

32 **Introduction**

33 The coastal natural lagoons are transitional ecosystems at the boundary between land and sea.
34 These vulnerable ecosystems are indirectly connected with the ocean and experience saline
35 intrusions (Schallenberg & Larned et al., 2010). In addition to water exchange with the ocean,
36 the status of a lagoon largely depends on the water quality of all inflowing rivers as well as
37 precipitation. Increased precipitation and river discharge would reduce salinity and enhance
38 eutrophication (Thompson et al., 2009a). Hydrodynamic characteristics are important controls
39 in coastal lagoons as well. Factors such as tidal changes, wind speed, and water density are
40 important drivers of water exchange in the lagoon ecology (Thompson et al., 2009b).
41 Plankton primary production is co-limited by phosphorus and nitrogen levels due to the
42 combined effects of water and high nutrient inputs from the boundary (Béjaoui et al., 2017).
43 Similarly, it has been reported that freshwater surges lead to short-term increases (1–2days) in
44 bacterial production as well as increases in the abundance of bacterioplankton and
45 picoeukaryotes (Fouilland et al., 2017). A study on the bacterioplankton in the Conceição
46 Lagoon, Southern Brazil, was carried out in winter and summer to characterize the bacterial
47 spatiotemporal distribution and heterotrophism. This study indicated that bacterial abundance
48 increased significantly ($p < 0.05$) in summer. Principal component analysis showed that salinity,
49 temperature, and light were the abiotic factors that better explained the temporal variability of
50 bacterial assemblages. Spatially, bacterial assemblages were influenced by nutrient gradients
51 and oxygen (Fontes & Abreu, 2010). The Rodrigo de Freitas Lagoon consists of fresh water
52 but has a connection with the ocean through a channel. Thus, research results showed that the
53 lagoon is affected by adjacent fresh water and the structure of the bacterial community had
54 both freshwater and marine characteristics when sampled from within the channel.

55 Prokaryotes are key components within lagoons, due to their role as primary producers (e.g.,
56 photoautotrophic bacteria). Prokaryotes are agents of organic matter remineralization and
57 particles degradation, cycling of biogeochemically relevant elements, pollutants degradation,
58 and transfer of matter and energy to higher trophic levels (Quero et al., 2017). In recent years,
59 bacterioplankton research has been given more attention for these reasons. Researchers have
60 carried out extensive research on coastal waters nationally and internationally. Coastal
61 lagoons are highly productive ecosystems characterized by chemical and physical gradients

62 that make these systems unstable and subject to fluctuating conditions (Manini et al., 2003).
63 They provide diverse ecosystem services, such as flood and erosion control, shoreline
64 stabilization, sediment and nutrient retention, local mitigation of climate change effects and
65 water purification, and they represent a reservoir of biodiversity and biomass (Danovaro,
66 Pusceddu, 2007). At the same time, coastal lagoons are vulnerable to a number of
67 anthropogenic disturbances such as agricultural, industrial, and tourist activities (Ghai et al.,
68 2012a; Ferrarin et al., 2015). They represent a transition zone between terrestrial, freshwater,
69 and marine interfaces (Newton et al., 2014) and act either as sinks for organic matter
70 accumulation (Pinhassi, Berman, 2003). In addition, they can act as reservoirs able to fertilize
71 the adjacent sea by exporting organic and inorganic nutrients (Marques et al., 2014a). The
72 balance between export and accumulation depends, in addition to physical and hydrological
73 factors, on degradation and utilization processes by planktonic and benthic microbes. This
74 data demonstrates the unique importance of studying the spatial and temporal dynamics of
75 lagoon microbes.

76 Shanghai Fengxian Bihaijinsha is located in the south of Hangzhou Bay, near the Yangtze
77 River estuary. As a typical offshore artificial lagoon, it was built in 2005 via coastal
78 reclamation. The sea area is about 2.30 km² and the average depth is 15 meters. The water of
79 this artificial lagoon, and its adjacent sea, were characterized by low salinity, muddy water,
80 and large sediment. The industrial waste water receiving area of Hangzhou Bay poses a threat
81 to this environment. The temporal and spatial changes of the microbial community in an
82 industrial effluent receiving area in Hangzhou Bay were investigated by 454 pyrosequencing,
83 and the bacterial community showed that proteobacteria dominated the bacterial communities
84 of all sediment samples tested (Yan et al., 2016). It was found that distribution of microbes in
85 the Yangtze River estuary had obvious seasonal variations. In summer, *Shewanella* and
86 *Pseudomonas* were the dominant species, while the highest abundance in winter was
87 *Acinetobacter* (Cao et al., 2011). Flow cytometry was used to examine the abundance and
88 distribution of different picophytoplankton groups (i.e., *Synechococcus*, *Prochlorococcus*, and
89 picoeukaryotes). As such, nanophytoplankton, heterotrophic bacteria and viruses were
90 examined in the Yangtze River estuary, China, and adjacent coastal waters during the autumn
91 of 2004. The results showed that picoeukaryotes were the most successful group among

92 picophytoplankton in nearshore eutrophic waters, whereas *Prochlorococcus* surpassed other
93 groups within the pico- and nanophytoplankton communities in the offshore oligotrophic
94 regions of the East China Sea Shelf (Pan , Zhang & Zhang , 2007).

95 In recent years, the Fengxian artificial lagoon has been mainly used to breed economic
96 shrimp such as *Penaeus monodon* and *Penaeus orientalis*. The water contained in it is restored
97 in March by local tides, and drained off the following January. The water column of the
98 Fengxian artificial lagoon has higher transparency than the adjacent open sea, and is affected
99 by the tide and precipitation levels. Due to aquaculture activities and the characteristics of
100 water exchanges, the water quality of the artificial lagoon is difficult to control. The dynamics
101 and diversity of bacterial communities are important indicators of ecosystem health and
102 function. Changes in microbial community structure can also provide useful information
103 about water environmental assessment and pollution control (Ghai et al., 2012b). In order to
104 explore the relationship between water quality and the microbial communities, the dynamics
105 and diversity of the microbial community in the surface water of the Fengxian artificial
106 lagoon was studied.

107 **Materials and methods**

108 **Site description and sampling**

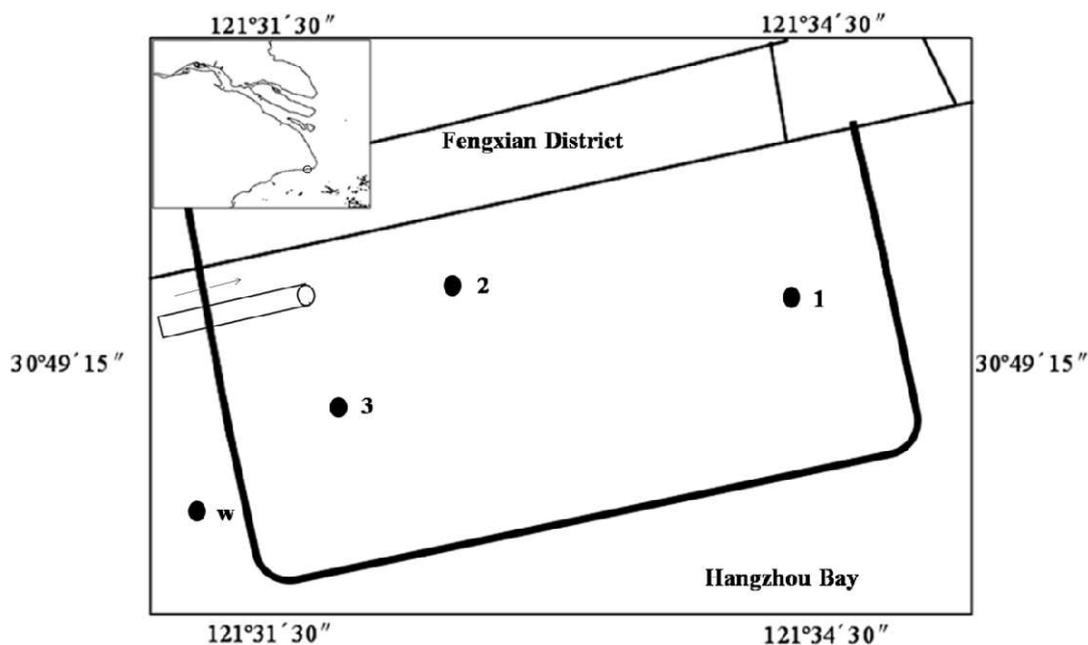
109 The survey was carried out every three months from April 2016 to January 2017, such as
110 April 22, 2016, July 13, 2016, October 16, 2016, and January 15, 2017. Due to rain, sampling
111 times were appropriately delayed. The sampling sites were located near 121°E 30°W, as shown
112 in Table 1 and Figure 1. Three liters of water from the upper 0.2 m of the sea surface were
113 taken, then transported to the laboratory (on ice). Water samples were pre-filtered through
114 3µm pore size filters to remove large organisms and particles. Freely living bacterioplankton
115 cells were collected through 0.22µm polycarbonate filters. Filters were frozen at -80°C until
116 DNA extraction. Water chemistry analysis such as temperature and DO were monitored with a
117 portable dissolved oxygen test (JENCO 9010, USA), while salinity was measured using a
118 salinity meter (HAS-10, Shanghai). NO₂-N, NO₃-N, NH₄⁺-N and PO₄³⁻-P were filtered with
119 0.22µm cellulose acetate membranes and analyzed with Skalar flow analyzer (Skalar San++,
120 Netherlands) (Londong & Wachtl, 1996). Chemical oxygen demand (COD) was determined by
121 potassium permanganate titration (Tian & Wu SM, 1992).

122 Table 1:

123 Location of the sampling site

site	longitude	latitude	Water depth(m)
1	121°34'00.78"E	30°49'23.87"N	5
2	121°32'46.69"E	30°49'35.48"N	7.8
3	121°31'39.91"E	30°49'10.68"N	5.5
w	121°30'46.32"E	30°48'41.76"N	2.5

124



125

126

Figure 1: Location of sampling sites in FengXian.

127

128 DNA extraction, PCR and illumine sequencing

129 After frozen filter membranes were ground in liquid nitrogen, then samples were thoroughly

130 mixed and centrifuged, and the environmental samples were extracted. DNA was quantitated

131 by 1% agarose gel electrophoresis and then subjected to PCR amplification. Amplification of

132 bacterial 16S rRNA gene fragments was conducted using barcode and adaptor added primer

133 515F (5'-GTGCCAGCMGCCGCGG-3') and 907R (5'-CCGTCAATTCMTTTRAGTTT-3')

134 (Xiong et al, 2012).Barcode sequences were ligated to the sequencing primer during the

135 process of primer synthesis, before PCR was performed. The reaction system consisting of 20

136 μL was assembled as follows: 5 \times FastPfu Buffer(4 μL), 2.5 mM dNTPs(2 μL), forward
137 primer(5 μM)(0.8 μL), reverse primer(5 μM)(0.8 μL), FastPfu Polymerase(0.4 μl), Template
138 DNA(10 ng).The PCR reaction was performed in triplicate under the following conditions: an
139 initial denaturation at 95°C for 3 min, 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for
140 45 s, and then a final extension at 72°C for 5min.After PCR amplification, the resulted PCR
141 products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA
142 Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using
143 QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol. Finally, the
144 Illumina platform library was constructed and sequencing started. Sequencing was carried out
145 on Illumina platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

146 **Sequence quality control and operational taxonomic unit(OTU) assignment**

147 The raw reads were processed following the pipeline of Mothur. According to the overlap
148 relationship between PE reads, the paired reads were merged into a sequence, and the quality
149 of reads and the effect of merge were quality-controlled. According to the sequence
150 information of the two barcode ends and primer sequences, samples were discriminated and a
151 valid sequence was obtained. Filtering parameters were: (i) minimum average quality score of
152 20; (ii) minimum overlap length of 10 bp; (iii) minimum mismatch rate of stitching sequence
153 of 0.2; (iv) barcode mismatch number of 0 and maximum primer mismatch number of 2.
154 Based on the similarity of the sequences, the sequence was classified as multiple OTUs and
155 these OTUs were analyzed by biological information at 97% similarity levels. Based on the
156 similarity of the sequences, the sequence was classified as multiple OTUs and these OTUs
157 were analyzed by biological information at 97% similarity.

158 **Data analysis**

159 Alpha diversity measures including richness estimator Chao 1 (Chao & Bunge, 2002),
160 diversity index Shannon (Magurran, 1988), and Good's coverage (Good, 1953), were
161 calculated at a 3% dissimilarity level in Mothur. Statistic Package for Social Science (SPSS)
162 software was used to analyze the diversity of the differences between the quarters. A

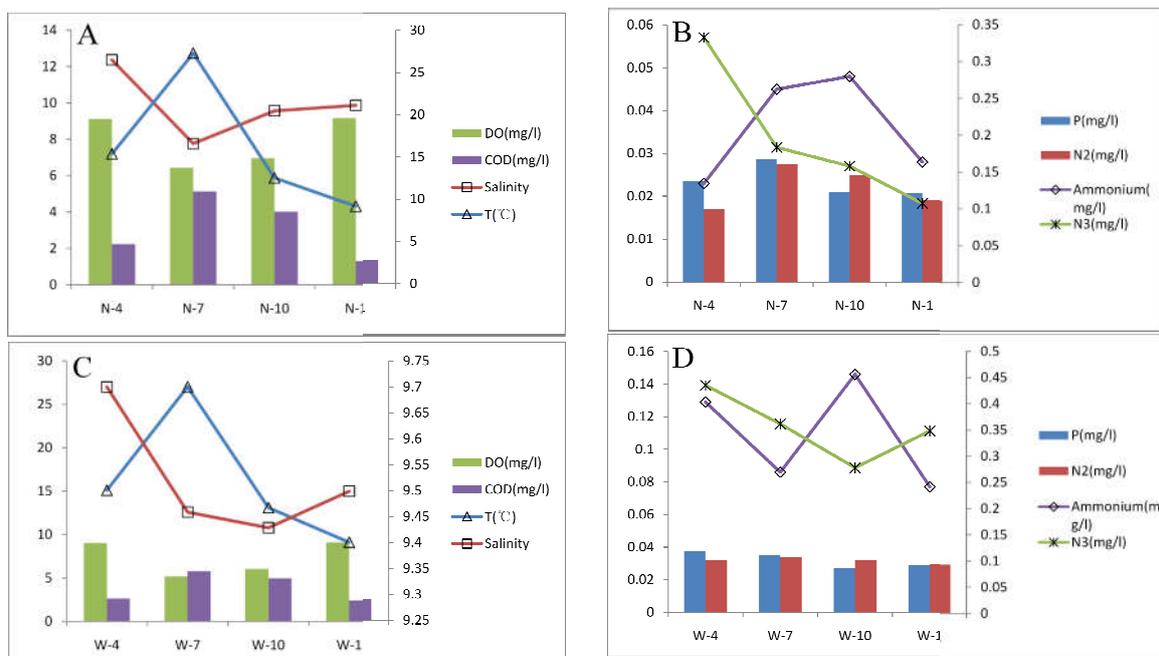
163 community barplot and heatmap were generated in the Programming Language (R) (version
 164 3.4.0) to compare the community composition of different groups at the Phylum and Genus
 165 levels. CCA with the Monte Carlo test was performed to calculate the relationship between
 166 bacterial clades and water properties at both the Order and Genus levels.

167 **Results**

168 **Environmental characterization**

169 The physical and chemical properties of sampling sites are showed in Figure 2. The
 170 temperature changed significantly over the four seasons, the maximum temperature can reach
 171 29.9°C in July, the lowest temperature reached 9.07°C in January. Change in dissolved oxygen
 172 (DO) concentration showed an opposite trend with temperature, which reached the lowest
 173 value in July and the highest value the following January. The concentration of nutrients on
 174 the artificial lagoon water surface also showed a significant gradient. The concentration of
 175 PO_4^{3-} , NO_2^- , and NH_4^+ reached the highest value in July, and the concentration of NO_3^-
 176 reached the highest value in April. The concentration of COD in each site increased at first
 177 and then decreased, with a maximum value PO_4^{3-} in July.

178 Figure 1: Location of sampling sites in FengXian.

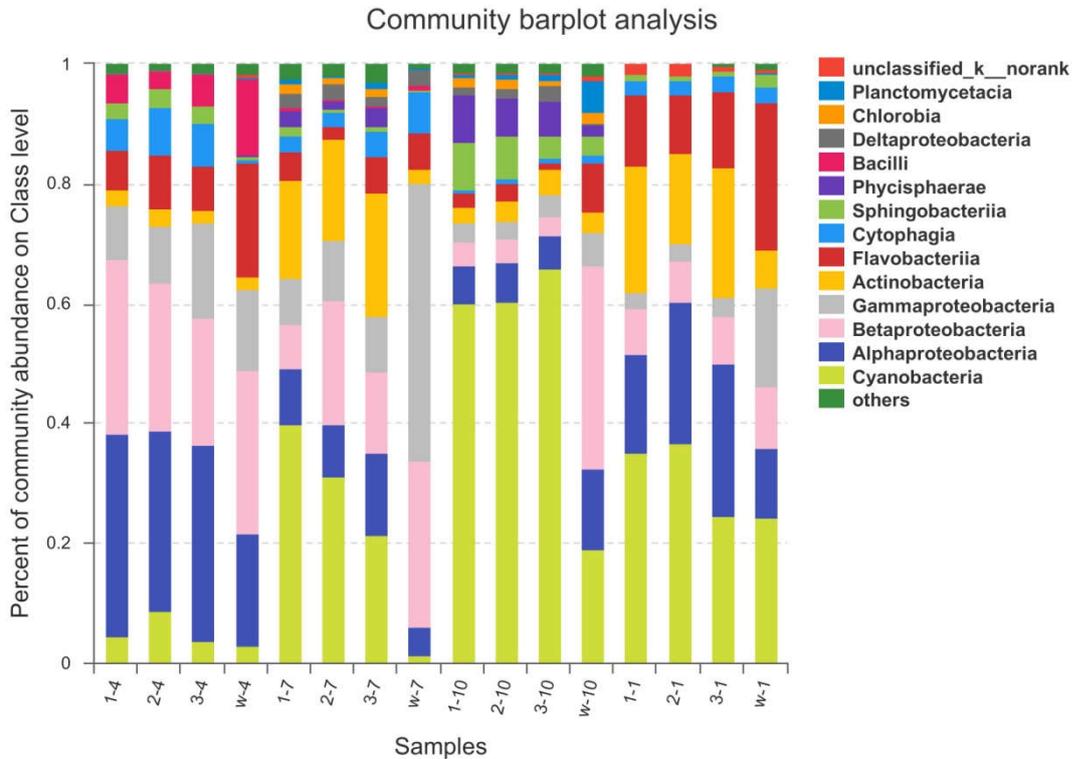


179 Figure 2: Environmental parameters of water samples.

180 Y-axis shows the concentration of environmental factors. "N" and "W" indicate lagoon and
 181 adjacent offshore respectively, and the numbers indicate the sampling month.
 182

184 **Richness and diversity estimators**

185 The total reads, ranging from 21998–44438 in each sample, were obtained for further
186 analyses. After random resampling, all sequences were fractionated at 3% dissimilarity levels,
187 ranging from 17918–39207. Data were further analyzed for diversity using Chao, Shannon,
188 and Coverage (Table 2). Among these analytical tests, which were grouped by season, there
189 was no significant difference in the Chao of all samples, but there were significant differences
190 between seasons in the closed lagoon. January, April, and July were significantly different
191 ($P < 0.05$). In addition, there was no significant difference between the Shannon diversity
192 seasons in all samples, and there was a significant difference between July, April, and October
193 ($P < 0.05$) in the closed lagoon. The coverage of all samples was above 99%, indicating that the
194 probability of the sequence being detected in the sample was extremely high and reflected the
195 real situation of microbes in the sample.

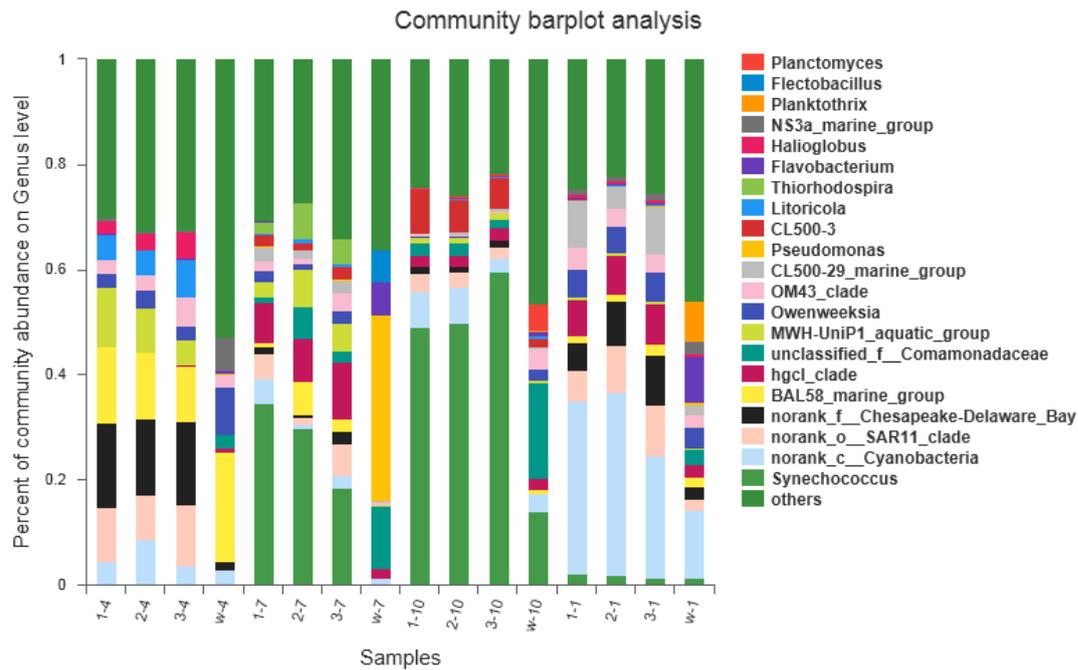


197 **Figure 3: Abundance distribution of the bacterial community at the phylum level.**

199 **Taxonomic assignment**

200 Thirty-four bacterial Phyla were found in this study. Among them, proteobacteria had the
201 highest abundance, followed by cyanobacteria (Figure 3). According to seasonal variations, it
202 was observed that the bacterioplankton community had obvious changes at the phylum level.
203 The bacterioplankton community of the artificial lagoon and the adjacent open sea were
204 significantly different, but no significant change was observed between the sampling sites
205 within the artificial lagoon.

206 *Synechococcus* appeared primarily in October and July, and the abundance of the open sea site
207 was significantly lower than that in the artificial lagoon. (Figure 4) The abundance of
208 Betaproteobacterial genes in seasonal abundance was quite different. The abundance of the
209 BAL58 marine group, primarily appearing in April, was higher in the offshore sites. However
210 MWH-UniP1, also appearing in April, was more abundant in the closed lagoon.
211 Comamonadaceae primarily appeared in the offshore samples with the highest abundance in
212 October. In addition, pseudomonas of Gammaproteobacteria was dominant in July in the
213 offshore sample and the abundance was very high. In addition, several genera appeared in
214 Actinomycetes, although the abundance was not high, but the time and space differences were
215 more obvious. The highest abundance of Actinobacteria was from the Hgcl-clade, appearing
216 in July, while the offshore abundance was lower than in the closed lagoon. Several other
217 genes were low in abundance, but there were also seasonal differences. For example, the
218 CL500-29 marine group of Actinobacteria appeared in January, and the *Owenweeksia* and
219 *Flavobacterium* of the Bacteroidetes possessed a high abundance in January and July samples
220 appearing in the lagoon and offshore sites, respectively.

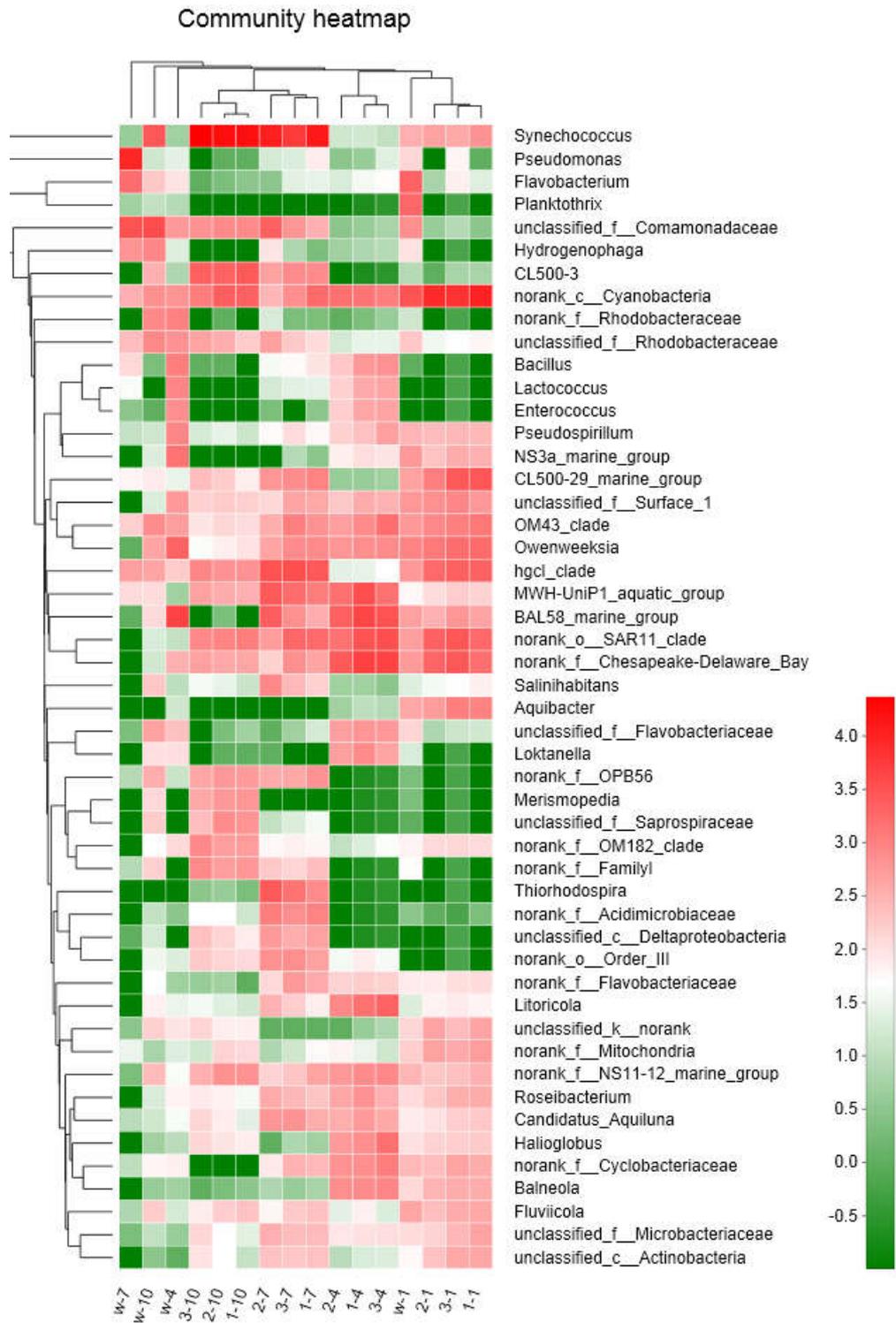


221

222 Figure 4: Abundance distribution of the bacterial community at the genes level.

223

224 Based on the composition and abundance of each genus, the similarity relationship between
 225 the samples was observed as seasonal, and the similarity between the samples was high.
 226 Samples were primarily clustered into two groups: January and April, and July and October
 227 (Figure 5). There was no obvious aggregation in the four seasons for the offshore samples.
 228 The similarity of offshore samples compared to the closed lagoon samples was higher in July,
 229 while lowest in July. Significant changes in abundance were observed at the genus level.
 230 Genes with similar kinship had similar seasonal and spatial variations. The abundance of
 231 Chesapeake-Delaware-Bay and SAR11, which belong to Alphaproteobacteria, showed
 232 similarity in space and time, appearing highest in abundance in April. Abundance in the
 233 lagoon was significantly higher than that of the open sea. Similarly, Owenweeksia and OM43,
 234 which belong to the Bacteroidetes, also showed similar abundance changes.



235

236

Figure 5: Heatmap of the top 50 genes according to abundance.

237

Phylogenetic relationships are shown on the right tree. The top tree shows the clustering relationship of the

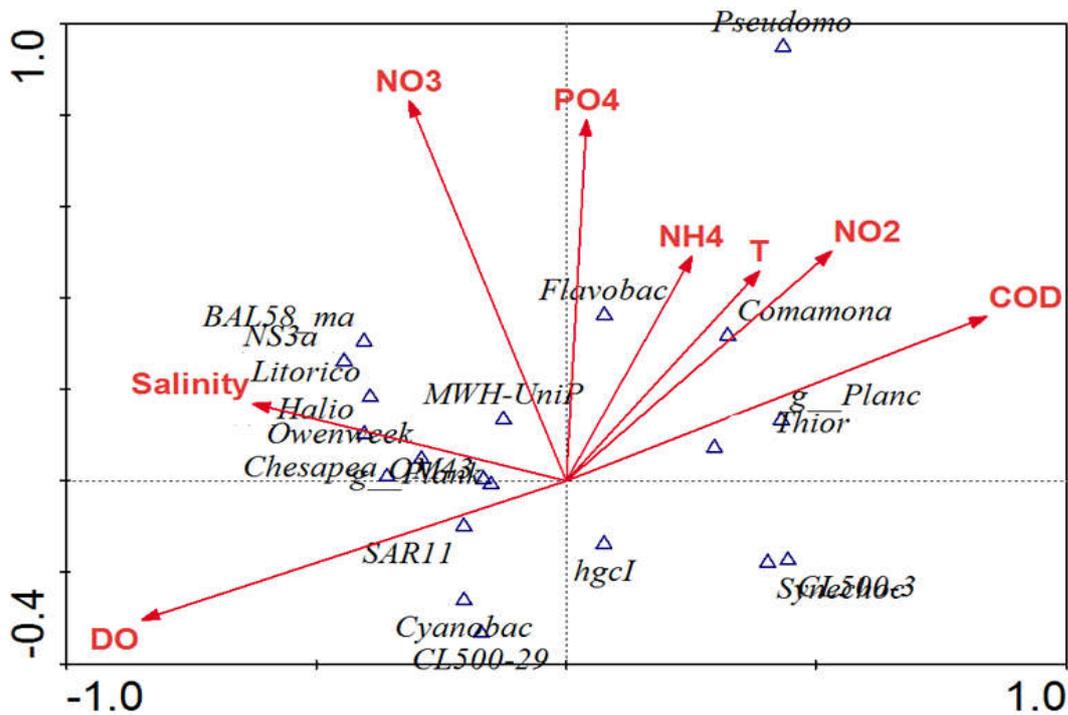
238

samples.

239

240 **The relationship between major bacterial clades and environmental factors**

241 The CCA across all samples was conducted to find the determinant environmental parameters
242 shaping bacterial groups (Figure 6). The first axis explained 24.36% of the total variance,
243 while the second axis explained 20.32%. Most of the environmental parameters contributed to
244 the heterogeneous distribution of major bacterial clades. For all samples, salinity played a
245 positive role in the aggregation of many Proteobacteria and Bacteroidetes. The results showed
246 that SAR11, affiliated with Alphaproteobacteria, was positively correlated with dissolved
247 oxygen and negatively correlated with COD. In addition, SAR11 was negatively correlated
248 with temperature and NH_4^+ and NO_2^- . The HgcI clade of Actinobacteria was negatively
249 correlated with salinity, NO_3^- , and PO_4^{3-} , but had no correlation with dissolved oxygen.
250 However, CL500-29 was negatively correlated with temperature and COD. Dominant species
251 in the offshore sea showed that Comamonadaceae of Betaproteobacteria, *Pseudomonas* of
252 Gammaproteobacteria, and *Flavobacterium* of Bacteroidetes were found to be positively
253 related to temperature and various nutrients, showing a higher correlation with NO_2^- , NH_4^+ , and
254 PO_4^{3-} . Affiliated with Gammaproteobacteria, *Thiorhodospiral* and Comamonadaceae, as well as
255 *Planktothrix* of Planctomycetes, were found to be negatively related to COD. Cyanobacteria
256 and CL500-29 were negatively correlated with temperature, NO_2^- , and NH_4^+ . *Synechococcus*
257 was negatively correlated with salinity, although not in an obvious manner, while
258 *Synechococcus* seemed to be negatively correlated with dissolved oxygen.



259
 260 Figure 6: The relationship between major bacterial clades and environmental factors.

261

262 **Discussion**

263 Coastal lagoons are unstable ecosystems characterized by chemical and physical gradients
 264 subject to anthropogenic disturbances. They represent a transitional zone between terrestrial,
 265 freshwater, and marine environments. The diversity of aquatic prokaryotes is shaped by an
 266 array of biotic and abiotic drivers. Advances in studying microbial dynamics have shown that
 267 their composition involves complex network interactions (Fuhrman, Cram & Needham, 2015).
 268 The importance of seasonality as a structuring factor for coastal bacterioplankton has recently
 269 emerged. In previous years, there were few studies on the dynamics of aquatic prokaryotes in
 270 the adjacent sea area of the East China Sea. Bacterioplankton production abundance was
 271 surveyed in a dilution zone of the Yangtze River estuary. The results showed that the average
 272 bacterioplankton production in spring was higher than autumn, and that the production at the
 273 surface was higher than the bottom in the surveyed area(Liu et al., 2001).The data presented
 274 here provided more information on the diversity of aquatic prokaryotes in the East China Sea
 275 and the nearby marine area. Previous microbiological studies in this and other lagoons were

276 restricted to sediment alone (Feng et al., 2009a; Wang et al., 2012). The present study
277 investigated differences in bacterial composition across multiple aquatic ecosystems (lagoon,
278 coastal sea) simultaneously over a seasonal cycle. It was observed that bacterioplankton alpha
279 diversity in July was lower in the lagoon than in the nearby sea. However, the
280 bacterioplankton alpha diversity in both sea areas was higher than in other seasons. In a study
281 that compared lagoons differing in primary productivity, higher bacterial richness was found
282 in the more productive lagoons. We speculate that there is a high diversity of planktonic
283 bacteria in the nearby sea because of the higher concentration of nutrients. Affected by the
284 discharge of land-based sources and man-made activities in Hangzhou Bay, the seawater near
285 the lagoon has maintained a high concentration of nutrients. Due to the impact of aquaculture
286 activities in the lagoon, nutrient concentrations in July were higher. In other months, due to
287 rainfall and large-scale seaweed breeding activities, nutrients had varying degrees of
288 reduction. The CCA plot of lagoon bacterioplankton showed that phosphates and nitrates were
289 also major environmental drivers.

290 Discovery of the mechanisms and drivers of community assembly is critical to understanding
291 the processes of microbial variation and maintenance, especially in coastal lagoons. The
292 present study showed that within each environment and domain investigated, temporal
293 variations were more important than spatial variations in structuring the assemblages. These
294 results highlighted the fundamental role played by seasonality in structuring coastal
295 bacterioplankton. Feng et al. (Feng et al., 2009b) reported seasonally driven changes in
296 sediment populations in the ChangJiang estuary and the coastal area of the East China Sea,
297 related to the hydrological regime. Boer et al. (Böer et al., 2009) identified time as the most
298 important factor affecting bacterial diversity in coastal sands. We hypothesize that the
299 seasonal variability observed in lagoon community composition is driven by seasonal changes
300 in environmental and trophic conditions. The heatmap plot of lagoon bacterioplankton
301 revealed a separation among the four seasons. This indicated that different environmental
302 variables could significantly explain the variance in community composition across the
303 different seasons. Proteobacteria was the dominant group, while Alphaproteobacteria was
304 generally abundant in marine waters (Kirchman, Dittel & Cottrell, 2005). It was also observed

305 that as the seasons changed, the abundance of Alphaproteobacteria had a relatively large
306 difference. Among them, the SAR11 population was characterized as oligotrophic. The
307 significant negative correlation between SAR11 and NH_4^+ , NO_2^- is shown in Figure 6.

308 Species and environment correlation analyses are increasingly used to explore large data sets
309 generated by DNA HTS technologies, in order to elucidate potential interactions between
310 microbial taxa (co-occurrence patterns), or between taxa and environmental variables across
311 spatial or temporal scales. The analysis of the network topological parameters indicated that
312 salinity showed positive and negative correlations with many bacterioplankton genera. In the
313 lagoon, negative correlations were found with HgcI. The HgcI clade is common and abundant
314 in a wide range of freshwater habitats (Warnecke, Amann & Pernthaler, 2004), and the
315 salinity of the water was reduced because of rainfall. Therefore, the abundance of the HgcI
316 clade in the summer lagoon was high. In lakes, members of the HgcI clade are often dominant
317 components of the bacterioplankton, where they have a competitive advantage in waters with
318 low DOC concentrations at low temperature (Glaser & Coker et al., 2000). This clade shows both
319 heterotrophic and autotrophic lifestyles, and a recent single cell genomic study showed that it
320 had a strong genetic ability to consume carbohydrate and N-rich organic compounds. In
321 addition, it also had the potential to utilize sunlight via actinorhodopsin which might promote
322 anoxygenic carbon fixation (Ghylin et al., 2014). However, bacteria in the HgcI clade remain
323 poorly characterized and their functional traits in marine/brackish environments are unknown
324 (Lindh et al., 2015). For *Synechococcus*, the influence of salinity was dominant. Research has
325 shown that for stable waters, a decrease in salinity and an increase in rainfall are favorable for
326 a high abundance of *Synechococcus* (R & Mitbavkar, 2013). As such, the high abundance of
327 *Synechococcus* appeared in July and October. Temperature interactions with a variety of taxa
328 were observed, including genera that are dominant in the lagoon (such as *Synechococcus*) and
329 also several minor members, such as CL500-29 and Comamonadaceae.

330 Compared with the enclosed lagoon, the dominant species of planktonic bacteria in the
331 offshore waters showed obvious differences (Figure 4). It is worth noting that
332 Comamonadaceae, affiliated with Comamonadaceae of Betaproteobacteria, and

333 *Pseudomonas*, affiliated with Pseudomonadaceae of Gammaproteobacteria were the two
334 dominant groups having the highest abundance in July in the offshore sea. Studies on the
335 removal of phosphorus from waste water indicated that Comamonadaceae was present when
336 the Bio-P activity was evident (Ge, Batstone & Keller, 2015a). Likewise, FISH analysis
337 combined with DAPI staining showed that bacterial cells of Comamonadaceae were arranged
338 in tetrads contained polyphosphate. These studies identified these species as the key
339 polyphosphate accumulating organisms (Ge, Batstone & Keller, 2015b). Microbial
340 community analysis indicated that organisms classified in the Comamonadaceae were
341 effective denitrifiers widely used in sewage treatment (Long et al., 2017). *Pseudomonas*
342 *aeruginosa* is a strain of non-fermentative DNPAOs (Denitrifying phosphate-accumulating
343 organisms) with strong nitrogen and phosphorus removal abilities. Studies on the metabolic
344 mechanisms suggested that intracellular PHB of *P.aeruginosa* plays dual roles, supplies
345 energy for phosphorus accumulation, and serves as a major carbon source for nitrification(Liu
346 et al., 2016).Simultaneous removal of nutrients (ammonium and phosphate) and COD was
347 investigated by the co-culture consortium of microalga *Chlorella vulgaris* and bacterium
348 *Pseudomonas putida*. The co-culture system showed higher removal of both nutrients and
349 COD than the each axenic culture, indicating that the nutrient uptake capability of *C. vulgaris*
350 was enhanced in the presence of *P. putida* (Mujtaba, Rizwan & Lee, 2017).Some studies have
351 shown that Gammaproteobacteria has the ability to degrade certain amounts of organic matter
352 in the ocean (Marques et al., 2014b; Brettar, Christen & Höfle, 2006). Similarly, microbial
353 high-throughput sequencing analysis showed that Gammaproteobacteria contain the main
354 PAH degradation genes used in the bioremediation of immobilized bacteria. As such, these
355 organisms could be used to remove complex and structurally related organic compounds in
356 the environment (Tian et al., 2016). *Thiorhodospiral* of Gammaproteobacteria also showed a
357 positive correlation with COD. Compared with the closed lagoon water, the accumulation of
358 dominant bacteria in the offshore waters may be due to the impact of higher concentrations of
359 Hangzhou Bay sewage discharge, nutrients, and COD.

360 **Conclusions**

361 The results of this research showed significant differences in the bacterioplankton community
362 and diversity between the artificial lagoon and the adjacent open sea. This study highlighted
363 the importance of seasonality in modulating planktonic assemblages in coastal lagoons and
364 the adjacent sea. The dominant bacteria in the lagoon were *Synechococcus* and *Cyanobacteria*.
365 The abundance of bacteria varied greatly with the seasons, primarily due to dissolved oxygen,
366 temperature, and salinity. The adjacent sea dominant bacteria such as bacteria in the family
367 Comamamonadaceae and *Pseudomonas* were affected by COD, PO₄, and NO₂. These results
368 indicated a negative impact of different human activities (seawater farming, land-based
369 pollution emissions) on coastal ecosystems. It was also found a dominant microbiota, which
370 can effectively remove nutrients such as nitrogen and phosphorus. These findings provided
371 new methods to improve breeding water quality. In addition, these findings may help to
372 restore the ecosystem in the future using large seaweed beds and local beneficial
373 microorganisms.

374 **Acknowledgments**

375 This study was sponsored by National Science and Technology Support Program
376 (2012BAC07B03) and State Oceanic Administration public welfare industry research project
377 (201105008).

378 We thank International Science Editing (<http://www.internationalscienceediting.com>) for
379 editing this manuscript.

380 **REFERENCCEES**

381 Béjaoui B, et al. 2017. 3D modeling of phytoplankton seasonal variation and nutrient budget
382 in a southern Mediterranean Lagoon. *Marine Pollution Bulletin* 114(2): 962-976.

383 <https://doi.org/10.1016/j.marpolbul.2016.11.001>.

384

385 Böer SI, et al. 2009. Time-and sediment depth-related variations in bacterial diversity and
386 community structure in subtidal sands. *The ISME Journal*, 3, 780–791.

387 <https://doi.org/10.1038/ismej.2009.29>.

388

389 Brettar I, Christen R, Höfle MG. 2006. *Rheinheimera perlucida* sp. nov., a marine bacterium
390 of the Gammaproteobacteria isolated from surface water of the central Baltic Sea.

391 *International Journal of Systematic & Evolutionary Microbiology* 56(9): 2177-2183.

392 <https://doi.org/10.1099/ijs.0.64172-0>.

393

394 Cao B, Yang H, Qiang-Hua XU, Liu RL. 2011. A study of the microorganism using
395 bio-molecular technology from the Yangtze Estuary based on the technology of 16S rRNA.

396 *Journal of Shanghai Ocean University* 20(2): 191-197.

397

398 Chao A, Bunge J.2002. Estimating the Number of Species in a Stochastic Abundance Model.

399 *Biometrics* 58(3): 531. <https://doi.org/10.1111/j.0006-341X.2002.00531.x>.

400

401 Danovaro R, Pusceddu A. 2007. Biodiversity and ecosystem functioning in coastal lagoons:
402 Does microbial diversity play any role? *Estuarine Coastal & Shelf Science* 75(1–2): 4-12.

403 <https://doi.org/10.1016/j.ecss.2007.02.030>.

404

405 Feng BW, et al. 2009. Bacterial diversity of water and sediment in the Changjiang estuary and
406 coastal area of the East China Sea. *Fems Microbiology Ecology* 70(2): 80-92.

407 <https://doi.org/10.1111/j.1574-6941.2009.00772.x>.

408

409 Ferrarin C, et al. 2015. Toward homogenization of Mediterranean lagoons and their loss of
410 hydrodiversity. *Geophysical Research Letters* 41(16): 5935-5941.

411 <https://doi.org/10.1002/2014GL060843>.

412

413 Fouilland E, et al. 2017. Significant Change in Marine Plankton Structure and Carbon

414 Production After the Addition of River Water in a Mesocosm Experiment. *Microbial Ecology*:

415 1-13. <https://doi.org/10.1007/s00248-017-0962-6>.

416

417 Fontes MLS, Abreu PC.2010. Spatiotemporal variation of bacterial assemblages in a shallow
418 subtropical coastal lagoon in Southern Brazil. *Microbial Ecology* 58(1): 140-152.

419 <https://doi.org/10.1007/s00248-009-9530-z>.

420

421 Fuhrman JA, Cram JA, Needham DM. 2015. Marine microbial community dynamics and
422 their ecological interpretation. *Nature Reviews Microbiology* 13(3): 133-146.

423 <https://doi.org/10.1038/nrmicro3417>.

424
425 Ge H, Batstone DJ, Keller J. 2015. Biological phosphorus removal from abattoir wastewater
426 at very short sludge ages mediated by novel PAO clade Comamonadaceae. *Water Research* 69:
427 173-182. <https://doi.org/10.1016/j.watres.2014.11.026>.
428
429 Ghai R, et al. 2012. Metagenomes of Mediterranean coastal lagoons. *Scientific Reports* 2(7):
430 490. <https://doi.org/10.1038/srep00490>.
431
432 Ghylin TW, et al. 2014. Comparative single-cell genomics reveals potential ecological niches
433 for the freshwater actI Actinobacteria lineage. *ISME Journal* 8(12): 2503.
434 <https://doi.org/10.1038/ismej.2014.135>.
435
436 Glaser CKner FO, et al. 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals
437 globally distributed phylogenetic clusters including an abundant group of actinobacteria.
438 *Applied & Environmental Microbiology* 66(11): 5053-5065.
439
440 Good IJ, 1953. THE POPULATION FREQUENCIES OF SPECIES AND THE
441 ESTIMATION OF POPULATION PARAMETERS. *Biometrika*.
442 <https://doi.org/10.2307/2333344>.
443
444 Kirchman DL, Dittel AL, Cottrell MT. 2005. Biogeography of Major Bacterial Groups in the
445 Delaware Estuary. *Limnology & Oceanography* 50(5): 1697-1706.
446 <https://doi.org/10.1111/j.1462-2920.2004.00561.x>
447
448 Lindh MV, et al. 2015. Consequences of increased terrestrial dissolved organic matter and
449 temperature on bacterioplankton community composition during a Baltic Sea mesocosm
450 experiment. *Ambio* 44(3): 402-412. <https://doi.org/10.1007/s13280-015-0659-3>.
451
452 Liu H, et al. 2016. Isolation of a non-fermentative bacterium, *Pseudomonas aeruginosa*, using
453 intracellular carbon for denitrification and phosphorus-accumulation and relevant metabolic
454 mechanisms. *Bioresource Technology* 211: 6. <https://doi.org/10.1016/j.biortech.2016.03.051>.
455
456 Liu ZL, et al. 2001. Bacterioplankton production in dilution zone of the Changjiang (Yangtze)
457 Estuary. *Acta Oceanologica Sinica* 20(1): 131-139.
458
459 Londong J, Wachtl P. 1996. Six years of practical experience with the operation of on-line
460 analysers. *Water Science & Technology* 33(1): 159-164.
461 [https://doi.org/10.1016/0273-1223\(96\)00168-0](https://doi.org/10.1016/0273-1223(96)00168-0).
462
463 Long M, Zhou C, Xia S, Guadisa A. 2017. Concomitant Cr(VI) reduction and Cr(III)
464 precipitation with nitrate in a methane/oxygen-based membrane biofilm reactor. *Chemical*
465 *Engineering Journal* 315: 58-66. <https://doi.org/10.1016/j.cej.2017.01.018>.
466
467 Magurran AE. 1988. *Ecological Diversity and Its Measurement*, Princeton University Pre.

468
469 Manini E, et al. 2003. Benthic microbial loop functioning in coastal lagoons: a comparative
470 approach. *Oceanologica Acta* 26(1): 27-38. [https://doi.org/10.1016/S0399-1784\(02\)01227-6](https://doi.org/10.1016/S0399-1784(02)01227-6).
471
472 Marques M, et al. 2014. Do lagoon area sediments act as traps for polycyclic aromatic
473 hydrocarbons? *Chemosphere* 111(4): 80. <https://doi.org/10.1016/j.chemosphere.2014.03.037>.
474
475 Mujtaba G, Rizwan M, Lee K. 2017. Removal of nutrients and COD from wastewater using
476 symbiotic co-culture of bacterium *Pseudomonas putida* and immobilized microalga *Chlorella*
477 *vulgaris*. *Journal of Industrial & Engineering Chemistry* 49: 145-151.
478 <https://doi.org/10.1016/j.jiec.2017.01.021>.
479
480 Newton A, et al. 2014. An overview of ecological status, vulnerability and future perspectives
481 of European large shallow, semi-enclosed coastal systems, lagoons and transitional waters.
482 *Estuarine Coastal & Shelf Science* 140(3): 95-122. <https://doi.org/10.1016/j.ecss.2013.05.023>.
483
484 Pinhassi J, Berman T. 2003. Differential Growth Response of Colony-Forming α - and
485 γ -Proteobacteria in Dilution Culture and Nutrient Addition Experiments from Lake Kinneret
486 (Israel), the Eastern Mediterranean Sea, and the Gulf of Eilat. *Appl Environ Microbiol* 69(1):
487 199-211. <https://doi.org/10.1128/AEM.69.1.199-211.2003>.
488
489 Pan LA, Zhang J, Zhang LH. 2007. Picophytoplankton, nanophytoplankton, heterotrophic
490 bacteria and viruses in the Changjiang Estuary and adjacent coastal waters. *Chinese*
491 *Biological Abstracts* 29(7): 187-197. <https://doi.org/10.1093/plankt/fbm006>.
492
493 Quero GM, et al. 2017. Seasonal rather than spatial variability drives planktonic and benthic
494 bacterial diversity in a microtidal lagoon and the adjacent open sea. *Molecular Ecology*.
495 <https://doi.org/10.1111/mec.14363>.
496
497 R KM, Mitbavkar S. 2013. Factors controlling the temporal and spatial variations in
498 *Synechococcus* abundance in a monsoonal estuary. *Marine Environmental Research* 92(12):
499 133-143. <https://doi.org/10.1016/j.marenvres.2013.09.010>.
500
501 Schallenberg M, Larned ST, Hayward S, Arbuckle C. 2010. Contrasting effects of managed
502 opening regimes on water quality in two intermittently closed and open coastal lakes.
503 *Estuarine Coastal & Shelf Science* 86(4): 587-597. <https://doi.org/10.1016/j.ecss.2009.11.001>.
504
505 Thompson JR, et al. 2009. Hydrological characteristics of three North African coastal lagoons:
506 insights from the MELMARINA project. *Hydrobiologia* 622(1): 45-84.
507 <https://doi.org/10.1007/s10750-008-9680-x>.
508
509 Tian LC, Wu SM. 1992. Determination of chemical oxygen demand in aqueous
510 environmental samples by segmented flow-injection analysis. *Analytica Chimica Acta* 261(1):
511 301-305. [https://doi.org/10.1016/0003-2670\(92\)80206-M](https://doi.org/10.1016/0003-2670(92)80206-M).
512

513 Tian WJ, et al. 2016. Application of cinder gel-beads/reeds combination strategy for
514 bioremediation of pyrene- and indeno(1,2,3-cd)pyrene-contaminated estuarine wetlands.
515 Environmental Science & Pollution Research 23(11): 10895-10902.
516 <https://doi.org/10.1007/s11356-016-6298-9>.
517

518 Wang JX, et al. 2012. A PRELIMINARY STUDY OF MICROBIAL DIVERSITY OF THE
519 SURFACE LAYER SEDIMENTS FROM THE EAST CHINA SEA. Oceanologia Et
520 Limnologia Sinica.
521

522 Warnecke F, Amann R, Pernthaler J. 2004. Actinobacterial 16S rRNA genes from freshwater
523 habitats cluster in four distinct lineages. Environmental Microbiology 6(3): 242-253.
524 <https://doi.org/10.1111/j.1462-2920.2004.00561.x>.
525

526 Xiong J, et al. 2012. Geographic distance and pH drive bacterial distribution in alkaline lake
527 sediments across Tibetan Plateau. Environmental Microbiology 14(9): 2457–2466.
528 <https://doi.org/10.1111/j.1462-2920.2012.02799.x>.
529

530 Yan Z, et al. 2016. Temporal and spatial changes of microbial community in an industrial
531 effluent receiving area in Hangzhou Bay. Journal of Environmental Sciences 44(6): 57.
532 <https://doi.org/10.1016/j.jes.2015.11.023>.