



# Structural and functional shifts of bacterioplanktonic communities associated with spatiotemporal gradients in river outlets of the subtropical Pearl River Estuary, South China



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## ABSTRACT

In this study, we used high-throughput sequencing of 16S rRNA gene amplicons, to investigate the spatio-temporal variation in bacterial communities in surface-waters collected from eight major outlets of the Pearl River Estuary, South China. *Betaproteobacteria* were the most abundant class among the communities, followed by *Gammaproteobacteria*, *Alphaproteobacteria*, *Actinobacteria*, and *Acidimicrobia*. Generally, alpha-diversity increased in winter communities and the taxonomic diversity of bacterial communities differed with seasonal and spatial differences. Temperature, conductivity, salinity, pH and nutrients were the crucial environmental factors associated with shifts in the bacterial community composition. Furthermore, inferred community functions that were associated with amino acid, carbohydrate and energy metabolisms were lower in winter, whereas the relative abundance of inferred functions associated with membrane transport, bacterial motility proteins, and xenobiotics biodegradation and metabolism, were enriched in winter. These results provide new insights into the dynamics of bacterial communities within estuarine ecosystems.

## 1. Introduction

Bacterial communities are integral in nutrient recycling and the degradation of organic contaminants in aquatic ecosystems (Downing et al., 2014; Wang et al., 2014). Estuarine surface waters are diverse habitats that are subject to dynamic environmental factors associated with water flow and are important ecosystems for understanding bacterial ecology. The diversity and distribution of bacterial communities within estuarine ecosystems dictate community and ecosystem functionalities, and structural variation of bacterial communities can result in functional shifts. Thus, understanding the spatio-temporal shifts in bacterial community structure can better inform the relationship between bacterial community characteristics and estuarine ecosystem functioning. Numerous investigations of bacterial communities in estuarine surface water have been described recently, and they generally indicate that bacterial community composition changes with environmental variation in estuaries (Lallias et al., 2015; Sakami et al., 2016; T. Zhang et al., 2016). Several environmental factors have been shown to be potential determinants that shape bacterial community composition, including salinity (Fortunato et al., 2012) in addition to concentrations of nitrogen (Jeffries et al., 2015), phosphorous (Jin et al., 2014), heavy metals (Besaury et al., 2014), and organic contaminants (Ling et al.,

2015). The above studies, among others, have investigated the spatial heterogeneity of bacterial diversity within estuarine ecosystems (Lallias et al., 2015; Sakami et al., 2016; T. Zhang et al., 2016), yet the relationship between seasonal changes in estuary environments and bacterial community diversity remains unclear. Seasonal homogeneity was observed for bacterioplankton diversity in Chesapeake Bay, USA (Kan et al., 2007). In contrast, bacterial community composition varied with sampling seasons in estuaries within the Zhejiang Province of China (Lu et al., 2016). Other than these few spatial and seasonal patterns, investigations of the longitudinal distribution of bacterial communities throughout entire outlets of estuaries remains under-explored. Moreover, the relationship between bacterial communities in estuarine surface waters and their ecological functions also remains uncertain.

The Pearl River is the second largest (about  $3.30 \times 10^{11} \text{ m}^3 \cdot \text{yr}^{-1}$  of water discharge) and third longest ( $2.32 \times 10^3 \text{ km}$ ) river in China. The river system consists of three major tributaries, namely Xi (“West”), Bei (“North”), and Dong (“East”) rivers, that form a complex river network system that discharges into the Pearl River Estuary from eight major outlets: Yamen (YAM), Hutiaomen (HTM), Jitimen (JTM), Modaomen (MDM), Hengmen (HEM), Hongqilimen (HLM), Jiaomen (JOM) and Humen (HUM) (X. Chen et al., 2013). The Pearl River Estuary is located

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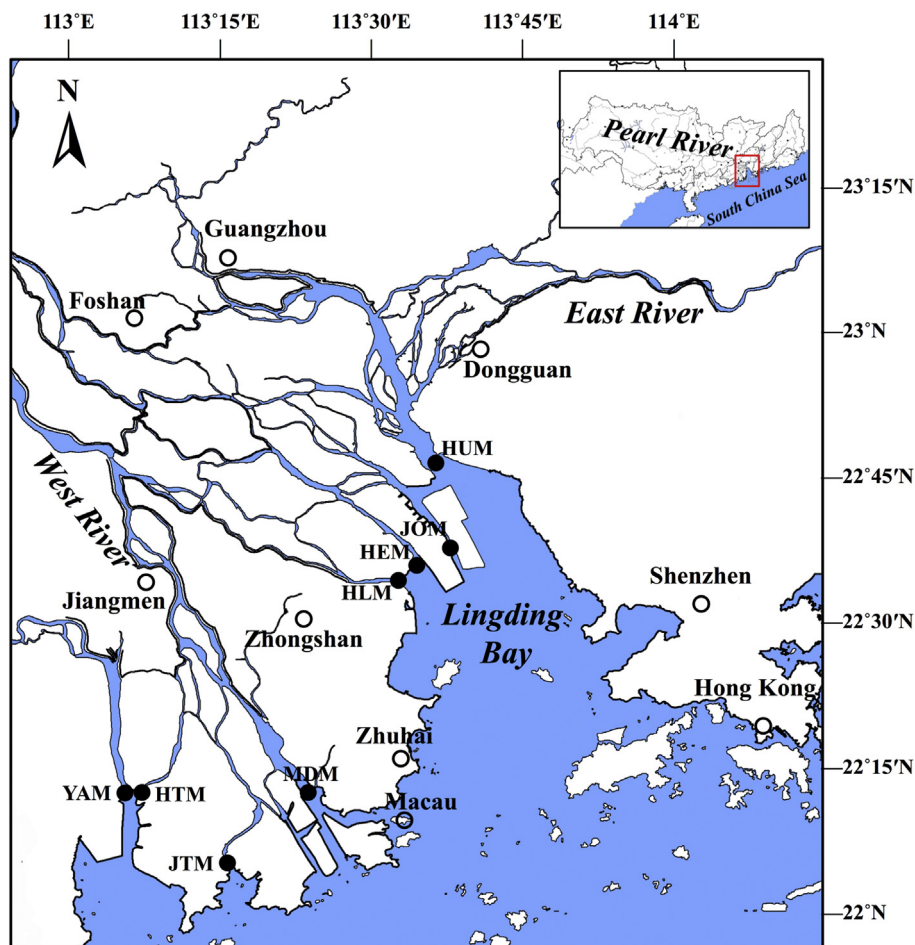


Fig. 1. Map of the Pearl River Estuary. Solid dots denote approximate location of sampling sites. Specific latitude and longitude information about each sampling site are shown as follows: YAM (22.20° N, 113.09° E), HTM (22.20° N, 113.10° E), JTM (22.05° N, 113.28° E), MDM (22.19° N, 113.41° E), HEM (22.58° N, 113.59° E), HLM (22.60° N, 113.60° E), JOM (22.64° N, 113.65° E) and HUM (22.79° N, 113.61° E).

in the Guangdong–Hong Kong–Macau Greater Bay Area, which is one of the most economically developed and densely populated regions in China. Consequently, the estuary suffers from severe heavy metal pollution (Ye et al., 2012; Zhang et al., 2015) in addition to pollution from organics (Li et al., 2014) that derive from riverine sewage and waste disposal. Most of the contaminants in the Pearl River Estuary include polycyclic aromatic hydrocarbons (PAHs) (Zhang et al., 2012) and petroleum hydrocarbons (Zhang et al., 2012) that enter the Pearl River Estuary via river outlets by upstream riverine runoff. These pollutants have been shown to considerably shift bacterial community compositional and functional diversity in some systems (Wu et al., 2014). Moreover, the overuse of chemical fertilizers and farming has led to the discharge of a large amount of nitrogen and phosphorus into the Pearl River Estuary over the previous several decades (R. Li et al., 2017; Shi et al., 2017). This is also particularly true as recent studies have shown high levels of antibiotic resistance genes (ARGs) in the waters and sediments of both the west and east portions of the Pearl River Estuary (B. Chen et al., 2013), indicating that severe antibiotic contamination may threaten the bacterial community diversity in this area as well as the overall aquatic ecosystem functioning. Thus, it is urgent to comprehensively investigate the bacterial community dynamics of all eight major outlets of the Pearl River Estuary, as these dynamics are likely to underpin the broad functionality of these systems.

Previous studies of Pearl River estuary ecology have focused on community dynamics of phytoplankton (Wang et al., 2010), zooplankton (Gao et al., 2008), macrozoobenthos (Peng et al., 2010) and larval fish (Shuai et al., 2016) in response to impact factors. In addition,

several recent studies have begun to investigate the bacterial community structure of sediments and waters from the Pearl River Estuary. Temporal succession patterns were observed between surface sediments and bottom water bacterial communities in the estuary (W. Zhang et al., 2014). The bacterial community compositions of surface and deep waters were also strongly correlated with environmental factors, but weakly correlated to geographic distance in the estuary (Y. Zhang et al., 2014). Further, salinity was identified as the primary factor that affects archaeal community structure and ecological function in the sediments of the Pearl River Estuary (Xie et al., 2014). River mouths can be significant sources or sinks of nitrogen (Larson et al., 2013). It has been documented that about  $1.73 \times 10^{10}$  metric tons of wastewater containing nutrients, antibiotics and other pollutants are discharged from the Pearl River Delta into the Pearl River Estuary and the South China Sea every year (Huang et al., 2003; Xu et al., 2014; Xu et al., 2013). This influx of pollutants has threatened the ecology and functioning of the Pearl River Estuary, and has also altered the diversity and distribution of bacterial communities in the estuarine ecosystem (Cao et al., 2012). However, pollution input effects estuarine environmental conditions at different river outlets differentially. For example, a large abundance of antibiotics is discharged into the Pearl River Delta via the Humen outlets and the main channel into the Pearl River Estuary, and ultimately, the coast. However, a relatively small ecological risk is posed by erythromycin, ciprofloxacin, and ofloxacin in other outlets of the Pearl River Estuary (Xu et al., 2013). Importantly, previous research on bacterial community composition in the Pearl River Estuary has only focused on the main channel from the Humen outlets to Lingding Bay,

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