Marine Pollution Bulletin xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Spatial and seasonal distributions of bacterioplankton in the Pearl River Estuary: The combined effects of riverine inputs, temperature, and phytoplankton

Jiajun Li[a,b], Xin Jiang[b], Zhiyou Jing[c], Gang Li[b], Zuozhi Chen[a], Linbin Zhou[b], Chunyu Zhao[b,d], Jiaxing Liu[b], Yehui Tan[b,d,*]

[a] Key Laboratory of South China Sea Fishery Resources Exploitation & Utilization, Ministry of Agriculture, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, China
[b] Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
[c] State Key Laboratory of Tropical Oceanography, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
[d] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Keywords:
Bacterioplankton
Pearl River Estuary
16S rRNA sequencing
Monsoon wind
Plume

ABSTRACT

In this study, we used flow cytometry and 16S rRNA gene pyrosequencing to investigate bacterioplankton (heterotrophic bacteria and picocyanobacteria) abundance and community structure in surface waters along the Pearl River Estuary. The results showed significant differences in bacterioplankton dynamics between fresh- and saltwater sites and between wet and dry season. *Synechococcus* constituted the majority of picocyanobacteria in both seasons. During the wet season, *Synechococcus* reached extremely high abundance at the mouth of the estuary, and heterotrophic bacteria were highly abundant ($> 10^6$ cells ml^{-1}) throughout the studied region. At the same time, bacterioplankton decreased dramatically during the dry season. Pyrosequencing data indicated that salinity was a key parameter in shaping microbial community structure during both seasons. Phytoplankton was also an important factor; the proportion of *Synechococcus* and *Rhodobacteriales* was elevated at the frontal zone with higher chlorophyll *a* during the wet season, whereas *Synechococcus* were markedly reduced during the dry season.

1. Introduction

Estuaries are subject to complex and dynamic influences of riverine inputs, which are always characterized by high nutrients, abundant suspended particles, and low salinity. The complex environmental variations along estuaries and between seasons make them ideal natural laboratories for studying the biological and biogeochemical effects associated with river inflows. A series of studies have evaluated the potential impacts of large rivers such as the Mississippi River, Yangtze River, and Pearl River (Amon and Benner, 1998; Cai et al., 2004; Harrison et al., 2008; Su et al., 2017; Wang et al., 2017).

Bacterioplankton (heterotrophic bacteria and picocyanobacteria) are key components of the microbial ecosystem, facilitating carbon delivery, organic matter decomposition, and contaminant removal. Additionally, different microbial groups have unique functions in the regulation of biogeochemical processes. Surveys of various estuaries have revealed that freshwater from rivers is the major determinant in structuring microbial communities (Crump et al., 1999; Selje and Simon, 2003; Crump et al., 2004; Bernhard et al., 2005; Liu et al., 2015; Xia et al., 2017a). *Actinobacteria* and *Betaproteobacteria* are the major freshwater taxa in river estuaries, whereas *Alphaproteobacteria* and *Gammaproteobacteria* have been found to increase as a function of salinity (Gilbert et al., 2009; Campbell and Kirchman, 2013; Liu et al., 2014; Staley et al., 2015; Angly et al., 2016). Moreover, the abundance of microbes is often much higher in freshwater than in salty waters. In addition to environmental factors, interactions between biological variables display also an essential role in shaping microbial distribution patterns. For example, organic matter derived from bloomed phytoplankton can provide habitats and resources for abundant numbers of bacteria and consequently affect community structure in aquatic ecosystems (Sarmento and Gasol, 2012; Taylor et al., 2014; Galand et al., 2015; Jiang et al., 2017; Li et al., 2017).

The Pearl River has the second largest discharge volume among rivers in China and receives large amounts of waste water from

* Corresponding author at: Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China.
E-mail address: tanyh@scsio.ac.cn (Y. Tan).

http://dx.doi.org/10.1016/j.marpolbul.2017.08.026
Received 22 December 2016; Received in revised form 8 August 2017; Accepted 13 August 2017

intensifying anthropogenic activities. The average annual discharge of the Pearl River is about 10,000 $m^3 s^{-1}$; and most of the discharge occurs disproportionately during the wet season (about 80% of it between April and September) (Dong et al., 2004; Ou and Zhang, 2009; Ye et al., 2017). During the warm and wet season, horizontal extension of the plume is advected eastward with high river discharge, southwesterly winds (wind speeds of approximately 6 m s^{-1}, Fig. S1) and coastal currents. In contrast, during the cold and dry season, the plume is advected westward and waters in the whole estuary are mixed well by strong northeasterly winds (wind speeds of 7–10 m s^{-1}, Fig. S1) and tide (Gan et al., 2009). Previous reports have suggested that the spatial movement of the coastal plume affected biological distribution patterns in the Pearl River Estuary (PRE) and adjacent coastal waters (Yin et al., 2004). Phytoplankton and productivity are more abundant in wet compared to dry seasons and algal blooms occur always in the vicinity of the estuary during the wet season. Some studies have also examined the factors that influence the distribution patterns of Pearl River microbial communities, primarily during the wet season (Zhang et al., 2009; Zhou et al., 2011; Liu et al., 2015). However, few studies of bacterioplankton communities have addressed the variability on both spatial and seasonal scale.

In the present study, we used flow cytometry and high-throughput sequencing to evaluate the abundance and community structure of bacterioplankton. Our goals were to determine the seasonal and spatial variations of bacterioplankton so as to gain insight into the environmental factors and physical processes that regulated microbial dynamics, and to better understand the potential interaction between phytoplankton and heterotrophic bacteria (HBA).

2. Materials and methods

2.1. Study site and sampling

Water samples were collected along the PRE from 16 June to 27 June 2015 and from 5 December to 15 December 2015 using 5-l Niskin bottles. Samples were collected from a total of 42 and 33 stations, respectively, during the wet and dry season (Figs. 1 and 2). Environmental variables such as salinity, temperature, pH, turbidity, and dissolved oxygen (DO), were monitored in situ using a YSI 6600 multiprobe sensor (Yellow Springs, OH, USA). Immediately upon retrieval of the Niskin bottles, DO samples were also analyzed by the Winkler spectrophotometric method. Subsamples for the determination of size-fractioned chlorophyll *a* (Chl *a*), picoplankton, total suspended matter (TSM), and dissolved nutrients were also taken at each station. Triplicate samples were collected for picoplankton, and the samples were fixed with formaldehyde at a final concentration of 2%, kept at room temperature for 10–15 min, and frozen in liquid nitrogen (Jiang et al., 2017). One-liter samples of surface (1-m depth) water from six sites along the salinity gradient (C1, C3, C7, C9, A3 and F4 for the wet season; C1, C3, C7, C9, A3 and E3 for the dry season, Fig. 1) were pre-filtered through 3-μm-pore size filters to remove large organisms and particles, and free-living bacterial cells were collected through 0.22-μm polycarbonate membranes (Millipore Corporation, Billerica, MA, USA). The filters were then flash-frozen in liquid nitrogen and stored at − 80 °C until DNA extraction.

2.2. Determination of dissolved nutrients, TSM, and Chl a

After returning to the laboratory, nutrients were analyzed using a QuikChem® 8500 flow injection analyzer (Lachat Inc., Loveland, CO, USA). TSM content was calculated using variations in pre-weighed Whatman GF/F filters. Micro- (> 20 μm), nano- (3–20 μm), and pico-Chl *a* (0.7–3 μm) were determined fluorometrically after grinding in 90% acetone, and total Chl *a* was calculated as the sum of the three size fractions (Parsons, 1984; Zhou et al., 2015).

2.3. Identification and enumeration of picoplankton

Picoplankton was analyzed within four weeks with a Becton-Dickinson FACSCalibur flow cytometer (Franklin Lakes, NJ, USA). Different populations, including *Prochlorococcus*, *Synechococcus,* and picoeukaryotes, were distinguished based on SSC (side light scatter), FL2 (orange fluorescence), and FL3 (red fluorescence) parameters (Olson et al., 1990). For HBA counts, samples were stained with SYBR®-Green I nucleic acid stain (1:10,000 final dilution; Molecular Probes, Eugene, OR, USA) and were discriminated according to their FL1 (green fluorescence) and side scatter properties (Marie et al., 1997).

2.4. DNA extraction, PCR amplification, and sequencing analysis of 16S rRNA gene amplicons

Total genomic DNA was extracted directly from 12 samples during two seasons using the E.Z.N.A.® Bacterial DNA Kit (Omega bio-tek, Norcross, GA, USA) following the manufacturer's protocol. DNA was stored at − 80 °C for subsequent analyses. The V3–V4 region of the 16S rRNA gene, which can yield accurate taxonomic information and shows little bias for various bacterial taxa, was amplified with the universal bacterial primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-

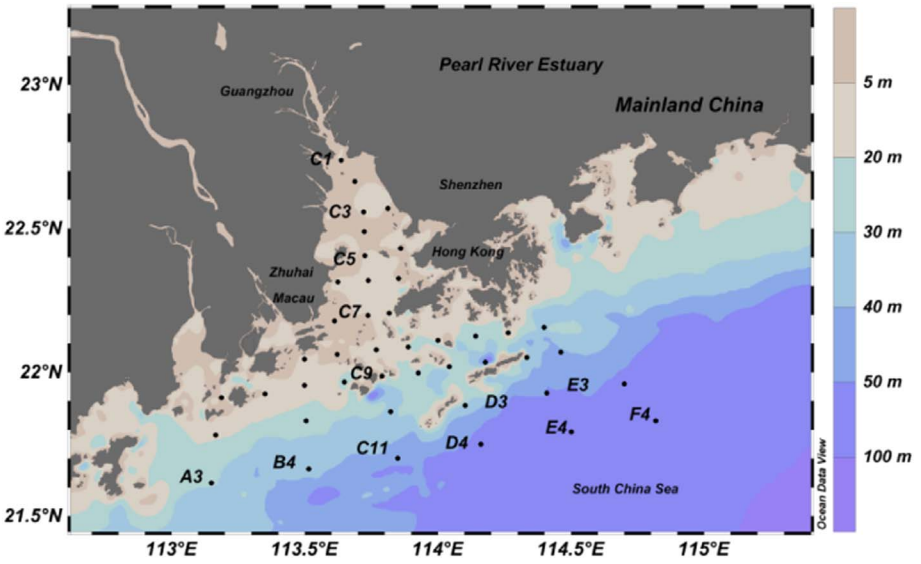

Fig. 1. Location and sampling sites in the Pearl River Estuary, South China Sea. The wet season cruise included 42 stations and the dry season cruise included 33 stations, as shown in Fig. 2.