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Variations of picoplankton abundances during blooms in the East China Sea

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ABSTRACT

The picoplankton distribution in the East China Sea was investigated during bloom events occurred in spring (June) and summer (August) 2011. In spring, there was no significant difference in picoplankton abundances between areas where bloom conditions were or were not established. In the bloom area, *Synechococcus*, picoeukaryotes and heterotrophic prokaryotes exhibited at only some stations that abundances were higher than those within the non-bloom area. In summer, the abundances of *Synechococcus* and picoeukaryotes were significantly higher inside the bloom area than outside. Among the picoplankton components, heterotrophic prokaryotes represented the highest carbon biomass.

Factors that most influenced picoplankton distribution under bloom conditions in the East China Sea varied with season. In spring, ciliates and salinity tended to be the main factors, whereas in summer, this role was played by temperature and chlorophyll *a* concentration.

1. Introduction

Marine picoplankton refers to a functionally diverse group of organisms which are microscopic in size ($\leq$ 2 μm in diameter). Picoplankton includes cyanobacteria of the genera *Synechococcus* (Johnson and Sieburth, 1979; Waterbury et al., 1979) and *Prochlorococcus* (Chisholm et al., 1988), a great diverse assemblage of picoeukaryotes and heterotrophic prokaryotes. Picophytoplankton has a ubiquitous distribution and contributes significantly to phytoplankton biomass and primary production in the ocean (Agawin et al., 2000; Bell and Kalff, 2001), and is suggested to contribute for about 1/3 to global phytoplankton biomass (Quere et al., 2005). In eutrophic coastal waters, picophytoplankton could also play important role with high abundance and biomass during certain seasons and conditions (Gaulke et al., 2010; Murrell and Lores, 2004). Heterotrophic prokaryotes are major decomposer of organic material, as well as utilizer of dissolved organic carbon (DOC) in marine ecosystems. In coastal waters, about 10–50% of all primary production passes through the heterotrophic prokaryotes (Fuhrman et al., 1980).

Phytoplankton blooms are characterized by high levels of abundances caused by increased reproduction (Garrison, 2009). Blooms are common but complex phenomenon in the ocean. They are important to the ecosystem productivity and carbon flux by providing more food to organisms higher up the food chain. Spring phytoplankton blooms could account for 1/3 of the annual phytoplankton production in some areas (Townsend et al., 1994).

The picoplankton distribution depends on both abiotic and biotic factors that affect the assemblage composition and cell abundances. Abiotic factors, known to drive a bottom-up control, include temperature, salinity, as well as light and nutrient availability. Biotic factors, assigned to top-down control, include predation by nano- and microzooplankton, as well as lysis by virioplankton. Studies on picoplankton distribution during blooms led to different patterns. Indeed, an abrupt abundance decrease for *Synechococcus* and/or picoeukaryotes was observed in mesocosm and field investigations, (Cunliffe et al., 2009; Larsen et al., 2004; Martínez-Martínez et al., 2006; Simek et al., 1995), whereas *Synechococcus* and picoeukaryotes could also maintain a "bloomer" growth strategy with higher abundance during bloom (Mackey et al., 2009). The DOC released by phytoplankton serves as

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an important substrate and energy source for heterotrophic prokaryotes in the ocean (Azam et al., 1983). It was commonly observed that heterotrophic prokaryotes exhibited higher abundance and biomass during bloom (Hyun and Kim, 2003; Larsen et al., 2004; Martínez-Martínez et al., 2006).

East China Sea (ECS) is the largest marginal sea in the western North Pacific. The picoplankton distribution in ECS was the subject of many studies, most of them addressing variations induced by seasons and hydrological conditions (Chiang et al., 2002; Jiao et al., 2005; Jiao et al., 2002; Le et al., 2012; Li et al., 2010; Pan et al., 2007). In this paper, we reported on picoplankton distribution in spring and summer ECS-blooms. The picoplankton abundance variations were exploited so as to gain insights into the factors and processes that regulated picoplankton development under bloom conditions in ECS.

2. Materials and methods

2.1. Sampling site and strategy

Two cruises were conducted in the ECS (25–33°N, 120–127°E) in spring and summer 2011. They were conducted on the R/V *Shiyan III* from 11 May to 7 June and on the R/V *Dongfanghong II* from 10 to 30 August, respectively. The present study only concerns 6 stations selected in each cruise as illustrated in Fig. 1.

At each station, seawater samples were collected with 5 dm^3 Niskin bottles carried by a "Sea-Bird" CTD (Conductivity/Temperature/Depth; Seabird19 Plus, Sea-Bird Electronics) rosette sampler. Seawater samples for picoplankton and Chlorophyll a (Chl *a*) were mainly collected at 4 (spring) or 5 (summer) depths from the surface to near bottom depending on the fine structure of CTD profiles and water depth (Table 1). At some stations, samples were also collected at the Chl *a* maximum depth (CMD). Samples for nutrients were also collected at surface layer (0 m).

2.2. Picoplankton abundance analyzed by flow cytometry

Seawater samples (4 cm^3) for picoplankton analysis by flow cytometry were fixed with paraformaldehyde (final concentration 1%) immediately after collection and then freeze-trapped in liquid nitrogen until analysis in the laboratory (Marie et al., 2000b).

Flow cytometry analyses were run with a FACS Vantage SE flow cytometer cell sorter (Becton Dickinson) equipped with a water-cooled Argon laser (488 nm, 1 W, Coherent). Protocols were adapted from literature (Marie et al., 2000a; Marie et al., 2000b). Fluorescent beads (2 μm, Polysciences) were used as the instrument internal standard (Olson et al., 1993). Flow cytometry data were recorded and analyzed with the CellQuest software (Version 3.3, Becton Dickinson).

For autotrophic picoplankton, the recording of forward scatter (FSC), side scatter (SSC), and two fluorescence (red, 695 ± 20 nm; orange, 585 ± 21 nm) signals was triggered on red (Chl *a*) fluorescence to discard signals from heterotrophic organisms and inorganic particles. *Synechococcus* and picoeukaryotes were distinguished on the basis of their scatter and fluorescence signals. The presence of *Prochlorococcus* was not detected.

For the analysis of heterotrophic prokaryotes, seawater samples were diluted 5 fold with TE buffer (Tris-EDTA, 100 mM Tris-Cl, 10 mM EDTA, pH = 8.0, Sigma), then stained with the nucleic acid dye SYBR Green I (Molecular Probes) (final dilution 10^{-4}, v/v) and kept in the dark at room temperature for 20° min before analysis. Heterotrophic prokaryotes were resolved into cell-groups on the basis of their green (530 ± 15 nm) fluorescence signal.

2.3. Chlorophyll a

For the determination of Chl *a* concentration, 50–200 cm^3 seawater samples were filtered onto GF/F glass-fiber filters (Whatman) under low vacuum. The filters were wrapped in aluminum foil and kept frozen at −80 °C until analysis. Upon return to the lab, Chl *a* was extracted with 90% acetone at 4 °C in the dark for 20 h. Chl *a*

Table 1
Information of sampling stations and the corresponding sampled depths for picoplankton and Chl *a*, both in spring and summer.

	Station	Latitude (°N)	Longitude (°E)	Depth (m)	Sampled depths (m)
Spring	C2	30.60	122.83	40	4, 10, 22, 36
	D2	29.18	122.41	39	0, 4, 9, 22, 36
	E1	28.21	121.80	23	4, 10, 20
	FE2	27.86	121.53	29	3, 8, 16, 26
	FE1	27.45	121.21	29	0, 7, 12, 26
	F1	27.26	120.81	25	4, 5, 12, 22
Summer	C3	31.89	123.02	36	3, 8, 15, 25, 32
	D3	31.02	123.05	55	3, 10, 20, 35, 52
	X1	30.50	122.94	57	3, 5, 10, 20, 35, 53
	E3	30.10	123.01	48	3, 10, 20, 30, 45
	F3	29.10	122.66	54	3, 10, 20, 35, 51
	G3	28.04	122.18	69	3, 22, 45, 65

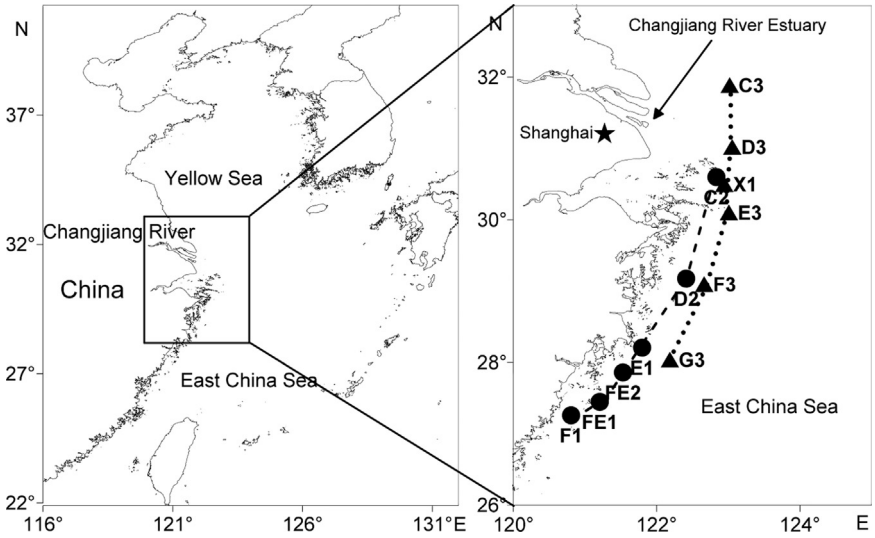

Fig. 1. Study area and sampling stations. —●—: Spring; …▲…: Summer.