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Impact of oyster culture on coral reef bacterioplankton community composition and function in Daya Bay, China

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ABSTRACT: Subtropical coral reefs along the coast are facing multiple pressures. Mariculture is one of the main sources of such pressure. Oyster culture has become a worldwide phenomenon in coastal ecosystems. Due to the high filtration efficiency of oysters, their culture has helped to purify some coastal waters. However, high-density oyster culture has also had negative effects on coastal ecosystems, including the loss of natural habitat, changes in hydrology, cross infection of corals with pathogenic bacteria, and changes to the structure and function of bacterioplankton communities. In this study, the effect of oyster culture on coral reefs was characterized based on variability in the structure and function of bacterioplankton communities. Using 16S rRNA gene sequencing, a comprehensive bacterioplankton reference database was constructed for coral reef habitats associated with oyster culture and subjected to different disturbance gradients. Small shifts in the surrounding coral reef environment caused by oyster culture disturbance were detected by comparing the structure and function of bacterioplankton communities with biogeochemical parameters. The measured chemical dynamics explained 71.15% of the bacterioplankton community variability between habitats. Oyster culture increased the richness and diversity of bacterioplankton communities. Species composition similarity was highest between the oyster culture area and the nearest coral reef habitat. The spatial turnover in the bacterioplankton community was characterized by less uniform community assembly patterns. The bacterioplankton function of reefs relatively far from anthropogenic disturbance differed from that of those closer to such disturbances. Our results also show that the variability in structure and function of bacterioplankton communities between oyster culture areas and coral reef areas was mainly driven by salinity and ammonium. Oyster culture can impact bacterioplankton community composition and dynamics around coral reef habitats. The results provide an important context for developing frameworks for managing ecological interactions among oyster cultures and coral reef habitats of concern.

KEY WORDS: Bacterioplankton · Coral reef · Oyster culture · 16S rRNA · Disturbance

1. INTRODUCTION

Coral reefs support a series of ecosystem services and goods that contribute to the production and maintenance of billions of organisms (Woodhead et al. 2019). Since the Anthropocene, human disturbance has led to changes in temperate coral reef ecosystems, resulting in novel dynamics (Magel et

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al. 2019, Williams & Graham 2019). The changes can manifest as transformation of morphological traits (Zawada et al. 2019), reef structural changes (Perry & Alvarez-Filip 2019), reshaping of coral–algal symbiosis (Tong et al. 2017), or compositional and functional shifts of coral-associated microbial communities (Sweet et al. 2017, Glasl et al. 2019). Once gametes are produced, conditions in the water column regulate the stone coral reproductive processes of fertilization, planktonic larval dispersal, larval settlement, post-settlement growth, and survival (Ceh et al. 2012). Bacterial communities in coral tissues may be acquired after larval settlement and development of the juvenile polyp (Sharp et al. 2010, Leite et al. 2017). Environmental changes can affect all life stages, but the early stages of marine organisms are generally more sensitive to environmental stress than the adult stages (Reichelt-Brushett & Harrison 1999, Styan & Rosser 2012, Guillemette et al. 2018). If these early life stages are disrupted, this can have negative impacts on recruitment and affect the survival and distribution of corals (Albright & Langdon 2011). The bacterioplankton community plays an important role in coral reproduction. It has been reported to be an important component of the microbiome in the early life stages of the brooder *Porites astreoides* and the broadcast spawner *Acropora millepora* (Damjanovic et al. 2020).

Oysters have a long history as a marine culture species in coastal ecosystems (van der Schatte Olivier et al. 2018, Wijsman et al. 2019). Bivalves graze on natural plankton communities and are valued for providing food and other cultural and ecosystem services (Smyth et al. 2016, Smith et al. 2018, van der Schatte Olivier et al. 2018). Over time, oyster cultivation technology developed different models and adapted to new environments, but these changes were not without consequences for the surrounding environment (Frawley 2017, de Souza Sampaio et al. 2019). The adverse effects of high-density oyster farming on coastal ecosystems have been studied and documented. These include habitat loss, changes in hydrological characteristics (Gaurier et al. 2011), biogenic deposits (Comeau et al. 2014, Shi et al. 2019), infection of corals by *Vibrio coralliilyticus* (Ushijima et al. 2018), and alteration of the structure and functioning of planktonic communities (Hulot et al. 2020).

When coral spawning takes place every year, the polyps and gametes are sensitive to surrounding environmental pressures. The gametes represent a vast amount of nutrients in sea water that may produce slicks (Jamodiong et al. 2018). The bacterioplankton community structure and function may fluctuate in response to a pulse of nutrients from the spawning event (Guillemette et al. 2018) and potentially pathogenic bacteria may reach outbreak levels (Haas et al. 2016). Short-lasting phytoplankton blooms may develop within a few days, accompanied by rising temperatures and eutrophication events (Wild et al. 2008). With their high filtration efficiency, oyster farms may play a purifying role and/or influence coral susceptibility to disease during early life stages. However, these roles are not yet well understood (Burge et al. 2016).

In this study, the structure and diversity of bacterioplankton community profiles from oyster farms and coral reefs in Daya Bay, China, were analyzed. The goal of the study was to gain insight into the response of bacterioplankton communities to oyster culture and the resulting changes in the nearby coral reef environment during the early life stages of the coral. As filter bivalves that process large volumes of seawater daily, oysters are especially susceptible to changes in bacterial community composition in the water (Burge et al. 2016). Moreover, bacterioplankton both contribute to and serve as indicators of coral health and function of the microbial community (Le Roux et al. 2016). This information will deepen our understanding of environmental interactions and changes in biogeochemical cycling between the different habitats.

2. MATERIALS AND METHODS

2.1. Study area

This study was carried out in western Daya Bay, east of the city of Shenzhen. This economically advanced city is responsible for various disturbances to the bay (Shi et al. 2019), such as longline oyster farming, urban runoff, and holiday resorts adjacent to coral reefs (Tong et al. 2019). Daya Bay is a semi-enclosed embayment in the northeast South China Sea (Fig. 1). The bay includes diverse habitats, coral reefs, and oyster and fish cage mariculture areas (Qin et al. 2019, Chen & Chen 2020). The average water depth in the oyster aquaculture areas is 5 m (range: 4–7 m). The coral reef habitats next to the oyster aquaculture areas have a discontinuous distribution along the coastline. The average water depth of the coral reef habitat is 2 m (range: 1–5 m). The annual mean seawater surface temperature is 28.0°C.

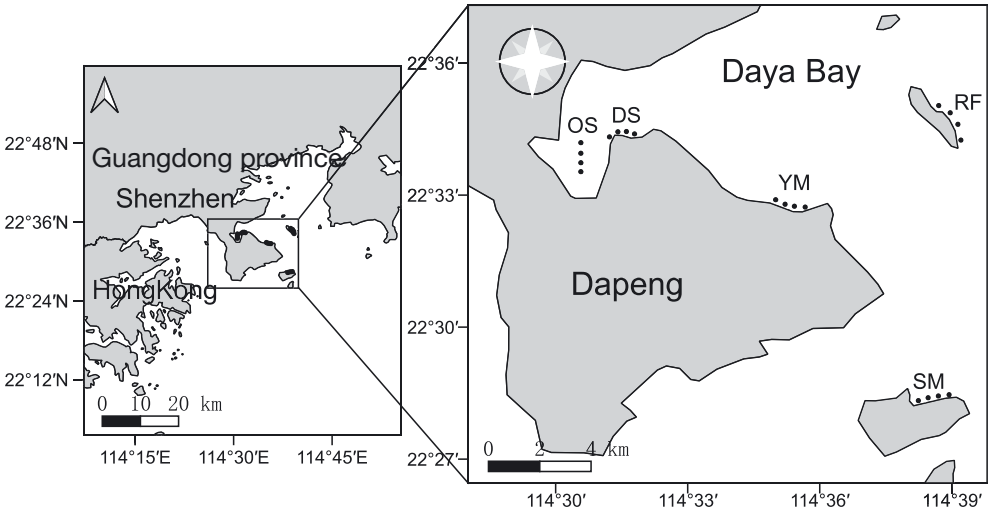

Fig. 1. Sampling sites (•) in the Daya Bay study area. DS: Dongshan; YM: Yangmei; SM: Sanmen; RF: reference; OS: oyster aquaculture area

2.2. Sample collection and environmental measurements

Samples were collected on 4 June 2018. Water samples were collected with a water extractor from the surface layer (0.5 m from the surface) at the oyster aquaculture area (OS), and 4 coral reef areas Dongshan (DS), Yangmei (YM), Sanmen (SM), and reference (RF) (Fig. 1). DS, YM, and SM were connected by alongshore currents. RF was facing the open sea, and it was less connected with other sites. OS was about 1, 8, 17, and 15 km from the DS, YM, SM and RF sites, respectively. Four samples (1–4) with 3 replicates each were collected at each site. Seawater samples for bacterioplankton analysis were pre-filtered (50 µm) to remove large particles and subsequently filtered (2 l) onto 0.2 µm Sterivex filters (Millipore) (Glasl et al. 2019). Samples were flash-frozen in liquid nitrogen and stored at −80°C prior to DNA extraction.

For environmental factor analyses, chlorophyll *a* (chl *a*) concentration was determined using a portable chl *a* fluorescence tester (Aquafluor, Turner Designs). Sea surface temperature (SST), pH, salinity, and dissolved oxygen (DO) were determined using a portable water quality analyzer (6600EDS, YSI). Within 24 h of sampling, a spectrophotometer was used to determine ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), silicate (SiO_3^{2-}), chemical oxygen demand (COD), and soluble reactive phosphate (SRP). The hydrodynamic model FVCOM (Unstructured Grid Finite Volume Community Ocean Model v. 2.7.1; http://fvcom.smast.umassd.edu/fvcom/) was used to evaluate the interactions among the research areas based on flow analysis.

2.3. PCR amplification and high-throughput MiSeq sequencing

Bacterioplankton DNA was extracted from filters using a Power Water DNA Isolation Kit (Mo Bio Laboratories). The 16S rRNA gene covering the V4 region was amplified with the universal bacterial barcoded primer pair 515FmodF (5′-GTG YCA GCM GCC GCG GTA A-3′) and 806RmodR (5′-GGA CTA CNV GGG TWT CTA AT-3′) using a thermal cycler PCR system (GeneAmp 9700, Thermo Fisher Scientific).

The PCR reactions were carried out in triplicate using a 20 µl mixture containing 10 ng of template DNA with 0.4 µl of FastPfu Polymerase (TransGen Biotech), 4 µl of 5 × FastPfu Buffer, 0.2 µl BSA, 0.8 µl of forward and reverse primer (5 µM), and 2 µl of 2.5 mM dNTPs. The reaction conditions were as follows: 3 min of denaturation at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s for annealing at 55°C, and then 45 s for elongation at 72°C, with a final elongation step at 72°C for 10 min and then 10°C until the reaction was halted by the user. An AxyPrep DNA Gel Extraction Kit (Axygen Biosciences) was used to further purify the PCR products (extracted from a 2% agarose gels). This was followed by quantification using a blue fluorescence quantitative system (QuantiFluor™-ST, Promega). In a second PCR assay, with customized primers including adapter complementary regions, purified amplicons were pooled in equimolar ratios and paired-end sequenced on an Illumina MiSeq platform (Illumina) according to the standard protocols of Majorbio Bio-Pharm Technology. All the sequences used in this study are publicly available at the NCBI sequence read archive (SRA) with BioProject accession number PRJNA611996.

2.4. Data processing and statistical analysis

Raw gene tags were quality-filtered by Trimmomatic (https://github.com/usadellab/Trimmomatic) and merged by FLASH (http://www.cbcb.umd.edu/software/flash) to obtain the effective tags (Shi et al. 2019). Next, Usearch software v7.0 (http://drive5.com/uparse/) was used to cluster valid sequences into operational taxonomic units (OTUs). Sequences not belonging to domain bacteria were subsequently eliminated. The RDP classifier Bayes algorithm was used to perform taxonomic analysis based on 97% or higher OTU similarity (Celikkol-Aydin et al. 2016). Samples of 16S rRNA gene sequences were aligned with the Silva reference database (Release132; www.arb-silva.de). All of the statistical analyses were performed with R v.3.5.3. The differences between sites were tested using 1-way analysis of variance (ANOVA) followed by a Tukey's honestly significant difference (HSD) test or Games-Howell post hoc test, with $p < 0.05$ considered to be significant (Hoffman et al. 2018). The data were analyzed with the statistical software SPSS 21.0. The data normality and the homogeneity of variances were confirmed using Kolmogorov-Smirnov and Levene's tests, respectively. Both assumptions were met by the data. The *t*-test was used to determine the differences in alpha diversity. Results were considered significant at $p < 0.05$. All values are presented as means ± SD. The diversity and phylogenetic data were analyzed on the online Majorbio Cloud Platform (www.majorbio.com). An inter-taxa relationship network was created for bacteria at the genus level using Spearman's correlation coefficients and a p-value threshold of 0.05. The co-existence relationships of environment factors and species were confirmed using Spearman's rank correlation coefficients and *t*-tests. The community functional predictions were analyzed via the Tax4Fun software package based on the Silva reference database (Aßhauer et al. 2015).

3. RESULTS

3.1. Environmental variables of the habitats

The distributions of NO_2^-, NO_3^-, NH_4^+, SRP, COD, SiO_3^{2-}, salinity, temperature, DO, chl *a* and pH in surface seawater are shown in Table 1. Chl *a*, NH_4^+, COD, and SRP contents were significantly different among the 5 habitats (1-way ANOVA, $F = 139.63$, df = 15, $p < 0.001$; $F = 9.50$, $p < 0.001$; $F = 4.54$, $p = 0.013$; $F = 4.50$, $p = 0.014$, respectively). The chl *a* content was significantly higher in OS than DS (Tukey HSD, $p = 0.023$), YM ($p < 0.001$), SM ($p < 0.001$), and RF ($p < 0.001$). The SRP content was higher in SM than OS ($p = 0.033$), RF ($p = 0.014$), and YM ($p = 0.038$).

Water currents flow from YM to DS to OS during rising tides (Fig. S1a in the Supplement at www.int-res.com/articles/suppl/q013p489_supp.pdf). Residual currents (Fig. S1b) also flow in this direction. During ebb tide, water currents flow from OS to DS to YM (Fig. S1c). These currents thereby create strong connectivity between OS, DS, and YM. There is only weak connectivity between SM and the other sites.

3.2. Alpha diversity analysis

The diversity indices revealed the variation in the bacterioplankton communities among different areas. OS harbored bacterioplankton communities with the highest richness. Bacterioplankton community richness was lowest at SM. Richness (Fig. 2a) was signif-

Table 1. Mean ± SD of water environmental factors at each habitat. SRP: soluble reactive phosphate; SST: sea surface temperature; COD: chemical oxygen demand; DO: dissolved oxygen; see Fig. 1 for definitions of site abbreviations

Parameters	OS	DS	YM	SM	RF
Salinity	34.01 ± 0.02	34.01 ± 0.02	33.99 ± 0.02	34.09 ± 0.03	33.95 ± 0.01
SRP (μmol l^{-1})	0.53 ± 0.06	0.59 ± 0.06	0.54 ± 0.10	0.89 ± 0.29	0.49 ± 0.14
NO_2^- (mg l^{-1})	0.0041 ± 0.0013	0.0042 ± 0.0009	0.0032 ± 0.0007	0.0045 ± 0.0003	0.0032 ± 0.0006
SST (°C)	29.80 ± 0.11	29.70 ± 0.14	28.75 ± 0.12	29.72 ± 0.00	29.10 ± 0.54
COD (mg l^{-1})	0.43 ± 0.09	0.37 ± 0.07	0.36 ± 0.13	0.61 ± 0.11	0.36 ± 0.11
NH_4^+ (mg l^{-1})	0.055 ± 0.008	0.054 ± 0.015	0.049 ± 0.005	0.024 ± 0.003	0.044 ± 0.003
DO (mg l^{-1})	4.74 ± 0.29	4.75 ± 0.24	6.02 ± 0.30	5.31 ± 0.11	6.65 ± 0.17
NO_3^- (mg l^{-1})	0.071 ± 0.028	0.065 ± 0.019	0.044 ± 0.010	0.049 ± 0.009	0.052 ± 0.009
Chl *a* (mg m^{-3})	0.82 ± 0.05	0.72 ± 0.02	0.30 ± 0.04	0.37 ± 0.02	0.33 ± 0.06
pH	8.15 ± 0.04	8.10 ± 0.04	7.87 ± 0.46	8.16 ± 0.01	8.12 ± 0.05
SiO_3^{2-} (μmol l^{-1})	5.09 ± 0.62	4.38 ± 0.46	4.71 ± 1.70	2.80 ± 1.91	4.49 ± 1.41

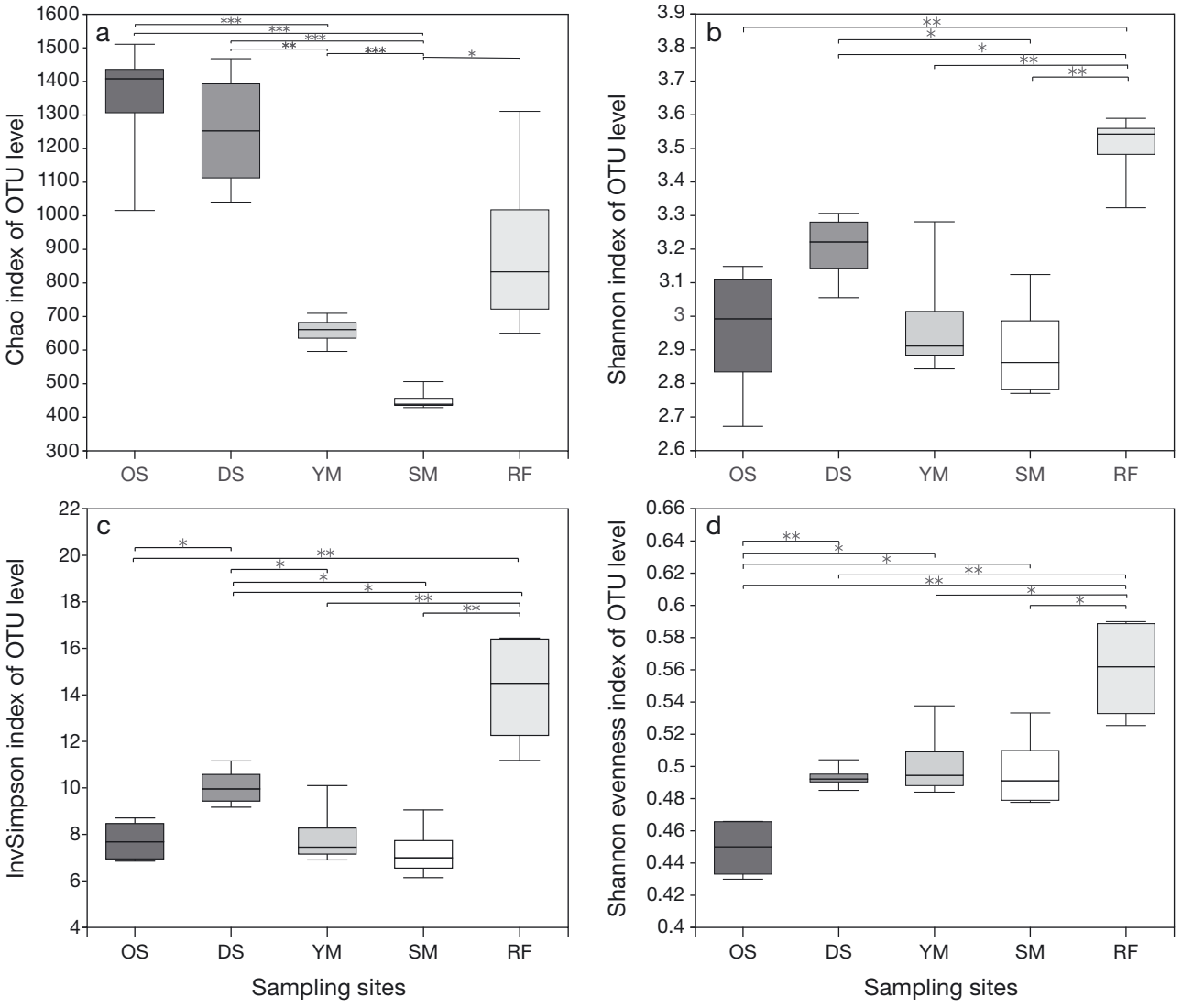

Fig. 2. Alpha diversity analysis of the operational taxonomic units (OTUs) (a) Chao index, (b) Shannon index, (c) inverse Simpson (InvSimpson) index, (d) Shannon evenness index. Boxes show the upper and lower quartile values, solid horizontal lines show median values, upper and lower whiskers are the maximum and minimum values, respectively. See Fig. 1 for definitions of site abbreviations. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

icantly different between OS and YM (t-test, $t = 7.97$, df = 6, $p < 0.001$) and SM ($t = 6.07$, df = 6, $p < 0.001$) sites. The bacterioplankton community diversity was significantly higher at RF than at other sites (Fig. 2b,c). The site with the lowest diversity was SM, which was significantly different from that at DS ($t = -2.96$, df = 6, $p = 0.025$ for the Shannon index; $t = 3.57$, df = 5, $p = 0.016$ for the inverse Simpson [InvSimpson] index). Community evenness of the 5 sites is shown in Fig. 2d. Evenness at RF was significantly higher than at other sites (1-way ANOVA, $F = 10.93$, df = 15, $p < 0.001$). Evenness at OS was significantly lower than at the other 4 sites. In summary, there was significant variation in richness, evenness, and diversity of the bacterioplankton communities between the oyster culture, coral reefs, and open area reference sites.

3.3. Species composition analysis

The tags were clustered into 2281 OTUs which were assigned to 1101 genera representing 40 different phyla. The highest number of OTUs (1490) was detected in the OS area and the lowest number (518) in the SM area. The DS and OS sites had the largest number of OTUs in common (943), while the SM and YM areas had the least (369). There were 287 OTUs (12.58% of the total OTUs) in common among the 5

ecosystems (Fig. 3). Proteobacteria were found most frequently (124 OTUs), followed by Bacteroidetes (80 OTUs). Cyanobacteria was the most abundant phylum (Fig. S2), accounting for 50.58% of the relative abundance in the 5 areas.

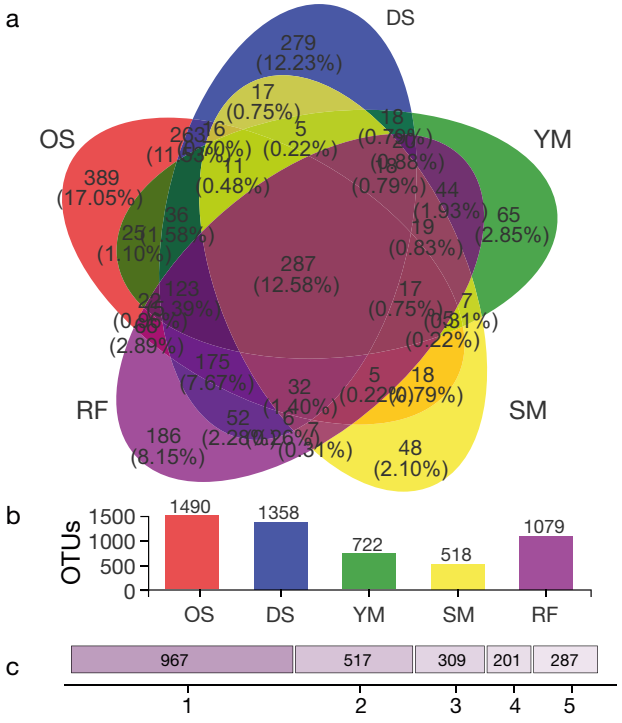

Fig. 3. (a) Numbers of shared and unique OTUs, (b) OTUs at each site, and (c) numbers of OTUs that were specific to 1 site or shared by 2 or more sites. See Fig. 1 for definitions of site abbreviations

Hierarchical clustering (Fig. 4) provided deeper insights into differences in the bacterioplankton community composition among the 5 ecosystems. OTUs with low proportional relative abundance (<0.02) at the genus level over all the sampling sites were removed for ease of data representation. Each sample from the same area clustered together except for the DS4 sample site. The data revealed that the bacterioplankton community structure was relatively unique among areas. The community structure similarity between OS and DS area was higher than between other areas. The SM area was different from the OS, DS, and YM areas, and the reference area was different from the other sites. *Synechococcus* sp. CC9902 was the dominant genus at all of the sites. The second most dominant genus varied among the study areas, being the NS4 marine group in YM and RF, and unclassified class *Alphaproteobacteria*, HIMB11, and the NS5 marine group for SM, OS, and DS, respectively (Fig. 4).

3.4. Spatial species turnover of bacterioplankton at different areas

The 15 most abundant genera were organized into 3 groups based on relative abundance (Fig. S3). The first group included 8 bacterioplankton genera that

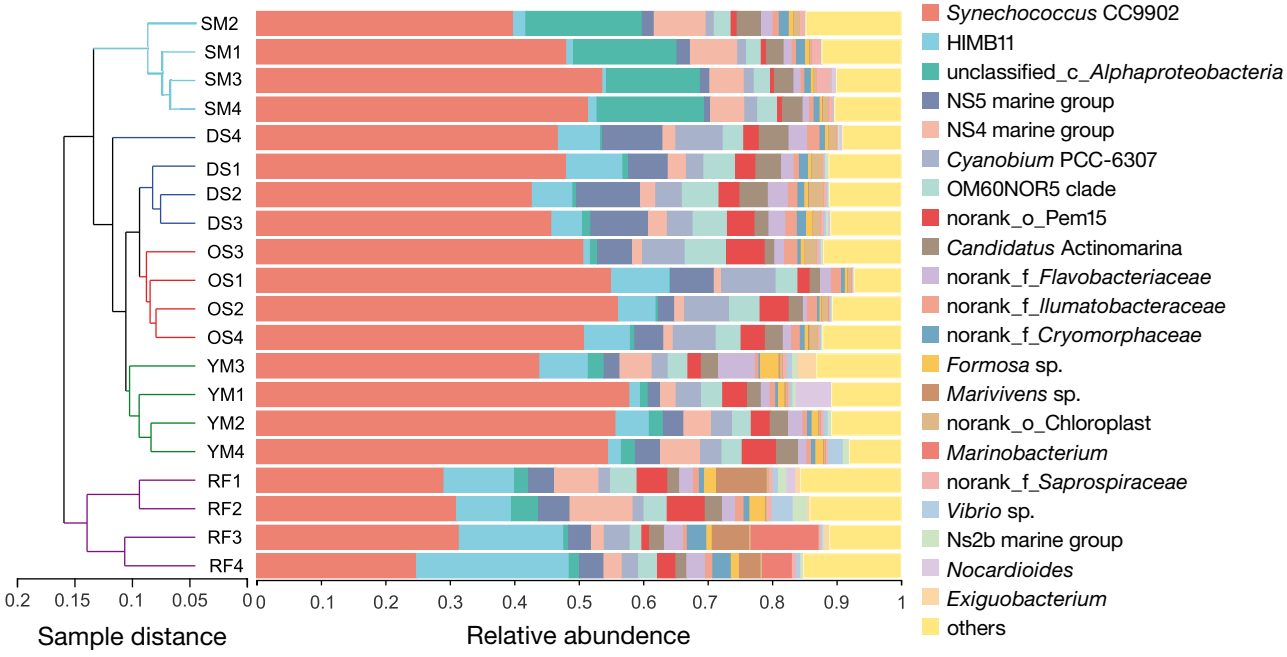

Fig. 4. Hierarchical clustering (Bray-Curtis distance) of samples according to relative abundance, showing abundance and distribution at the genus level. See Fig. 1 for definitions of site abbreviations; c: class; o: order; f: family

were significantly different between SM and the other areas: *Ilumatobacter* sp. (p = 0.014), Sva0996 marine group (p = 0.005) and norank order PeM15 of *Actinobacteria* (p = 0.022), NS5 marine group (p = 0.014) and the NS4 marine group of *Bacteroidetes* (p = 0.019), *Luteolibacter* sp. of *Verrucomicrobia* (p = 0.023), norank family *Flavobacteriaceae* of *Bacteroidia* (p = 0.026), and unclassified class *Alphaproteobacteria* (p < 0.001). The second group included 5 genera: HIMB11 (p = 0.023) and norank order SAR86 clade of *Proteobacteria* (p = 0.044), NS2b marine group (p = 0.021) and norank family *Cryomorphaceae* of *Bacteroidetes* (p = 0.044). and *Synechococcus* sp. CC9902 of *Cyanobacteria* (p = 0.005). Significant differences in this group were mainly between RF and the other areas. The third group included 2 genera: norank family *Ilumatobacteraceae* (p = 0.035) of *Actinobacteria* and *Cyanobium* sp. PCC-6307 of *Cyanobacteria* (p = 0.005). The relative abundance of these bacterioplankton was highest in the OS area. The similarities in bacterioplankton communities between areas declined with geographic distance.

3.5. Environmental factors driving fluctuations in bacterioplankton community ecoclades

Each of the samples from the same area showed similar results (Fig. 5). The bacterioplankton communities from the DS and OS areas had similar distribution patterns and responses to environmental fluctuations. The planktonic bacterioplankton communities

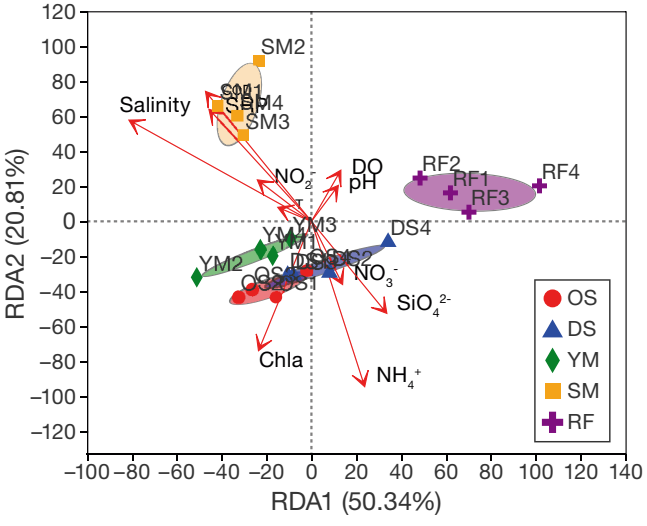

Fig. 5. Redundancy analysis (RDA) showing genus-level bacterioplankton communities and sensitivity to environmental parameter fluctuations. For definitions of site and parameter abbreviations, see Fig. 1 and Table 1

from the SM area clustered far from the others. The environmental factors on the first 2 axes explained 71.15% of the bacterioplankton community variability. The community structures were mainly driven by salinity and NH_4^+, followed by chl *a*, COD, and SRP.

Environmental factors drove the α-diversity fluctuations of the bacterioplankton communities. According to the linear regression, the Chao index showed a close correlation between bacterioplankton communities and chl *a* (R^2 = 0.607). The Shannon and Simpson indices showed a close correlation between bacterioplankton communities and salinity (Table 2).

Correlations between bacterioplankton classification at the genus level and environmental variables were detected using a hierarchical clustering heatmap (Fig. S4). The bacterioplankton clustered into 4 main branches based on the correlations between environmental factors and relative abundance. The first group (including *Nocardioides* sp., *Synechococcus* sp. CC9902, norank family *Microbacteriaceae*, *Candidatus* Actinomarina, *Candidatus* Aquiluna, *Erythrobacter,* and norank family *Cryomorphaceae*) was positively correlated with chl *a*, SST, NO_3^-, SRP, COD, NO_2^-, and salinity.

The second group (*Tropicibacter* sp., norank family AEGEAN-169 marine group, norank family *Hyphomonadaceae*, unclassified family *Hyphomonadaceae*, CL500-3, norank family SAR116 clade, norank family NS9 marine group, *Balneola* sp., norank order SAR86 clade, NS4 marine group, norank family *Flavobacteriaceae*, norank family PS1 clade, and norank class OM190) was negatively correlated with NH_4^+, SiO_3^{2-}, NO_3^-, chl *a*, and SST.

The third group (*Ascidiaceihabitans* sp., *Bacillus* sp., *Exiguobacterium* sp., *Formosa* sp., HIMB11, *Ilumatobacter* sp., *Luteolibacter* sp., *Marinobacterium* sp.,

Table 2. Linear regression correlating α-diversity of bacterioplankton communities with environmental factors. See Table 2 for definition of parameter abbreviations

Parameters	Chao		Shannon		Simpson	
	p	R^2	p	R^2	p	R^2
Salinity	0.094	0.148	0.001	0.462	0.000	0.522
SRP	0.038	0.217	0.097	0.146	0.055	0.189
NO_2^-	0.863	0.002	0.056	0.188	0.092	0.150
SST	0.062	0.176	0.289	0.094	0.282	0.091
COD	0.065	0.181	0.189	0.062	0.196	0.064
NH_4^+	0.024	0.251	0.687	0.009	0.415	0.037
DO	0.003	0.399	0.573	0.018	0.447	0.033
NO_3^-	0.166	0.104	0.267	0.068	0.595	0.016
Chl *a*	0.000	0.607	0.506	0.025	0.654	0.011
pH	0.420	0.037	0.506	0.025	0.720	0.007
SiO_3^{2-}	0.040	0.214	0.863	0.002	0.820	0.003

Marivivens sp. norank family *Flavobacteriaceae*, norank family NS11-12 marine group, norank family *Propionibacteriaceae*, norank order *Gaiellales*, norank order PeM15, NS2b marine group, *Ruegeria* sp., *Thalassobius* sp., unclassified family *Rhodobacteraceae*, and *Vibrio* sp.) was negatively correlated with SRP, NO_2^-, salinity, COD, and pH.

The fourth group (*Cyanobium* sp. PCC-6307, *Luminiphilus* sp., norank family *Ilumatobacteraceae*, NS5 marine group, OM60NOR5 clade, *Salinihabitans* sp., and Sva0996 marine group) was positively correlated with NH_4^+, SiO_3^{2-}, NO_3^-, chl *a*, and SST but negatively correlated with DO, SRP, pH, and salinity.

3.6. Co-existence relationship of species

An inter-taxon relationship network for those taxa whose relative abundance was greater than 0.05% at the genus level among the 5 sampling sites is shown in Fig. 6. A total of 536 nodes were included in the network. *Balneola* sp., *Cyanobium* sp. PCC-6307, and unclassified family *Flavobacteriaceae* (20 nodes) were highly correlated with the other phyla. In contrast, *Candidatus* Actinomarina and *Synechococcus* sp. CC9902 (single nodes) were poorly correlated with the other phyla. *Vibrio* sp. was observed to be most negatively associated with *Candidatus* Aquiluna and most positively associated with the NS2b marine group. At the phylum level, bacterioplankton genera belonging to *Proteobacteria* and *Bacteroidetes* were highly correlated with the other phyla. The network showed relationships between species that might lead to co-existence or competitive exclusion in the habitats.

3.7. Bacterioplankton functional annotation and distribution in different areas

The hierarchical clustering heatmap showed similarities and differences between areas with predicted functions (relative abundance in the top 50). The 5 areas clustered mainly into 2 main branches (Fig. S5). In the predictive analysis of functional pathways, SM differed from the other sites in that the predicted relative abundance was significantly enriched (1-way ANOVA, $F = 139.63$, df = 15, $p < 0.001$) in genes related to arginine and proline metabolism, bacterial chemotaxis, bacterial secretion system, butanoate metabolism, carbon fixation pathways in prokaryotes, *Caulobacter* cell cycle, citrate cycle (TCA cycle), fatty acid biosynthesis, flagellar assembly, glyoxylate and dicarboxylate metabolism, histidine metabolism, mismatch repair, oxidative phosphorylation, peptidoglycan biosynthesis, ribosome, 2-component system, and valine, leucine, and isoleucine degradation.

In contrast, the predicted relative abundance of SM was diminished in ABC transporters, amino sugar

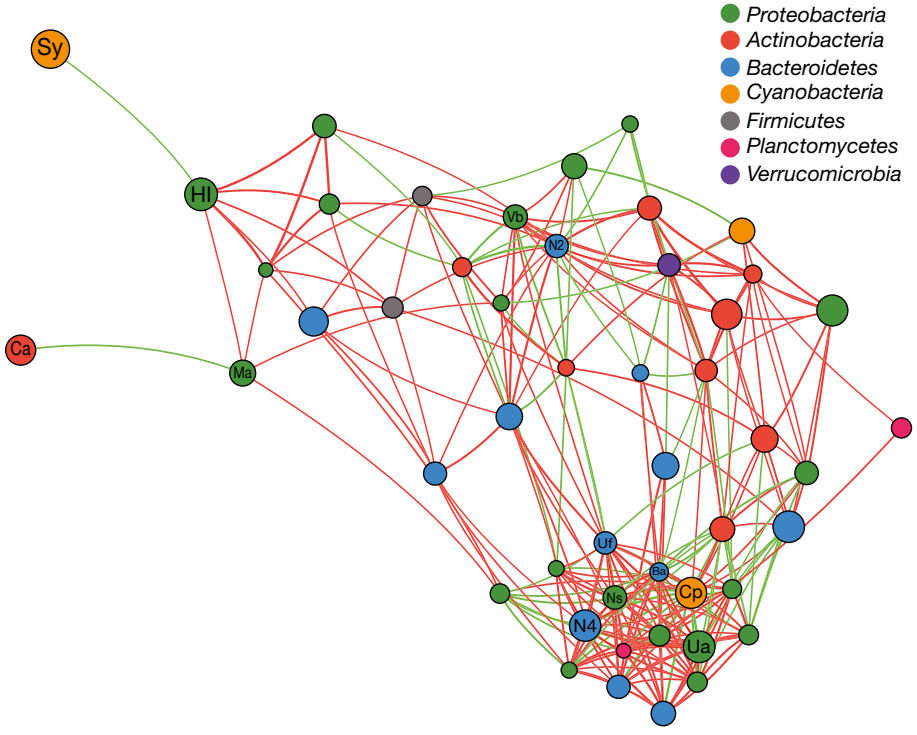

Fig. 6. Co-existence relationships (red line: positive; green line: negative) of bacterioplankton at the genus level (the top 50 in terms of relative abundance were used to construct the network). The size of the node depends on the abundance. The larger nodes (no. nodes ≥18), nodes with only 1 connection, and key genera are indicated by abbreviations. Cp: *Cyanobium* sp. PCC-6307; Ba: *Balneola* sp.; Uf: unclassified family *Flavobacteriaceae*; N2: NS2b marine group; N4: NS4 marine group; Ua: unclassified class *Alphaproteobacteria*; Ns: norank order SAR86 clade; Ca: *Candidatus* Actinomarina; Sy: *Synechococcus* sp. CC9902; HI: HIMB11; Ma: *Marivivens* sp.; Vb: *Vibrio* sp.

and nucleotide sugar metabolism, galactose metabolism, glycolysis or gluconeogenesis, methane metabolism, non-ribosomal peptide structures, pantothenate and CoA biosynthesis, phenylalanine metabolism, and starch and sucrose metabolism. Areas OS and DS were similar with respect to their dominant KEGG categories. Beyond this, they clustered with YM and RF. Compared to the SM habitat, a convergence of metabolic function was observed among the OS, DS, YM, and RF habitats.

4. DISCUSSION

Given the importance of bacterioplankton community complexity in mediating the responses of reefs to disturbances, such as coral disease (Ceh et al. 2012), understanding the factors driving such communities will be critical for the preservation of reefs under future scenarios of environmental change. In this study, the bacterioplankton community structure of 5 different habitats was examined.

The richness of bacterioplankton communities was significantly higher in the OS area than in the YM and SM areas. Oyster culture significantly increased species richness, which is consistent with the variation in abundance in the microbial communities of Xiangshan Bay (Jiang et al. 2019). It has been suggested that the environment in oyster culture areas is more suitable for these bacterioplankton. This is consistent with the higher NO_3^-, NH_4^+, and chl *a* contents in the OS and DS sites. The larger bacterioplankton in the OS area experience grazing by filter-feeding bivalves, but the smaller bacterioplankton do not (Cotner et al. 1995). This is consistent with previous findings that highlighted the selective feeding of phytoplankton and protozoans, which may be affected by bivalve feeding. The reduction in phytoplankton might reduce nutrient competitiveness with bacterioplankton (Fulford et al. 2007), and the reduction in protozoans might directly reduce bacterioplankton grazing. In other words, bacterioplankton abundance is controlled by protozoan predation (Thingstad & Lignell 1997). This 'top-down' control of richness would theoretically work even if all of the bacterioplankton and phytoplankton communities were limited by the same substrate (Torsvik et al. 2002). However, the OS and DS areas were intensely disturbed by human activities and runoff from the adjacent areas. This additional NO_3^-, NH_4^+, and SiO_3^{2-} could support a higher abundance and diversity of bacterioplankton (Cotner et al. 1995, Cárdenas et al. 2018). Furthermore, longline oyster farms reduce the speed and flow of currents, increasing the residence time of the bacterioplankton (Fortunato et al. 2013, Pagano et al. 2017).

However, the Shannon and Simpson diversity indices of the OS area were markedly lower than those of the RF area (which were similar to those of the YM and SM areas). These results are inconsistent with previous findings that highlighted human disturbance as contributing to increased bacterial diversity (Wainwright et al. 2019). This inconsistency might be due to the high filter-feeding activity by the oysters (Torsvik et al. 2002). This result is supported by the evenness (Shannon evenness was significantly lower in OS than in other areas). Notably, Simpson diversity was higher in the DS areas than in OS, YM, and SM. These slight fluctuations were detected by the Simpson index but were not detected by the Shannon index. The Simpson index is more sensitive to slight changes in species richness and evenness, whereas the Shannon index is more sensitive to other slight changes (Gorelick 2006). The higher diversity might be due to the edge effect (Aiken & Navarrete 2014). The coral reef area of DS was adjacent to the oyster culture areas (OS). These habitats could connect easily through hydrodynamics (Lopez-Joven et al. 2018). Tide is an important environmental factor of the ocean, directly affecting the transport and diffusion of nutrients and gametes. Furthermore, coral spawning always occurs on an astronomical full moon high tide. The Venn diagram (Fig. 3) further illustrates the good connectivity between OS and DS, the areas that had the largest number of OTUs in common. Bacterioplankton community diversity was lowest in the SM area, which is consistent with increased human disturbance contributing to increased bacterial diversity (Auguet et al. 2010, Cárdenas et al. 2018, Wainwright et al. 2019). The coral reef of SM had less human disturbance than the other areas.

Proteobacteria was the phylum with the most species in the bacterioplankton communities in the 5 study areas; this may indicate an important trophic interaction between autotrophic phytoplankton and heterotrophic bacterioplankton (Schada von Borzyskowski et al. 2019). A similar situation has been observed in Dongzhen reservoirs (Nyirabuhoro et al. 2020), in the Caribbean (Guillemette et al. 2018), and in Ofunato Bay (Reza et al. 2018). However, *Cyanobacteria* was the most abundant phylum (Fig. S2), accounting for 50.58% of the common relative abundance in the 5 study areas. This is consistent with several previous studies indicating that *Cyanobacteria* abundance is driven by competition rather than

the grazing pressure associated with oyster culture (He et al. 2017).

In the current study, *Synechococcus* sp. CC9902 (*Cyanobacteria*) was the dominant genus at all of the sites, and it was more abundant along the nearshore habitats than in offshore areas. This may have been due to nutrient pollution increasing the abundance of this *Cyanobacteria*. This is consistent with the correlations between bacterioplankton classification and environmental variables. This genus was positively correlated with chl *a*, SST, NO_3^-, and SRP. At the same time, the oysters feed on nutritive algae such as diatoms, and this may reduce competition with *Synechococcus* sp. (Gobler et al. 2011, Zaneveld et al. 2016). On the other hand, the *Synechococcus* sp. could take advantage of multiple nitrogen sources. Therefore, it was the dominant genus at the survey sites (Lesser et al. 2004, Zaneveld et al. 2016). *Synechococcus* sp. is toxic and may cause harmful prokaryotic blooms during hot months (Gobler et al. 2011, Hamilton et al. 2014). In addition, the autotrophic prokaryotic algae on the coral surface may compete with the *Symbiodinium* in the coral for photosynthetic resources (Koh 1997). However, *Cyanobacteria* can be inhibited by antibacterial active substances extracted from a variety of scleractinians (Koh 1997). Furthermore, *Cyanobacteria* may also affect the success of larval settlement and recruitment. Biofilm is the basis for coral larval colonization, and *Synechococcus* sp. may compete with other probiotics (such as *Roseobacter* sp. of *Proteobacteria*) in biofilms (Sharp et al. 2015). At the same time, *Synechococcus* sp. exists in some tissues of corals, and these may have beneficial effects for corals, such as providing nutrients (Lesser et al. 2004, Neulinger et al. 2008), In addition, late-stage larvae can utilize *Synechococcus* sp. as a coral-associated bacterial community (Lema et al. 2014). In summary, the free-living bacterioplankton community is a repository of the early stages of broadcast-spawning coral microbiota in the water column (Barott & Rohwer 2012). The larvae can selectively ingest bacterioplankton from the environment, and changes in the microbiome communities at different life stages of the coral indicate direct and indirect interactions among the environment, bacteria, and corals (Barott & Rohwer 2012, Franzenburg et al. 2013).

The second most common genus differed from area to area. *Cyanobium* sp. PCC 6307 was the second most common genus in the OS area, and this genus may induce cyanobacterial blooms as with *Synechococcus* sp. (Genuário et al. 2016). This is consistent with the correlations between bacterioplankton classification level and environmental variables. The genus was positively correlated with chl *a*, SST, NO_3^-, SRP, and NH_4^+. In addition, the morphology of *Cyanobium* sp. can be transformed after being grazed on by protists (Jezberová & Komárková 2007). In the present study, *Cyanobium* appeared to be directly and indirectly controlled by oysters via their high efficiency filtration capacity. The NS5 marine group (*Flavobacteriales*) of *Bacteroidia* represented the second most common genus in the DS area. This group has enzymes for catalyzing phytoplankton-derived macromolecules, which are associated with phytoplankton blooms (Seo et al. 2017). This characteristic is consistent with the correlations between bacterioplankton classification and environmental variables. Suitable habitat for the NS5 marine group has similar environmental characteristics to that of *Cyanobium* sp. PCC 6307. The HIMB11 strain (*Rhodobacteraceae*) of *Alphaproteobacteria* was the second most common genus in the YM and RF areas. This genus represents a group that was negatively correlated with SRP, NO_2^-, and salinity. This strain has been reported to play an important role in the biogeochemical cycling of carbon and sulfur (Durham et al. 2014), as it can obtain energy from sulfur oxidation (Neulinger et al. 2008). Furthermore, as a unique sublineage within the *Roseobacter* clade (Durham et al. 2014), the HIMB11 strain might produce antimicrobial compounds such as tropodithietic acid to defend against pathogens (Raina et al. 2016). The co-existence relationships (Fig. 6) also showed a strong negative relationship between HIMB11 strain and *Synechococcus* sp. CC9902 that indicates inhibition activity of the HIMB11 strain. The second most common genus of YM differed from those in the other 4 habitats, and was identified as an unclassified class of *Alphaproteobacteria*. The unclassified groups of bacterioplankton may reveal their physiological characteristic responses to the specific habitat environmental regime. The dominant genera play important and unique roles in the early life history stages of corals, including production of activity-inhibiting compounds, induction of settlement, metamorphosis, and attachment, provision of nutrients to larvae, and promotion of direct uptake of nutrients by larvae (Rosenberg et al. 2007, Patten et al. 2008, Tran & Hadfield 2011, Lema et al. 2014, McDevitt-Irwin et al. 2017).

The dominant genus differed from area to area. Nonetheless, these differences were not strong enough to blur the powerful effect of local environmental selective factors on bacterioplankton community structure. As shown in Fig. 4, the genus-based

approach revealed similar community composition patterns driven by specific environment factors, suggesting that samples from the same habitat had roughly the same diversity and abundance, and clustered together (Auguet et al. 2010). This is consistent with previous studies of the Pearl Estuary (Liu et al. 2015), Sargasso Sea (Nelson & Carlson 2012), and Magnetic Island (Glasl et al. 2019). However, knowledge of the interactions between dominant species of bacterioplankton and the early stages of corals is limited. Monitoring local dominant species of bacterioplankton in combination with oyster culture disturbance studies will help to identify potential opportunistic pathogens and provide a more complete understanding of the interactions between the microbiome and coral at the early stages.

Analysis of the relative abundance of species among habitats provided a thorough understanding of the similarities and differences between bacterioplankton communities along a spatial gradient (Fig. S3). The top 15 abundant genera that differed among habitats belonged to *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*. Previous studies have found that *Actinobacteria* is dominated by strains originating from terrestrial runoff (Bull & Stach 2007), which may explain its relatively low abundance in SM. *Bacteroidetes* has been reported to have a specialized ability for degrading polymers with peptidases, glycoside hydrolases (GHs), and glycosyl transferases. This phylum prefers to grow while attached to particles, surfaces, and algal cells. As degraders of particulate matter, they may be affected by the filter-feeding activity of the oysters. The relatively low abundance of *Bacteroidetes* in the OS area further supports this. The phylum *Proteobacteria* was the largest and phenotypically most diverse phylogenetic lineage. An enormous diversity of morphology, physiology, and metabolism has been observed in this phylum (Kersters et al. 2006). This diversity has helped the phylum to adapt to a wide variety of environments. Genera belonging to *Proteobacteria* showed different fluctuation characteristics among the 5 habitats, suggesting different sensitivity levels of the different bacterioplankton communities to specific environmental factors (Fig. 5). At the class level, the members of *Alphaproteobacteria* and *Gammaproteobacteria* were the most abundant. This observation is consistent with studies in Ofunato Bay (Kobiyama et al. 2018) and the Columbia River coastal margin (Fortunato et al. 2012).

Previous studies have shown that despite heterogeneity in the environmental characteristics of different habitats, bacterioplankton communities within each environment are relatively homogenous (Hewson & Fuhrman 2004). This study was consistent with these results. In addition, the bacterioplankton communities of each individual environment could be further clustered across spatial scales. The OS and DS areas were very similar, probably due to their close proximity to one another, which facilitated hydrologic exchange between these communities. Consistent with the study of Lopez-Joven et al. (2018), pathogenic free-living or plankton-related communities could spread rapidly via hydrodynamic means and the motility of the organisms themselves. In contrast, the SM area was closer to the open ocean with less connection with the other sites. Samples from this area were distinct from inner bay samples. Similar results have been found for Chesapeake Bay (Kan et al. 2005) and Amvrakikos Gulf (Pavloudi et al. 2017).

The redundancy analysis identified salinity, NH_4^+, chl *a*, COD, and SRP as the most important environmental factors affecting variability. These factors varied due to runoff and the presence of marine culture, which drives the differences in bacterioplankton communities. Similar results were seen in Oregon and the Washington coast (Fortunato & Crump 2011), the Pearl Estuary (Liu et al. 2015), the Columbia River coastal margin (Fortunato et al. 2012), and Chesapeake Bay (Pavloudi et al. 2017). Collectively, the bacterioplankton communities are broadly distributed across a gradient of different environments, but each group supports a different combination of these bacterioplankton, creating distinct community structures within each environment. There was 'bottom-up' control of bacterioplankton community diversity between the habitats (Torsvik et al. 2002).

Previous studies have paid more attention to *Vibrio* sp., a group that can be pathogenic in free-living or plankton-related communities (Jones & Oliver 2009). A co-existence relationship of species at the genus level was detected in the network (Fig. 6). The *Vibrio* sp. were most negatively associated with *Candidatus* Aquiluna and most positively associated with the NS2b marine group. These results may be due to *Vibrio* sp. having significant negative correlations with salinity, SST, SRP, and NO_2^-, whereas *Candidatus* Aquiluna had significant positive correlations with these factors. The results of this study were inconsistent with previous research that found that higher temperatures significantly increased the relative abundance of *Vibrio* sp. (Green et al. 2019). However, it is expected that many factors will influence *Vibrio* sp. abundance, not just temperature. This is in agreement with the studies of Lopez-Joven

et al. (2018) and Vezzulli et al. (2015), who observed (1) an important contribution of plankton community diversity to *Vibrio* sp. dynamics in the water column and (2) higher abundances of specific *Vibrio* sp. at lower temperatures. Furthermore, the details of a cross-infection mechanism between corals and oysters for *Vibrio* sp. require further study (Ushijima et al. 2018).

The Tax4Fun predictions showed differences in the clustering between functional categories and taxonomic community structure, indicating that mechanisms leading to the convergence of metabolic function do not necessarily lead to a convergence of taxonomic composition (Louca et al. 2017). In addition, the results reflect functional redundancy of the community. The functionally redundant rare species could become abundant in response to stress. This is in agreement with the replacement scenario in which the function of the community could be complex, highly context-dependent, and dependent on biotic interactions. The SM habitat was different from the other sites. Clustering showed that the differentiation of the sampling sites based on microbial function profiles followed the Chao richness gradient described earlier. Compared to the closed oyster culture site, some KEGG pathways of the SM bacterioplankton community indicated attenuation in glycolysis and amino sugar and nucleotide sugar metabolism, but enrichment in cell motility (e.g. bacterial chemotaxis and flagellar assembly) and carbon fixation pathways. However, the metabolism of terpenoids and polyketides, as well as xenobiotic biodegradation and metabolism were enriched in the inner bay habitats, suggesting an increase in multiple stress-resistance responses to negative environmental stress (Coclet et al. 2019). These habitat-specific selective pressures could explain a significant proportion of the variance. This study is consistent with previous studies indicating that different geophysical and geochemical characteristics and biological interaction processes drive variation in functional structure and composition between habitats, that in turn shape independent and complementary 'axes of variation' (Smith et al. 2015, Louca et al. 2016).

In conclusion, the bacterioplankton communities of oyster culture areas and coral reefs were significantly different during this study, regardless of the community structure or the community functional diversity between the habitats. Similarities in community structure and function increased as the spatial distance decreased. Although biogeochemical dynamics explained part of the variation between habitats, other comprehensive influencing factors also need to be identified. Further analyses are needed to better understand the effects of oyster culture on bacterioplankton communities in natural coral habitats. Particularly during the early life stages of corals, direct and indirect interactions between oyster culture and coral reef habitats should be closely monitored.

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