 \leq p < 0.05$); ** ($0.001 \leq p < 0.01$); *** ($p < 0.001$).

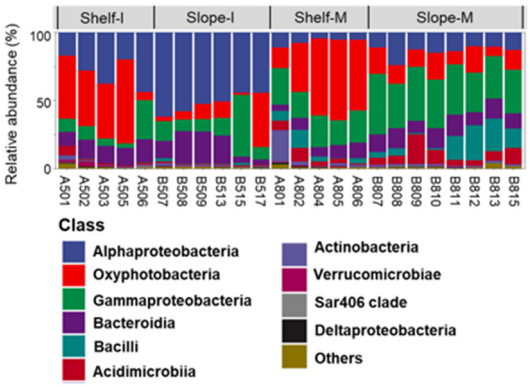

Figure 3: Relative abundance of ASVs per Class for bacteria across each site. "I" means Intermonsoon period, "M" means Monsoon period

Bacteria are mainly composed of Proteobacteria (48.4%, α- and γ- Proteobacteria), Cyanobacteria (25%, Oxyphotobacteria), Firmicutes (12.8%, Bacilli), Acidobacteria (7%, Acidimicrobiia) and Acitinobacteria (5.6%). Compared with the intermonsoon period, The relative abundance of α-Proteobacteria during the monsoon period decreased largely, The relative abundance of γ-Proteobacteria increased drastically. In terms of regional differences, compared with slope, the relative abundance of α-Proteobacteria in the shelf is low, while Oxyphotobacteria is high (Figure 3).

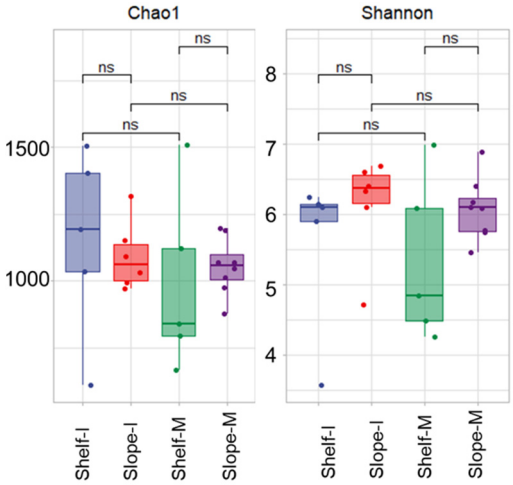

Figure 4: Chao1 and Shannon index of bacteria, between intermosoon and monsoon as well as shelf and slope based on ASV matrix. Significance by Wilcox test: ns ($p \geq 0.05$); * ($0.01 \leq p < 0.05$); ** ($0.001 \leq p < 0.01$); *** ($p < 0.001$).

Regarding Chao1 and Shannon index (Figure 4), there is no significant spatiotemporal difference in bacterial community.

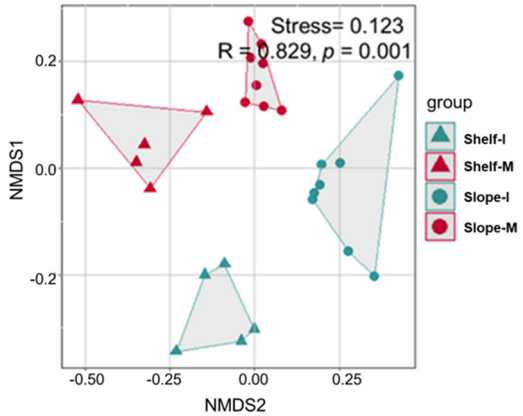

Figure 5: Non-metric multidimensional scaling (nMDS) analysis at the basis on Bray-Curtis dissimilarity index for bacteria

The NMDS (Figure 5) and ANOSIM results were used to display the differences between the four groups of bacteria (Stress = 0.123, R = 0.829, p = 0.001), the shelf and the slope regions during the monsoon and intermonsoon periods.

According to the results of PERMANOVA (Table 1), there are significant seasonal differences (F = 7.21, adj.P.Value = 0.012) of bacteria in the slope area, and significant spatial differences in the intermonsoon period (F = 4.31, adj.P.Value = 0.006) monsoon period (F = 5.06, adj.P.Value = 0.006)

Table 1: Quantitative effect of different bacterial groups on variations at the basis of the permutational multivariate analysis of variance (PERMANOVA).

pairs	F.Model	R^2	adj.P.Value
Shelf-I vs Slope-I	4.31	0.28	**0.006**
Shelf-M vs Slope-M	5.06	0.31	**0.006**
Shelf-I vs Shelf-M	2.42	0.23	0.054
Slope-I vs Slope-M	7.21	0.34	**0.012**

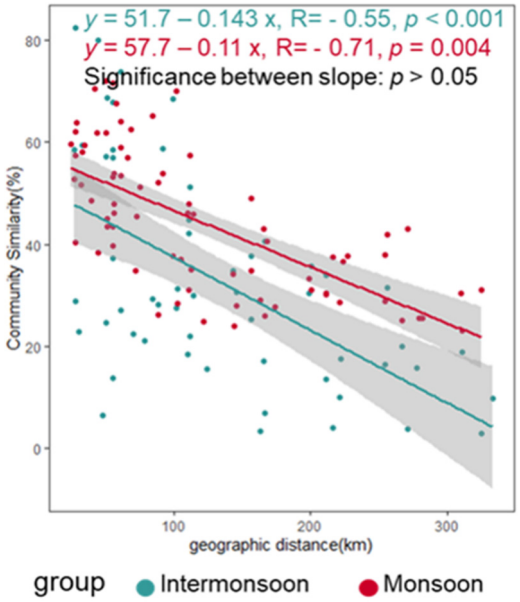

Figure 6: Distance decay relationships based on the community dissimilarity of bacteria northeast SCS during Intermonsoon and Monsoon.

During the intermonsoon period (May) and the monsoon period (August), the community similarity of bacteria was significantly correlated with geographical distance (Figure 6). Moreover, There is no significant difference in slope between the two seasons.

Table 2: Mantel tests for the correlations between environmental factors (Euclidean distance) and β-diversity of Bacteria (bray-curtis dissimilarity) with 999 permutations. Significance by Pearson: * ($0.01 \leq p < 0.05$); ** ($0.001 \leq p < 0.01$); *** ($p < 0.001$).

		Intermonsoon	Monsoon
Environment	R	0.5**	0.63**
Temperature	R	0.6**	0.71***
Salinity	R	-0.2	0.34
Chorophyll *a*	R	0.56**	0.86***
Dissovled O_2	R	0.47**	0.57*

Mantel test showed that the overall sea surface environment factors was significantly correlated with the bacteria in both seasons (Table 2). During both intermonsoon and monsoon periods, it is significantly correlated with temperature, chlorophyll *a*, and dissolved

O_2, but not with salinity; Moreover, the correlation coefficient R between bacteria and above three environmental factors increases during the monsoon period.

4 DISCUSSION

In our study, there are significant distinction from shelf to slope in bacteria, which may be due to the dominance of Oxyphotobacteria (mainly *Synechococcus*) on the continental shelf which had been proved in the previous study[28]. Meantime, there are seasonal differences in bacterial communities in the slope area, which may be due to the significant changes in the relative abundance of alpha proteobacteria and Bacilli.

Many studies have reported the distance decay relationship of bacteria[6],[12]. However, monsoon did not change the biogeographic pattern of bacteria significantly via the freshwater from Pearl River and wind-driven currents which may change water connectivity and shape stronger spatial distribution pattern. This could be partly explained that small passively dispersing plankton has lower losses of sinking[3] and the greater survival times of resting stages[7] which allows their populations to travel more distant than larger size plankton. And it also could be explained by spatial scale which is not large enough[12]. But this underlying mechanism between intermonsoon and monsoon needs further studies.

5 CONCLUSION

This paper evaluates the distribution differences of bacteria on the shelf and slope of the northeast South China Sea and between monsoon and inter monsoon periods. community structure between the shelf and slope are significantly different, as well as between monsoon and monsoon periods, and bacteria also exhibit significant spatial distribution patterns between the two seasons. Besides, all environmental factors except salinity during both seasons had significant impact on bacteria. However, the monsoon has not changed the alpha diversity and spatial distribution pattern of bacteria. Although we have discussed the potential mechanisms in discussion part, it still needs more works and studies to support our research.

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