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Spatial and temporal variations of picoplankton in three contrasting periods in the Pearl River Estuary, South China

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ABSTRACT

The distribution characteristics and biomass composition of three picophytoplankton (PP) groups (*Synechococcus*, picoeukaryotes and *Prochlorococcus*) were identified using flow cytometry in three contrasting periods (August 2010, January 2011 and August 2011) in the Pearl River Estuary (PRE), South China. To eliminate the overestimation of heterotrophic bacteria (HBA), HBA were identified by epifluorescence microscopy and flow cytometry. The average biomass in the three observations was as follows: 22.80, 21.04 and 18.72 μg C/L of HBA, and 12.92, 0.62 and 15.42 μg C/L of PP. The biomass ratio between the PP and HBA measurements increased along the estuarine axis, which suggested the dominance of PP in the outer estuary and HBA in the nearshore waters. The HBA biomass was not related to chlorophyll *a* or even exhibited a negative correlation with chlorophyll *a* in the two summer observations; this implied that dissolved organic carbon from other sources or some environmental factors, like suspended solids concentration (SSC), also affected bacterial growth and obscured the relationship between HBA and phytoplankton by shaping phytoplankton distribution. The HBA linked tightly with suspended particles and was mainly shaped by the SSC. We considered that most of the HBA were attached to riverine-originated particulates and consequently exhibited a decreasing trend from the upper estuary to the open shelf waters in the PRE. The low *Synechococcus* and picoeukaryotes biomass and the undetectability of *Prochlorococcus* in the winter were probably attributed to high turbidity and low water temperature. The sharp decrease in river flow in the summer of 2011 may have exerted less pressure on *Prochlorococcus* and resulted in biomass elevation and a further upward distribution scale. Furthermore, *Synechococcus* and *Prochlorococcus* exhibited similar distribution patterns and were relevant to the river input. Meanwhile, picoeukaryotes were the least abundant groups among the PP community in our investigations and showed a distinct distribution pattern from that of *Synechococcus* and *Prochlorococcus*.

1. Introduction

Picoplankton, generally defined as plankton in the size of 0.2 to 2 μm, consist mostly of heterotrophic bacteria and picophytoplankton [cyanobacteria (*Prochlorococcus* and *Synechococcus*) and picoeukaryotes]. Heterotrophic bacteria (HBA) play a critical role in the conversion of dissolved organic carbon into the particulate pool, therefore acting as both remineralizers of organic carbon and trophic intermediaries within aquatic ecosystems (Gasol et al., 1997; Lønborg and Søndergaard, 2009). Among picophytoplankton (PP), *Synechococcus* (Syn) is ubiquitous in both oligo- and mesotrophic oceanic and coastal areas (Partensky et al., 1996; Liu et al., 2004; Li and Li, 2012). *Prochlorococcus* (Pro) has been found to be more abundant in oligotrophic than in eutrophic waters (Partensky et al., 1999). Picoeukaryotes (Peuk) are generally less abundant, although they can be large contributors to biomass and production (Campbell et al., 1994; Li, 1994). It is generally accepted that phytoplankton, especially pico-fractioned phytoplankton, are the principal source of organic carbon for bacteria (Baines and Pace, 1991). Many investigators have observed significant correlations between bacteria and chlorophyll *a* (chl. *a*) in various ecosystems, including the Chesapeake Bay (Ducklow et al., 1999), the Mississippi River Plume (Liu et al., 2004), the Danshui Estuary (Tsai et al., 2011), the East China Sea (Pan et al., 2005) and the northern South China Sea (Yuan et al., 2011), indicating that primary production provides most of the carbon needed for HBA production in these settings. In many estuarine environments, however, the relationship between HBA and primary production is weak or non-existent (Shiah and Ducklo, 1994a; Kelley et al., 1998) because dissolved organic carbon is abundant or bacterial growth depends mostly on other non-phytoplanktonic sources of carbon (Fouilland and Mostajir 2010).

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Although a large set of data have been accumulated on the abundance of the different picoplankton populations throughout the world ocean, few studies address coastal ecosystems. Moreover, it is not yet clear what kind of environmental signals (e.g., light condition, water temperature, nutrient availability and grazing pressure) are responsible for picoplankton community structure and dynamics. Previous studies focused mainly on PP (Qiu et al., 2010; Lin et al., 2010) and HBA abundance or distribution (Yuan et al., 2011; Zhou et al., 2011) in the Pearl River Estuary (PRE), rather than simultaneously observing the four picoplankton groups. PRE is created by the flow of freshwater from the largest river system in southern China into the South China Sea. It has nutrient-replete and turbid waters in the inner estuary and P-depleted, clear open-shelf waters in the outer region (Yin et al., 2004a). It is thus an ideal field for ecological studies on temporal variations and spatial distribution of the picoplankton that contribute significantly to carbon biomass in these diverse hydrographical and physicochemical conditions.

We report here on the community structure and biomass composition of three PP groups and heterotrophic bacteria using a combination of flow cytometric protocols as well as microscopic analyses in three contrasting periods in the PRE. Moreover, the purpose of this paper is to develop a more comprehensive picture of spatial variability of PP and HBA in a complicated system along the salinity and nutrient gradient from the upper section to the outer estuary. The associated environmental variables shaping their distribution are analyzed, and the possible mechanisms controlling their abundances are discussed.

2. Materials and methods

2.1. Study area

The Pearl River Estuary, which lies in southern Guangdong Province, was created by inflows of the Pearl River to the South China Sea (SCS) through 8 entrances. Around 53% of the river runoff empties into the estuary through the four western outlets, namely Humen, Jiaomen, Hongqimen and Hengmen.

The Pearl River system is composed of the three main rivers, Xijiang (77.90% of the total basin area), Beijiang (10.30%) and Dongjiang (5.96%), as well as some small rivers (5.84%) draining the Pearl River Delta. The annual average freshwater discharge from the Pearl River into the PRE is 10,524 $m^3\ s^{-1}$ (Zhao, 1990). A large difference in river discharge between the dry and wet seasons has been observed, with roughly 80% of the annual discharge being delivered to the SCS during the wet season (April–September) and 20% entering during the dry season (October–next March) (Zhao, 1990). During the high-flow season, saline water is pulled into the estuary at the bottom with fresher outflow at the surface; hence, the PRE is highly stratified and the circulation has a two-layer structure in the entire estuary. However, the PRE was relatively homogeneous throughout the water column, as vertical mixing is quite good in low-flow winter seasons.

2.2. Station locations and sampling

Three surveys were conducted during August 2010 (the river's high-flow season, summer), January 2011 (the river's low-flow season, winter), and August 2011 (extreme drought events occurred) in the PRE (Fig. 1). The whole study area is under strong fluvial runoff impact and freshwater–marinewater interactions. Vertical profiles of temperature and salinity were recorded *in situ* using YSI6600 (Yellow Springs Instrument Co., USA). Water samples were collected 0.5 m below the surface and 0.5 m above the bottom at all stations, using 5 L Niskin bottles. All

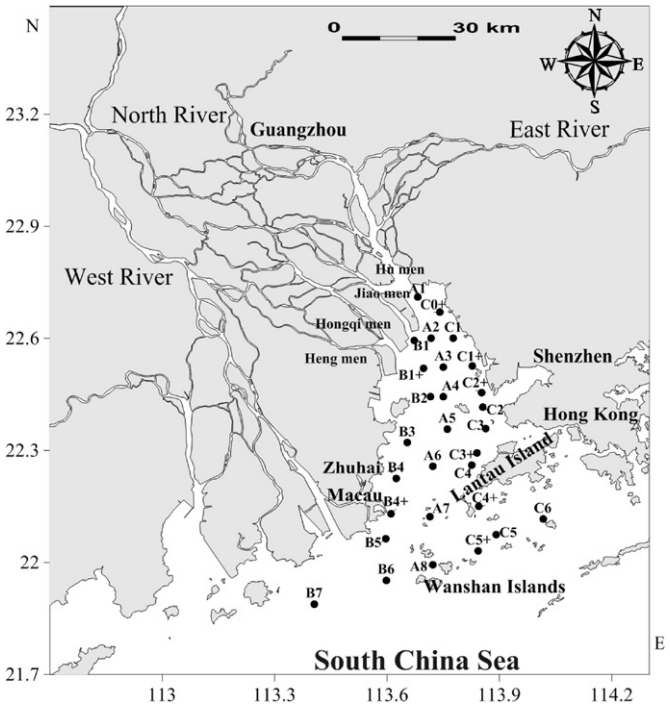

Fig. 1. The study area and sampling stations in the three seasons. A, B and C indicate stations along the three transects situated in the Middle Shoal, West Shoal and East Shoal, respectively. The summer 2010 cruise included 21 stations (A1–A8, B1–B7 and C1–C6), the winter 2011 cruise included 19 stations (A1–A8, B1–B6 and C1–C5) and the summer 2011 cruise included 20 stations (A1–A8, B1–B4, B1+, B4+, C1, C3 and C0+–C5+).

of parameters (except temperature and salinity) were the average value between the surface and bottom layers.

Total suspended solid (TSS) and chlorophyll *a* (chl. *a*) samples were acquired through pre-weighed Whatman GF/F fiber filters (0.7 μm, ∅25 mm). The filters for TSS were dried and weighed to determine the amount in mg/L of sample. Chl. *a* content was measured using a Turner Designs Model 10 Fluorometer. Armstrong et al.'s (1967) methods were used to measure phosphate concentrations. Dissolved inorganic nitrogen was the sum of the concentrations of nitrate, nitrite and ammonia. Silicate was only sampled on two cruises in 2011, also using the methods of Armstrong et al. (1967).

To measure dissolved organic carbon (DOC) and particulate organic carbon (POC), approximately 0.5–1.5 L of seawater was filtered through a pre-combusted 47 mm Whatman GF/F membrane and stored frozen (−20 °C). POC concentrations were quantified with an Elemental Analyzer (Elementar, Vario EL-III, Germany). The DOC filtrates were acidified with 50 μL of 50% H_3PO_4 to pH 2 to drive off the inorganic carbon. The acidified samples were purged with ultra-high-purity nitrogen immediately prior to analysis for approximately 10 min to remove the inorganic carbon. Milli-Q water was used as the carbon-free distilled water blank. The DOC concentrations were measured with a liquiTOC II analyzer.

2.3. Analysis of HBA and PP

The HBA were counted with an epifluorescence microscope (OLYMBUS BX51, Japan) with a 100 W high-pressure mercury burner for epifluorescence illumination after staining with DAPI (4, 6-diamidino-2-phenylindole dihydrochloride, Sigma) for at least 5 min in the dark (Porter and Feig, 1980). Stained bacterial cells were counted at 1000 × magnification under UV excitation, and at least 10–20 random fields (minimum of 400 cells) were