

Biogeography and co-occurrence patterns of bacterial generalists and specialists in three subtropical marine bays

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Abstract

Recent research has greatly expanded our understanding of microbial metacommunities in aquatic ecosystems. However, patterns at the mesoscale are still poorly understood. We present the first simultaneous analyses of the biogeography and co-occurrence patterns of generalists and specialists marine bacteria from three subtropical bays of China and test for signals of ecological processes (i.e., stochastic and deterministic processes) in biogeography and community assembly. Results showed that compared to specialists, bacterial generalists were less diverse, and were more widely dispersed in the three subtropical bays. Network analysis indicated that both habitat generalists and specialists showed non-random co-occurrence patterns, and specialists had a more complex co-occurrence pattern than generalists. Further, specialists co-occurrence network exhibited distinct robust structure compared to generalists, indicating that the both taxa showed different network stability. Our null models indicated that the generalists and specialists were primarily shaped by deterministic processes - such as variable selection. However, deterministic processes played a greater role in the community variation of specialists (84%) than generalists (56%). The study has broadened our understanding of generalists and specialists distribution in the bacterioplankton; further revealing the dominant roles of similar ecological mechanisms (deterministic processes) in shaping the community assembly. The differences in complex and stable co-occurrence pattern between generalists and specialists could be driven by deterministic processes. By considering the roles of species traits and ecological processes, we provide a deeper mechanistic understanding of bacterial biogeographical and co-existence patterns.

Unraveling the deep mechanisms that structure community biogeography is a central issue in understanding the responses of communities to environmental changes (Hanson et al. 2012; Székely et al. 2013). Traditionally, the biogeography of large plants and animals has been extensively investigated, with microorganisms only contributing a small amount of data to our understanding of biogeography and other aspects of ecology (Hanson et al. 2012). However, new methods mean that this is starting to change. Microbial ecology arguably became a distinct discipline around the 1960s, and from the 1990s increasingly made use of rapidly developing molecular methods (Atlas and Bartha 1998). A key question has been to what extent are the ecological patterns and

processes seen in microbial ecology similar to those seen in better studied large organisms (Andrews 1991). Recently, we have been able to more accurately observe and describe bacterial biogeography with unparalleled depth by high-throughput sequencing, even for the rare bacteria (e.g., Liu et al. 2015). Numerous studies have explored biogeographical patterns based on 16S rRNA gene sequences from different ecosystems, such as the surface ocean (Ruiz-González et al. 2019), boreal freshwater ecosystems (Ruiz-González et al. 2015), and wastewater treatment plants (Wu et al. 2019). This growing body of work provides evidence that bacterial communities display biogeographical patterns at various scales.

Over the last decade biogeographical studies have revealed that differences in microbial assemblages could be explained by a combination of stochastic and deterministic processes (Logares et al. 2013; Liu et al. 2015; Mo et al. 2018). Stochastic processes (neutral theory based processes) assume that

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community composition is driven by dispersal limitation, mass effects and random changes in birth/death rates, but tend to ignore interactions among species and their response to environmental factors (Hubbell 2001; Chave 2004). Deterministic processes (niche theory based processes), often include selection driven by the abiotic environmental conditions and biotic factors such as species interactions (Liu et al. 2019a). Recently, some studies have identified co-occurrence patterns in bacterial communities across different ecosystems (Barberán et al. 2012; Milici et al. 2016).

Co-occurrence patterns are key in understanding microbial community structure, which could provide new insights into the interactions of complex microbial networks, revealing shared niches among community members in the real world (Faust and Raes 2012). Correlation-based network analysis has been widely applied to explore the co-occurrence patterns among complex microorganisms in a variety of habitats, including marine (Cram et al. 2015), freshwater (Liu et al. 2019b), soil ecosystems (Ma et al. 2016), and the human gut environment (Faust et al. 2012). These studies found that non-random co-occurrence patterns were generally observed among microbial assemblages, indicating that deterministic processes (i.e., species–species interactions and environmental filtering) play important roles in regulating the assembly of the microbial community.

The roles of stochastic and deterministic processes in bacterial community assembly imply that different aspects of bacterioplankton subcommunities (i.e., habitat generalists and specialists; abundant and rare subcommunities) may be assembled by different mechanisms (Liu et al. 2015; Lindh et al. 2016). For instance, as with better studied macroorganisms, some bacteria are remarkably ubiquitous and have wider habitat preferences (habitat generalists), whereas others appear very specific with narrow environmental tolerances (habitat specialists) (Pandit et al. 2009). Note that although generalist is the common term used within much of ecology, within microbiology the term “versatility” has sometime been used instead (McArthur 2006). Compared to generalists, niche-based models predict that specialists have a faster worldwide decline (Thuiller et al. 2005), with specialists decline promoting the functional homogenization in biodiversity (Clavel et al. 2011). This homogenization could change ecosystem functioning and thus endanger ecosystem services, and community-level specialization can be regarded as an indicator of the influence of global changes (such as climate and habitat disturbances) on biodiversity (Clavel et al. 2011). Furthermore, some generalists with wider niches exhibit a rapid rate of niche evolution (Lavergne et al. 2013). The fast niche evolution is related to lower population decline. To some degree, this means that the change of niche breadth can reflect extinction risk. Habitat generalists and specialists become increasingly important for conservation biogeography in light of global biotic homogenization (Clavel et al. 2011). Normally, abundant taxa play a key role in carbon cycling (Pedrós-Alió 2012), rare taxa could harbor huge diversity and

conduct specific metabolic functions in ecosystems (Pester et al. 2010). Overall, generalist and specialist taxa play different roles in the ecosystems and ecology studies compared to the rare and abundant taxa. Our previous study has clearly revealed that neutral processes and environmental selection explained the similar biogeographical patterns of abundant and rare bacteria, with a large proportion of unexplained variation in the rare taxa (Mo et al. 2018), whereas the mechanisms affecting the distribution of habitat specialists and generalists are largely unknown in bacterial community across space and time. In a recent study, deterministic processes were found to shape the habitat specialists, whereas stochastic processes played a major role in structuring generalists in coastal sand microbial communities of southern China (Hu et al. 2019). Therefore, to distinguish the mechanisms affecting the distribution of habitat specialists and generalists is important to better understand bacterial community assembly.

In this study, we used a high-throughput sequencing approach to investigate the marine bacterioplankton community along a latitudinal gradient ranging from 20 to 25°N across three subtropical bays (Shenhu Bay, Dongshan Bay, and Beibu Gulf) in southern and southeastern China; a dataset previously analyzed by Mo et al. (2018) in another context. Our previous studies have found that marine eukaryotic microorganisms (i.e., planktonic ciliate; tintinnid ciliates; microeukaryotic plankton communities) were particularly susceptible to environmental change (Wang et al. 2014a, 2014b; Zhang et al. 2017), however, research on distribution of habitat generalists and specialists in marine bacterioplankton in subtropical regions has been lacking. Here, we used distribution in multiple samples across three subtropical bays to provide new insights into the importance of environmental conditions and ecological mechanisms in shaping habitat generalists and specialists community assembly and co-occurrence patterns. Therefore, for the first time, we used network analysis and null model to explore bacterial co-existence in these subtropical bays and quantify the relative importance of ecological processes in shaping the habitat generalists and specialists subcommunities.

Specifically we tested the following three hypotheses: (1) habitat generalists and specialists of the bacterioplankton community in three subtropical bays exhibit different biogeographical patterns; (2) compared to generalists, specialists show a more complex non-random pattern, and they have different network stability; and (3) deterministic processes play a greater role in the biogeography and assembly of habitat specialists than generalists.

Materials and methods

Sample collection and Illumina sequencing

Samples were collected in the South China Sea from Shenhu Bay in May 2012, and from Dongshan Bay and Beibu Gulf in August 2011 (Fig. S1) (Mo et al. 2018). A total of

22 samples were obtained from the surface (0.5 m) and bottom (3–41 m) layers of the epipelagic waters, that is a surface and bottom sample from 11 different sampling stations. All samples were collected by Niskin bottles mounted on a CTD (conductivity, temperature, and depth) oceanic profilers (SBE-917). In order to eliminate the metazoan and larger debris, seawater samples were pre-filtered through a 200 μm mesh, and about 800 mL seawater was subsequently filtered on a 0.2- μm -pore-size polycarbonate membrane (diameter 47 mm; Millipore, Billerica, Massachusetts). Afterward, the membranes were put in sterile 2 mL microcentrifuge tubes and were stored at -80°C in the laboratory until further analysis. DNA extraction, PCR, and Illumina sequencing were performed according to our previous procedure following Mo et al. (2018).

Sequence processing

Paired-end Illumina V4 region of 16S rRNA gene sequences was processed using VSEARCH (Rognes et al. 2016). Pairs of reads were merged by using FLASH (Magoč and Salzberg 2011). The chimeras and singletons were removed with default settings in VSEARCH. The unnoise3 algorithm was used to identify operational taxonomic units (OTUs) at a 97% sequence similarity level (Edgar 2010). Representative sequences were classified by the VSEARCH using the syntax algorithm with a cutoff value of 0.8 against the Greengenes database (DeSantis et al. 2006). All eukaryotic, chloroplast, archaeal, mitochondrial and unassigned sequences were removed before the downstream analyses. To standardize sequencing effort, a subset of 7624 reads at 97% threshold were randomly subsampled from per sample based on MOTHUR v.1.33.3 (Schloss et al. 2009). The total dataset obtained 167,728 reads.

Physicochemical analysis

The environmental characteristic of each station was assessed using a conductivity-temperature-depth (CTD) oceanic profiler. The pH, dissolved oxygen (DO), chemical oxygen demand (COD), total nitrogen (TN), nitrite nitrogen ($\text{NO}_2\text{-N}$), nitrate nitrogen ($\text{NO}_3\text{-N}$), ammonium nitrogen ($\text{NH}_4\text{-N}$), total phosphorus (TP), soluble reactive phosphorus (SRP) and dissolved silicon (DSi) were measured according to the standard methods defined in the Offshore Marine Chemical Survey Technical Regulations (Office of the State Oceanic Administration 2006). All physicochemical parameters are described in detail in Fig. 1 and Table S1.

Identification of habitat generalists and specialists

To identify habitat specialization, the "Levins niche breadth" approach (Levins 1968) was applied using the formula:

$$B_j = \frac{1}{\sum_{i=1}^N P_{ij}^2}$$

where B_j represents niche breadth and P_{ij} is the relative abundance of OTUs belonging to species j present in a given

habitat i (i.e., each of the 22 samples was considered a "habitat"). The higher niche breadth values indicate a wider range of habitats, implying OTUs with high and low niche breadth values could be defined as generalists and specialists, respectively (Pandit et al. 2009; Luo et al. 2019). In this study, OTUs with mean relative abundance $< 2 \times 10^{-5}$ were not used because they could potentially be misidentified as specialists (Pandit et al. 2009). Further, OTUs with niche breadth ≥ 5 were arbitrarily regarded as generalists, whereas those with niche breadth < 1.2 were classified as specialists (Fig. S2). Niche breadth ≥ 5 and niche breadth < 1.2 were artificially selected as there were within the area outlier of the niche breadth distribution (see Fig. S2a). In addition, niche breadth < 1.2 was chosen because it is close to 1, the smallest possible niche breadth value. Pandit et al. (2009) found that generalists and specialists species each included a mixture of abundant and rare species in a rock pool (tide pool) ecosystem. In order to explore whether both generalists and specialists obtained a similar result in our study, we defined the abundant and rare bacteria according to our previous study (Mo et al. 2018): locally abundant OTUs (OTUs with an abundance of $\geq 1\%$ within a sample) and those OTUs that exhibited a mean relative abundance of $\geq 0.1\%$ in all samples were defined as abundant OTUs. Rare OTUs were defined as those locally rare OTUs (OTUs with an abundance of $< 0.01\%$ within a sample) and the OTUs with a mean relative abundance of $< 0.001\%$ in all samples.

The abundant or rare taxa were defined based on their locally and regionally relative abundances, whereas generalist and specialist taxa were identified by their niche breadth (Fig. S2a). Obviously, generalist and specialist taxa differ from simply being abundant or rare taxa previously defined in our earlier paper (Mo et al. 2018).

Diversity and ordination

Alpha-diversity indices (OTU richness and Shannon-Wiener index) and rarefaction curves were calculated using the vegan package in R software (version 3.6.1) (R Core Team 2018). Tukey's honestly significant difference (Tukey HSD) post-hoc test was used to test the significant differences among environmental variables of the three bays in an R environment. Bacterial composition was visualized using the principal coordinate analysis (PCoA) with CANOCO for windows version 5.0.

Network construction and C-score

We used network analysis to examine co-occurrence networks in generalists and specialists. To reduce noise and complexity of the datasets, we remained OTUs with sequences ≥ 25 for network analysis. In total 1999 OTUs were involved in the network analyses, including 104 OTUs of generalists and 503 OTUs of specialists (Table S2). Spearman's rank coefficients (r) between those OTUs were calculated pairwise by the "picante" package in an R environment. Only

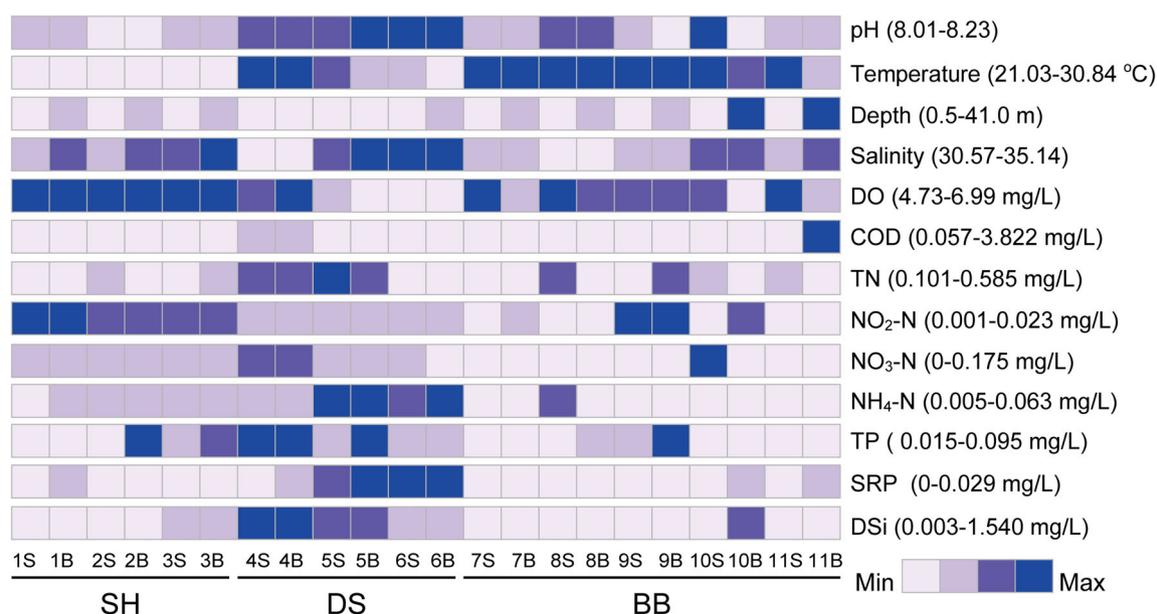


Fig 1. Heat map displaying change in environmental parameters among the three subtropical bays in China. Color scale for each environmental parameter varies between minimum and maximum values. DO, dissolved oxygen; COD, chemical oxygen demand; TN, total nitrogen; NO₂-N, nitrite nitrogen; NO₃-N, nitrate nitrogen; NH₄-N, ammonium nitrogen; TP, total phosphorus; SRP, soluble reactive phosphorus; DSi, dissolved silicon. SH, Shenhu Bay; DS, Dongshan Bay; BB, Beibu Gulf.

robust (Spearman's $|r| > 0.75$) and statistically significant (p -value < 0.01) correlations were considered in the network analyses. Network visualization was made with the Gephi version 0.9.1 and Cytoscape version 3.6.1. Based on species connectivity as described previously (Guimerà and Amaral 2005), connectors linking different modules in co-occurrence network were identified. All connector nodes were considered as potential key species in co-occurrence networks (note: there are no hubs in our analysis) (Shi et al. 2016). In addition, 1000 Erdős-Rényi random networks were obtained in the "igraph" R package, which had the same number of nodes and edges as the real networks, with each edge appearing with the same probability of being assigned to any node (Erdős and Rényi 1960). Topological characteristics of both real and random networks were calculated and compared, including clustering coefficient, modularity, and average path length. Groups of highly interconnected nodes in the co-occurrence networks were generated using the MCODE application with standard parameters (Bader and Hogue 2002). Network stability was assessed by natural connectivity, the nodes were removed in the network to evaluate how quickly robustness degraded (Peng and Wu 2016).

The checkerboard score (C -score) was calculated to evaluate the real distributions for non-randomness of OTUs. The OTU sequence table was transformed into a binary matrix of presence (1) and absence (0), and then was calculated under the null model (Gotelli and McCabe 2002). The checkerboard score, which is significantly larger than expected by chance, indicates non-random species distributions between stations

(Sanders et al. 2007). The standardized effect sizes (SES) for C -score were calculated: $SES = (\text{observed } C\text{-score} - \text{mean simulated } C\text{-score}) / \text{standard deviation of simulated } C\text{-scores}$ (Gotelli and McCabe 2002). Based on a burn of 30,000 simulations and sequential swap randomization algorithm, the C -score and C_{var} -score (variance of C -score) were calculated with the "EcoSimR" package in R version 3.6.1 (R Core Team 2018).

Relationships between community composition and environment variables

Spearman's rank correlations were calculated to analyze the relationship between environmental parameters and bacterial community composition and diversity. Additionally, we used Spearman correlations to determine the relationship between the OTUs of each main module and environmental parameters with the "picante" package in R version 3.6.1. Only robust ($|r| > 0.6$) and statistically significant (p -value < 0.01) correlations were selected in this analysis.

Stegen null model

To quantify the contributions of different ecological processes (stochastic vs. deterministic) to bacterioplankton community structure and biogeography, a null model (Stegen et al. 2013) was used. The beta nearest taxon index (βNTI) is the difference between observed βMNTD and mean of the null distribution of βMNTD normalized using its standard deviation. The mean nearest taxon distance metric (βMNTD) was calculated to evaluate pairwise phylogenetic turnover between communities within "picante" R package (Webb et al. 2002;

Stegen et al. 2013). We further calculated the Bray-Curtis based Raup-Crick metric (RC_{bray}) index to describe pairwise comparisons with $|\beta\text{NTI}| < 2$ (Stegen et al. 2013). Specifically, we considered a significant deviation (i.e., $|\beta\text{NTI}| \geq 2$) to indicate the importance of deterministic processes, and less than expected phylogenetic turnover (i.e., $|\beta\text{NTI}| < 2$) to indicate the dominance of stochastic processes (Dini-Andreote et al. 2015). When βNTI were < -2 and ≥ 2 , we suggested that homogeneous selection and heterogeneous selection affected community turnover, respectively. However, the $|\beta\text{NTI}| < 2$ and $RC_{\text{bray}} < -0.95$ or the $|\beta\text{NTI}| < 2$ and $RC_{\text{bray}} \geq 0.95$ indicated that community turnover was dominated by homogenizing dispersal and dispersal limitation, respectively. When the $|\beta\text{NTI}| < 2$ and $|RC_{\text{bray}}| < 0.95$, the “undominated fraction” was indicated, which represents community shifts because of ecological drift, weak selection, and weak dispersal (Stegen et al. 2013).

Results

Comparison of environmental factors between the three subtropical bays

All of the 13 environmental parameters measured had wide ranges and showed some differences between the three

subtropical bays (Fig. 1; Table S1). The key differences are summarized here. The concentration of dissolved oxygen in Shenhui Bay (6.56–6.99 mg L⁻¹) was higher than Dongshan Bay (4.73–6.49 mg L⁻¹) or Beibu Gulf (4.83–6.49 mg L⁻¹). Both temperature and NH₄-N showed a significant difference between the three studied bays. Temperature showed the highest value in the Beibu Gulf (29.55°C) compared to other bays. Mean concentration of NH₄-N in Dongshan Bay (0.04 mg L⁻¹) was higher than in the two others. It is of note that there was no consistent difference between the bay sampled in 2012 (Shenhui Bay) and the samples from other two bays.

Biogeographical pattern of bacterial community

A total of 8622 OTUs were identified based on 97% sequence similarity level. After deleting OTUs with mean relative abundances $< 2 \times 10^{-5}$, we remained 7850 OTUs for classification of niche breadth. Among these, 160 (2.0%) habitat generalist OTUs and 3059 (39.0%) habitat specialist OTUs were identified and they represented 4.1% and 29.0% of the total sequences, respectively (Fig. S2b). Occasionally, similar to specialists, there were few overlaps between generalists and regionally abundant OTUs in our dataset (Fig. S2b).

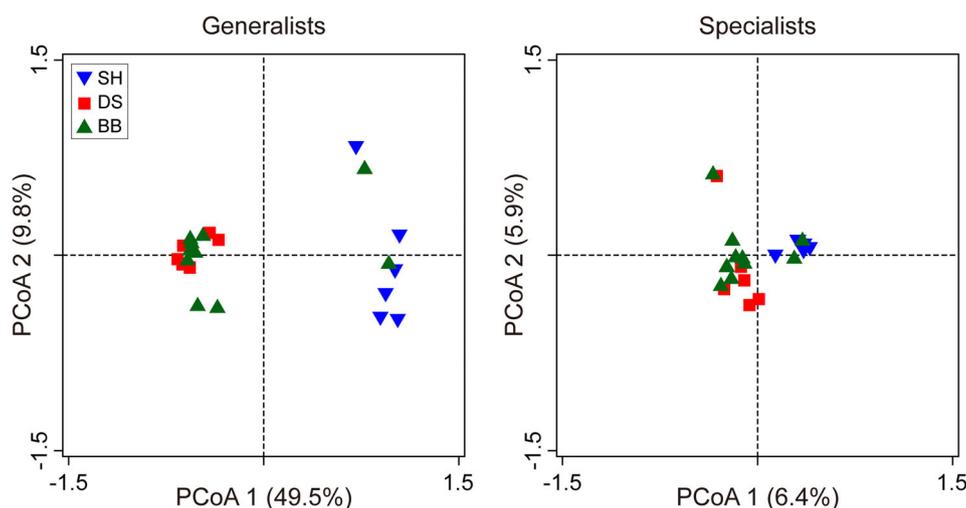


Fig 2. Principal coordinate analysis (PCoA) of bacterial generalist and specialist sub-communities using Bray–Curtis similarity. SH, Shenhui Bay; DS, Dongshan Bay; BB, Beibu Gulf.

Table 1. Observed C-scores and C_{var} -scores, mean metric values under null models, and standardized effect sizes (SES) for marine bacterioplankton subcommunities of 22 samples. If SES values are positive, it will show less co-occurrence than expected by chance, implying the predominance of segregation within communities and vice versa for negative values, implying the predominance of facilitation. If co-occurrences are not different from what is expected by chance, values of SES should fall between -2 and 2 .

Taxa	Number of OTUs	C-score			C_{var} -score		
		Obs.	Mean null	SES	Obs.	Mean null	SES
Generalists	104	14.10	11.72	34.76	272.17	58.13	49.84
Specialists	503	2.82	2.79	12.02	5.02	4.71	8.43

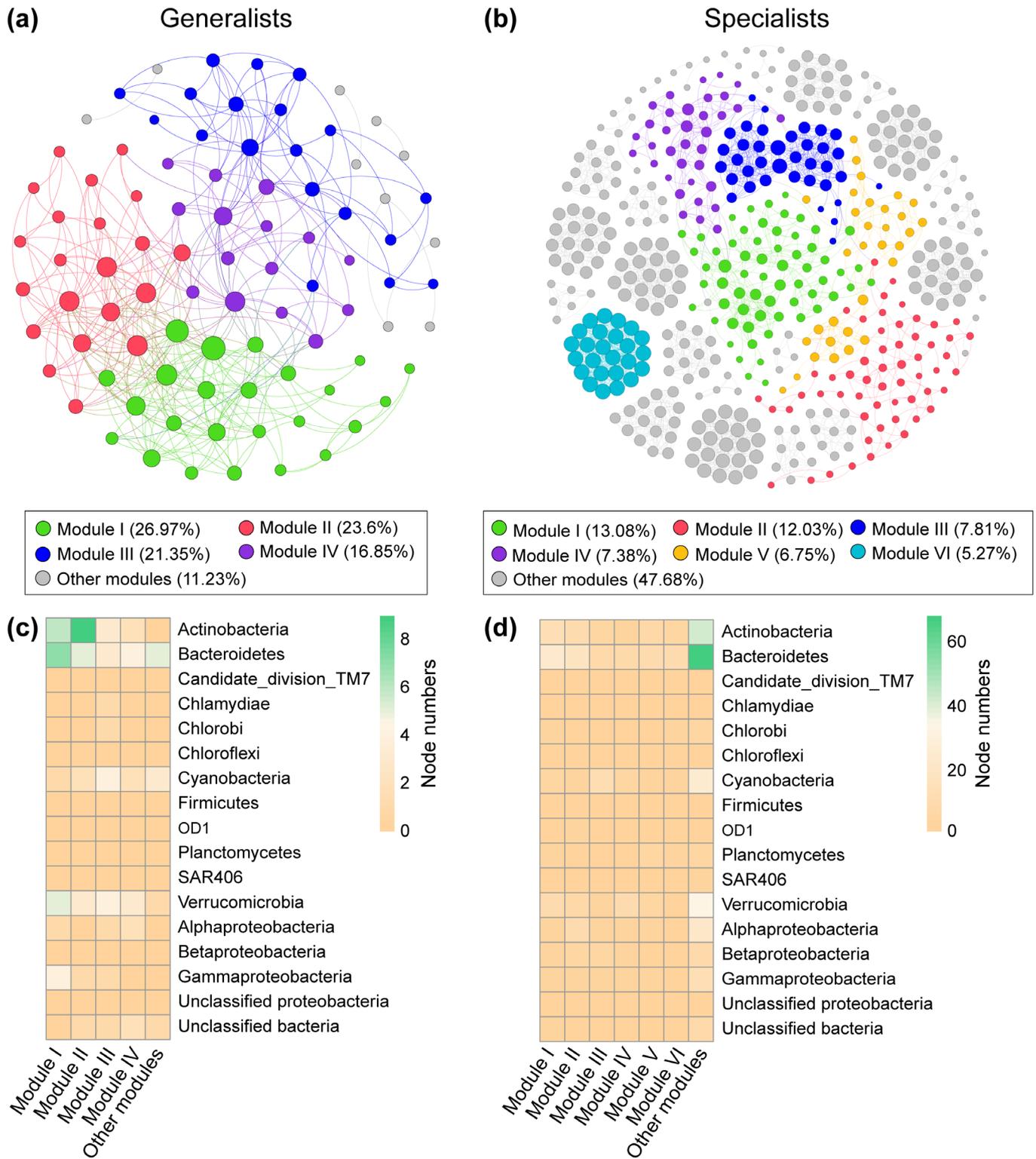


Fig 3. The co-occurrence patterns among generalists (a) and specialists (b) revealed by network analysis, respectively. The nodes were colored according to different types of modularity classes. Components of bacterioplankton biodiversity in each module of the co-occurrence network for generalists (c) and specialists (d) at the phylum/class-level, respectively. A connection stands for a strong (Spearman’s $|r| > 0.75$) and significant (p -value < 0.01) correlation. The size of each node is proportional to the number of connections (i.e., degree).

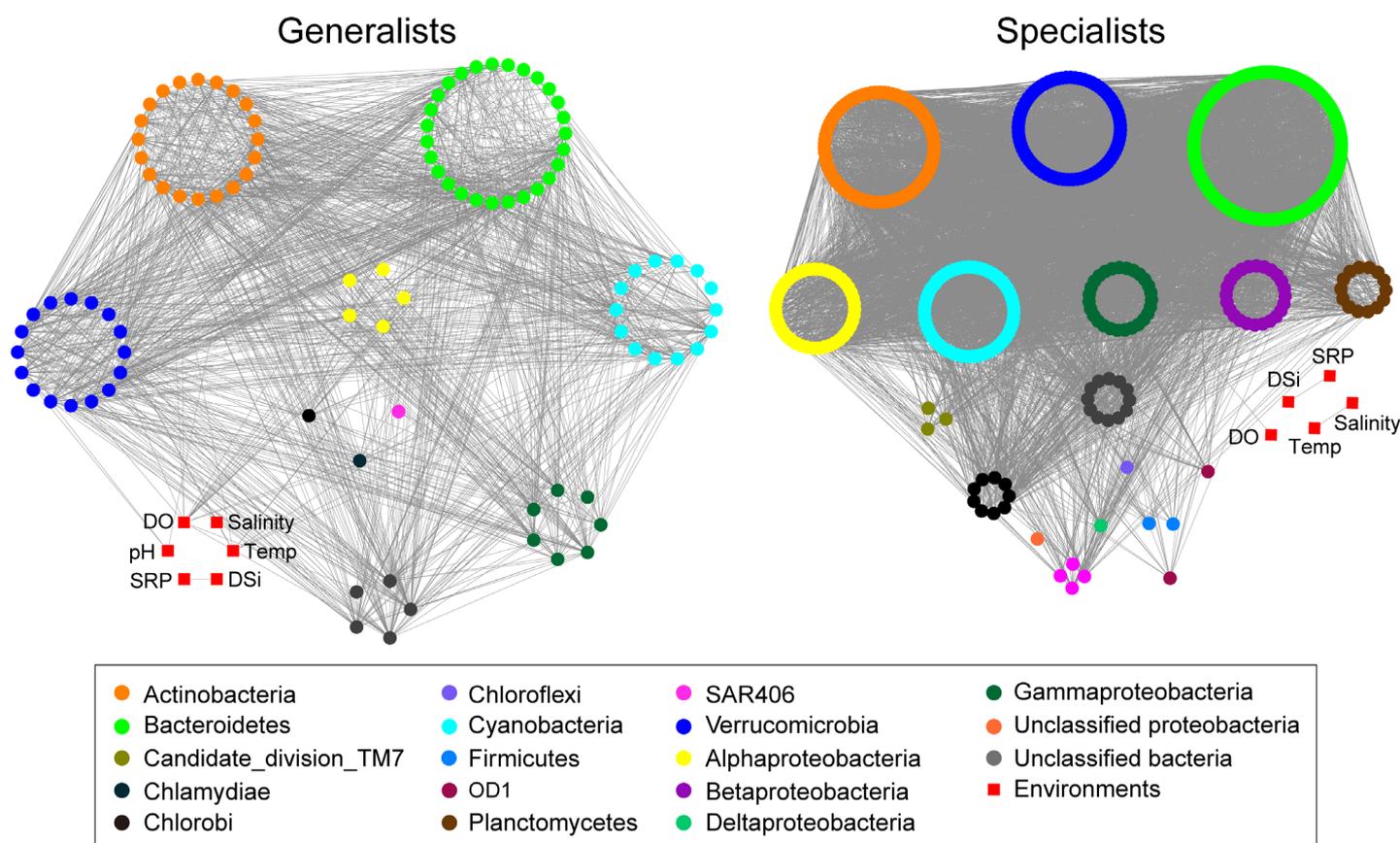


Fig 4. Species–species and species–environment association network. A connection stands for a strong (Spearman's $|r| > 0.6$) and significant (p -value < 0.01) correlation. Temp, temperature; DO, dissolved oxygen; SRP, soluble reactive phosphorus; DSi, dissolved silicon.

Specifically, we did not find a mixture of abundant and rare OTUs in generalists, and specialists did not contain any rare OTUs but only included an abundant OTU based on combined their local and regional relative abundances. Almost all generalists and specialists were moderate OTUs, and most generalists and specialists were in taxa identified as of intermediate abundance (Fig. S2b). The rarefaction curves of bacterial communities from the three bays showed a full rarefaction saturation for each of three bays (Fig. S3). A higher number of taxonomic groups was found for bacteria classified as specialists compared to generalists (Fig. S4). The results of PCoA based on Bray–Curtis similarity revealed that the distribution patterns were different between generalists and specialists across three subtropical bays. Compared with specialists, generalists were less clustered (Fig. 2).

Network of whole community

The whole network included 1909 nodes (OTUs) and 22,325 edges (Fig. S5-left; Table S3), and it was clearly separated into six modules. The co-occurrence network showed a scale-free distribution (power-law: $R^2 = 0.609$, Table S3), suggesting that the network structure had non-random

characteristics. Further, the observed modularity, average clustering coefficient and average path length were much higher compared to their corresponding values from Erdős–Rényi random networks (Table S4), implying the bacterial network exhibited a "small-world" property and modular structure. The majority of the generalists OTUs were in modules II and III, whereas the most of the specialists OTUs were in modules I, IV, V, and VI (Fig. S5-right).

Co-occurrence patterns of the generalists and specialists

The C -score results demonstrated that the co-occurrence patterns of bacterial generalists and specialists were non-random and exhibited significant segregated distributions; because the observed C -score values were higher than the C -score for expected by null models, and we always can observe standardized effect size values > 2 for the real networks (Table 1). They also showed small-world properties (their small-world coefficient > 1) (Table S4). Network topological features showed that the co-occurrence pattern in the specialists totally differed from the generalists network (Table S4). Generalists formed a smaller network with less nodes (89) and edges (360) than the specialists network (nodes: 474, edges: 2381). Modularity values,

clustering coefficient and closeness centrality were higher for specialists compared to generalists (Table S4).

Modular structure of the co-occurrence network was compared between generalists and specialists (Fig. 3a–d). The network of generalists parsed into four major modules, which accounted for 88.77% of the generalists network. However, specialists formed six network modules, which accounted for 52.32% of the specialists network (Fig. 3a,b). The four major modules were primarily occupied by Bacteroidetes and Actinobacteria for generalists, whereas Bacteroidetes, Actinobacteria, Cyanobacteria, Verrucomicrobia, and Alphaproteobacteria had higher network nodes in the

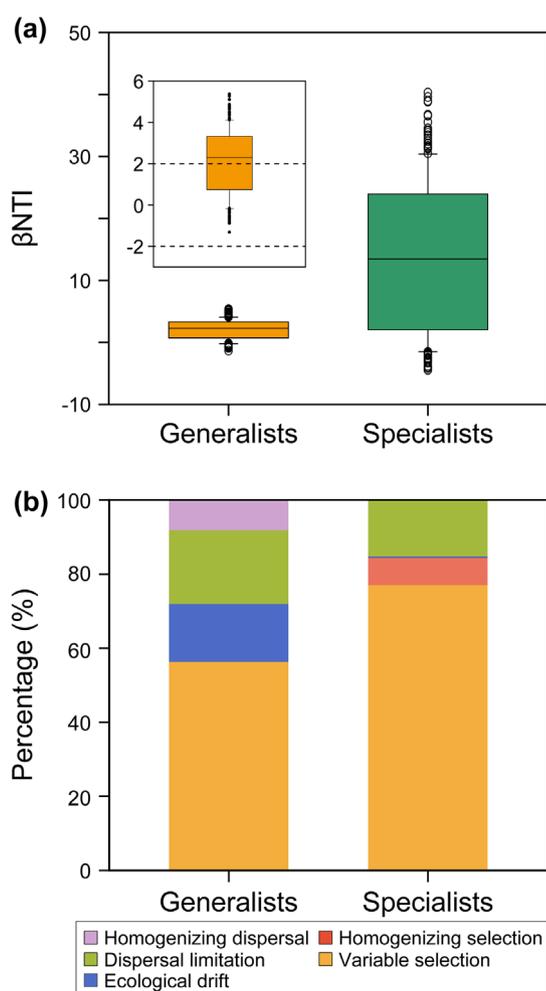


Fig 5. Community assembly processes of bacterial community from three subtropical bays. **(a)** Community assembly processes inferred using phylogenetic distance. Patterns of the beta nearest taxon index (β NTI) for generalists and specialists, and the horizontal dashed lines represent upper and lower significance thresholds at β NTI = +2 and -2, respectively. **(b)** The variation of bacterial generalists and specialists subcommunities governed primarily by deterministic processes. The between-community mean-nearest-taxon-distance (β MNTD) metric is used to quantify the phylogenetic turnover between communities. The beta nearest taxon index (β NTI) is the difference between observed β MNTD and the mean of the null distribution of β MNTD normalized using its standard deviation.

six modules of specialists network (Fig. 3c,d). The results of MCODE analysis showed that generalists generated six significant clusters with network scores ranging between 3 and 7.8, and the top six significant clusters were found from 48 sub-networks of specialists with network scores ranging from 17 to 25 (Fig. S6a,b).

In addition, the natural connectivity in the specialists network was different from that of generalists (Fig. S7). The natural connectivity of the specialists was lower than that of the generalists during initial period, indicating that the generalists network was more stable. Yet, when the proportion of removed nodes was greater than 21%, the generalists network was less stable than specialists. Based on connectivity analysis, 20 OTUs were defined as keystone species for generalists, including Actinobacteria (5 OTUs), Bacteroidetes (6 OTUs), Cyanobacteria (2 OTUs), Verrucomicrobia (3 OTUs), Gammaproteobacteria (1 OTU), and Unclassified bacteria (3 OTUs) (Table S5). For specialists, only 2 OTUs belonged to keystone species, including Bacteroidetes and Betaproteobacteria taxa (Table S5).

Factors related to the generalists and specialists

The relative abundance of generalists was significantly correlated with pH, temperature, and DO, while the diversity of specialists was significantly correlated with pH, temperature, DO, and $\text{NO}_2\text{-N}$ (Fig. S8).

The co-occurrence networks showed significant pairwise correlations among OTUs or between OTUs and environmental factors (Spearman's $|r| > 0.6$, $p < 0.01$) (Fig. 4). The pH, temperature, DO, and salinity were significantly correlated with network connectivity of generalists, of which temperature and DO exhibited a higher degree compared to other variables in the network (Fig. 4). For the specialists, DO (there were DSi-SRP and salinity-temperature relationships, but these environmental factors did not show any significant correlation with OTUs) was only driver of network connections, and it showed a low degree of connectivity in the network (Fig. 4).

Ecological processes influencing bacterial community assembly

The values of β NTI varied partly from -2 to 2 but mostly > 2 for the generalists, and specialists exhibited a higher value of β NTI (> 2) in three subtropical bays (Fig. 5a). Stochastic processes contributed approximately 44% to the community assembly of generalists, and the communities of habitat generalists were mainly driven by deterministic processes (56%). Interestingly, the deterministic processes were also largely responsible for the community assembly of specialists (84%), of which the greatest contributor was variable selection (77%, Fig. 5b).

Discussion

The previous analysis of these data focused on patterns in abundance from rare to abundant taxa (Mo et al. 2018). Given the difficulties in assigning “generalist” and “specialist” status

to microorganisms this raised an obvious question namely, has the current study identified patterns different from those shown in the abundance study or have the two approaches simply identified the same abundance based on patterns using different approaches? The fact that for both generalists and specialists the majority of taxa were of moderate abundance shows that these new analyses are picking up different patterns, which require explanation. As analyses such as ours (assigning specialist and generalist classifications and comparing these with abundance data) are rare in microbial ecology any interpretations have to be tentative. While it is impossible to rule out the possibility that these patterns are artifacts of the way we have classified these data it is possible to suggest an ecological interpretation. One would not expect a direct mapping of abundant and rare onto generalist and specialist as the abundance of a generalist or specialist microorganism will depend on local conditions (Andrews 1991; McArthur 2006); a pattern also seen in macroorganism such as birds in Corvidae where specialists have a more limited range but can be reasonably common in the right environment (Wilkinson 2006). Although one might expect many specialists to be rare it is possible that in these data the OTUs classified as rare were mainly organisms which were not an active part of the microbial community but had been washed in from elsewhere. As generalists are expected to be outcompeted by specialists under conditions that suit that particular specialist it is also likely that generalists will often not be the commonest taxa in a local community.

Biogeographical patterns of generalists and specialists

Our results suggested that a higher diversity was identified in the specialists than generalists taxa (Fig. S4); a result that matches that often found in macroscopic organisms (Andrews 1991). Reasonably, most bacteria are divided into specialists, because taxa have been identified in which specialize on particular resources (Hunt et al. 2008). This is consistent with previous observations showing that specialists dominate in species-rich communities (Logares et al. 2013). For example, it is shown that habitat specialists dominate microbial communities in sediments or aquatic environment (Székely and Langenheder 2014; Mariadassou et al. 2015). However, our results differ from recent research which found many habitat generalists and few specialists in aquatic bacterial communities (Muscarella et al. 2019). One possibility is that generalists, multivorous bacterial communities have a fitness advantages when resources are essentially substitutable (Egli 2010). An additional problem is that some of the taxa identified as specialists may not be functioning parts of the community samples but vagrants, washed in from elsewhere. Because Levin's niche breadth emphasizes high-probability occurrences (rather than the rare) it is considered reasonably insensitive to this issue—as long as these vagrants are not too common (Southwood and Henderson 2000). In addition, we removed the OTUs with the lowest relative abundances from our dataset (see Materials and methods) to avoid or reduce this

problem. Thus, although at our current state of knowledge it is impossible to rule out a substantial effect on vagrant taxa on our results, we are inclined to think this is not a significant problem.

Our results also revealed that generalists and specialists within the marine bacterial community exhibited distinct biogeographical patterns, where specialists were more clustered in our ordinations than generalists across the three subtropical bays (Fig. 2). This result is consistent with Liao et al. (2016) who found that generalist and specialist bacteria had distinct biogeographical patterns in 21 plateau lakes of China. More importantly, the biogeographical analyses yielded distinct patterns, implying that underlying assembly mechanisms, to some extent, exhibit differences between generalists and specialists. In previous study, however, we found similar biogeographical patterns between abundant and rare bacteria subcommunities in three subtropical bays of China (Mo et al. 2018).

Co-existence patterns of generalists and specialists

Network structure has important implications for the co-occurrence of species as well as for their stability (Bascompte et al. 2003). Our results clearly support the view that non-random community construction may be a common characteristic in bacterioplankton communities, as has been previously reported (Horner-Devine et al. 2007). For example, the whole bacterial community, as well as generalists and specialists in our networks followed a non-random assembly pattern (Tables 1, S3). This means that the biogeographical pattern can be predicted for the majority of bacterial community (Yao et al. 2019).

However, the network structure of specialists was more complex compared with generalists (Figs. 3a–d, S6; Table S4). In general, a more complex network structure may indicate more stable co-existence patterns. To some extent, stable co-occurrence pattern mirrors the less dynamic characteristic (Costa et al. 2006), as Thébaud and Fontaine (2010) demonstrated that a high connectivity promoted community stability in mutualistic networks. However, in this study, although the specialists had more network nodes and edges leading to more complex network structure than generalists, the network stability of specialists is not always greater than that of generalists (Fig. S7). The difference in network complexity and stability between generalists and specialists might be attributed to two reasons. On the one hand, differences were found in composition between specialist and generalist networks. The network of generalists was primarily constructed by the interaction between Actinobacteria and Bacteroidetes groups (Fig. 3c), while the network structure in the specialists consisted primarily of the interaction among five taxonomic groups (Fig. 3d). In addition, the results of MCODE analysis indicated that the network clusters with greatest scores of generalists included only five phyla/classes, while seven taxonomic groups frequently dominated the largest specialists clusters, with a greatest cluster score from specialists (score = 25) that is much higher than that of generalists (score = 7.8) (Fig. S6). This suggests that stronger pairwise

interactions among OTUs were found in specialists than in generalists. Different intensities of interactions among different taxonomic groups could shape networks with different degrees of complexity and stability. Our result is in accordance with a previous study which found that different taxonomic compositions possibly affected bacterial generalists and specialists network structure observed in soil ecosystem, so that shaped different bacterial network complexity and stability (Barberán et al. 2012). Interestingly, besides dominant groups mentioned above (included Actinobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia), one Gammaproteobacteria also belonged to potential key species for generalists, and one Bacteroidetes OTU and one Betaproteobacteria OTU were defined as potential key species for specialists networks across the three subtropical bays (Table S5). The loss of these species may lead to the breaking apart of the ecological networks and modules (Guimerà and Amaral 2005). Therefore, these potential key species might be important in maintaining the stability of generalists and specialists subcommunities.

On the other hand, these modules from both generalists and specialists did not necessarily mirror taxonomic classification. That is, major bacterial interactions were stronger between phyla/classes compared to within a phylum/class (Figs. 3, S6), providing evidence that the bacterial community structure is shaped by environmentally driven functional characteristics rather than phylogeny (Burke et al. 2011). The results demonstrated that non-random co-occurrence of generalists and specialists across the three subtropical bays, and thus these network structures may be driven by deterministic processes (Banerjee et al. 2016). Indeed, dissolved oxygen significantly associated with major groups of specialists, whereas more environmental variables significantly influenced generalists network structure, in which dissolved oxygen and temperature had maximum number of associations and acted as the most important determinants of generalists network we investigated (Fig. 4). The different environmental factors, or the interactions among different environments, drove the formation of different complex and stable networks. Previous studies have highlighted the importance of interdependencies between temperature and bacterioplankton networks in the Atlantic Ocean (Milici et al. 2016). Recent findings demonstrated that temperature is major environmental factor in protist network structure in marine intertidal sediments (Pan et al. 2020). However, Banerjee et al. (2016) did not find that temperature influenced bacterial network construction in Canadian agroforestry systems. The difference probably resulted from different ecosystems with different environmental gradients, because their samples came from soil ecosystem rather than from aquatic ecosystem.

Strong deterministic processes driving biogeographical distribution

In many studies, specialists appear to respond mostly to deterministic processes, whereas habitat generalists may be predominantly affected by stochastic and/or deterministic

processes (Liao et al. 2016; Lindh et al. 2016). However, generalists are sometimes primarily influenced by deterministic processes possibly because competitive interactions between specialists and generalists promote a preference for suboptimal habitats in generalists (Jacob et al. 2018). This means that deterministic processes were important mechanisms for the generation of biogeographical distribution in bacterial community. Similar results were found by Logares et al. (2013), who investigated the biogeography of bacterial communities exposed to progressive long-term environmental change in coastal lakes.

Indeed, in our study, the distinct biogeographical pattern could be mainly explained more by deterministic processes than stochastic processes. For instance, the results of Stegen null model indicated that deterministic processes had stronger roles for generalists and specialists subcommunities assembly than stochastic processes, because deterministic processes explained the large fraction (56% for generalists; 84% for specialists) of the community variability (Fig. 5a,b). In fact, environmental factors such as pH, temperature, and DO had a significant influence on variations of generalists abundance, while pH, temperature, DO, and NO₂-N showed a significant relationship with habitat specialists richness in our study (Fig. S8). It appeared that deterministic processes were predominantly responsible for both generalists and specialists, whereas the stochastic processes could partially influence the assembly of them.

Moreover, deterministic processes were stronger contributors for explaining specialists community variation compared to generalists. On the one hand, this difference may depend on certain traits of functional or taxonomic groups (Lindström and Langenheder 2012). Taking aquatic invertebrates as an example, a previous study had shown that habitat generalists vs. specialists with different lifestyles are prone to be affected by deterministic processes with different strengths (Pandit et al. 2009). Microorganisms such as bacteria also have similar life history traits, for example, dormancy strategies are often common for bacterioplankton in marine ecosystem (Gasol et al. 1995). Wu et al. (2018) suggested bacterial taxa with wider niche breadth may be due to their greater potential for dormancy, and thus habitat generalists with wide niche breadth are more susceptible to dormancy compared to specialists reducing the impact of the environmental conditions. This suggests that deterministic processes are important for both generalists and specialists, whereas the processes involved in structuring specialists are more important. On the other hand, many habitat generalists have evolved broad habitat tolerance and strong functional plasticity (Székely et al. 2013) to resist the environmental disturbances avoiding extinction risk. For example, generalists could normally experience larger geographical ranges than specialists (Sheth and Angert 2014), which has been further supported by recent study that certain marine bacteria could live in freshwater ecosystem (Comte et al. 2014). By contrast, habitat specialists

with narrow environmental tolerance tend to be particularly sensitive to environmental change and variability. As a result, habitat specialists may not respond to new environmental selection because of lack of plasticity, causing them to be vulnerable to extinction (Sheth and Angert 2014). Therefore, our results support that deterministic processes have a greater impact on specialists subcommunity than generalists. However, our finding differed from another study which found that bacterioplankton community assembly is governed by environmental selection for habitat generalists but not specialists in the Baltic Sea (Lindh et al. 2016). The difference may be attributed to sizes of geographical distance among distinct study sites, because Lindh et al. (2016) studied bacterioplankton community based on the small scale (one hundred kilometers transect), whereas our study at the mesoscale (tens to thousands of kilometers) (Mo et al. 2018).

Conclusion

Our study provides the first example of research revealing both biogeographical and co-occurrence patterns of habitat generalists and specialists from marine bacterioplankton communities at a mesoscale. The fact that our analyses find differences between the taxa defined as generalist and specialist and those defined as common and rare suggests that our spatial approach to identifying generalists and specialists is likely being successful (and not confounding abundance with niche breadth). This helps address a wider issue in ecology—namely to what extent is the ecology of microorganisms similar to the much better known ecology of macroorganisms? Distinct biogeographical patterns were found between generalist and specialist bacteria, suggesting that both groups exhibited differences in responses to environmental gradient to some extent. More importantly, this study highlights that deterministic processes appear to play more vital roles in the assembly of both generalists and specialists subcommunities than stochastic processes, yet the impact of deterministic processes on the specialists is more important than that of generalists. Specifically, pH, temperature, and dissolved oxygen were the three most important environmental factors in shaping bacterioplankton subcommunity assembly. Network analysis showed that habitat generalists and specialists exhibited non-random characteristics, but the bacterial co-occurrence pattern in generalists was different from specialists in complexity and stability. The bacterial co-occurrence pattern is simultaneously affected by the interaction of species and environmental filtering. Overall, our results revealed different biogeographical patterns of habitat generalists and specialists were mainly driven by deterministic processes. To obtain a comprehensive and full understanding of marine bacterial community biogeography and co-occurrence patterns, both generalists and specialists should be distinguished in future studies.

Data availability statement

All 16S rRNA gene sequences data from this study have been submitted to the NCBI database (<http://www.ncbi.nlm.nih.gov/>) under the accession number SRP109151 and BioProject accession number PRJNA380540.

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Conflict of Interest

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