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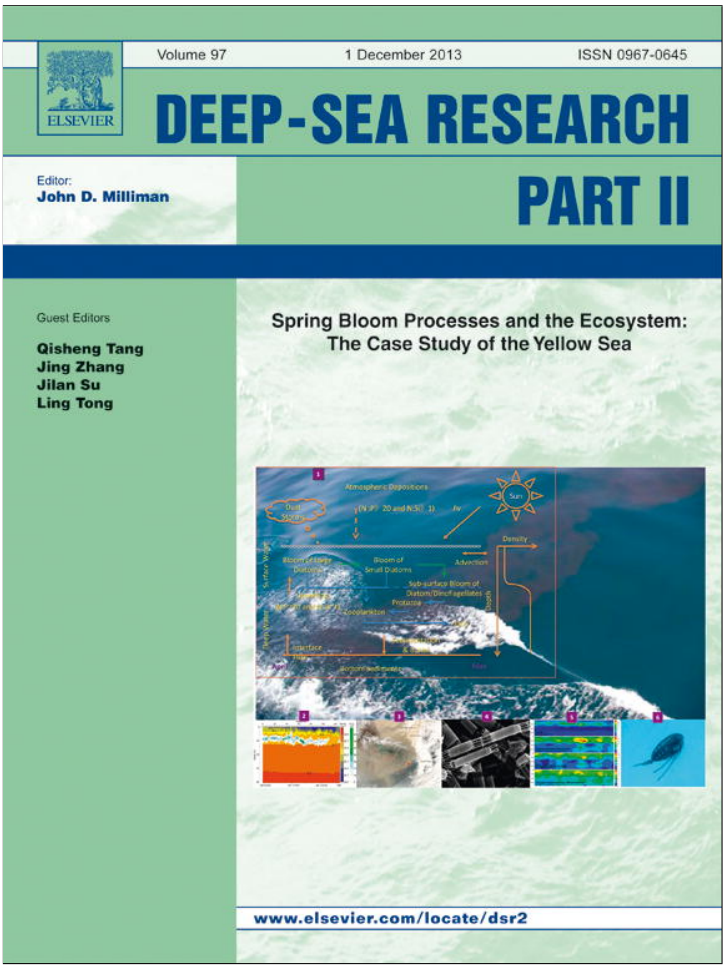

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Temporal variation of picoplankton in the spring bloom of Yellow Sea, China

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ABSTRACT

Temporal variation of *Synechococcus*, picoeukaryote and heterotrophic bacteria abundance and depth integrated biomass during three spring blooms in 2007 and 2009 were investigated in the Yellow Sea, China. *Synechococcus* and picoeukaryote responded differently to different types and course of spring blooms. During the diatom blooms of 2007 and Bloom B20 in 2009, *Synechococcus* and picoeukaryote abundances decreased sharply during the bloom period. However, during a mixed dinoflagellate and diatom bloom of Bloom B23 in 2009, *Synechococcus* and picoeukaryote increased in abundance and biomass along the bloom. During all three spring blooms, heterotrophic bacteria biomass had a similar increasing trend. Ciliate and heterotrophic nanoflagellate grazing could be responsible for *Synechococcus* and picoeukaryote abundance and biomass decrease during the spring blooms.

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1. Introduction

Marine picoplankton refer to a functionally diverse group of organisms which are microscopic in size (≤2 μm in diameter), but very abundant in the marine water column. Picoplankton include cyanobacteria of the genera *Synechococcus* (Johnson and Sieburth, 1979; Waterbury et al., 1979) and *Prochlorococcus* (Chisholm et al., 1988), a great diverse assemblage of picoeukaryote and heterotrophic bacteria which do not carry out oxygenic photosynthesis. Picoplankton have drawn much research attention due to its essential role in oceanic processes such as carbon production, biomass and energy transfer (Stockner, 1988; Li et al., 1983; Azam et al., 1983). Cyanobacteria are very important primary producers among autotrophic picoplankton. Generally, *Synechococcus* are present in inshore or coastal waters (Jochem, 1988) and can account for 20% of marine total biomass and 60% of total primary productivity (Caron et al., 1991). In some marine environments, picoeukaryote are major contributors in terms of biomass (Worden et al., 2004). Heterotrophic bacteria play an indispensable role in the carbon flux in aquatic ecosystems. They are decomposer of organic material, as well as utilizer of dissolved organic carbon (DOC) in marine ecosystems (Lefèvre et al., 1996; Hansell and Carlson, 1998). Thus heterotrophic bacteria are at the foundation of the microbial loop (Fuhrman and Azam, 1980).

Spring phytoplankton bloom is one of the most important seasonal patterns in pelagic food webs (Townsend et al., 1994). The Yellow Sea, located between mainland China and the Korean peninsula, is a typical semi-closed marginal sea of the Pacific Ocean, with depths ranging from 90 m in the central trough to less than 20 m within 50 km off the coast. Several typical hydrological features are present in the Yellow Sea. In spring, with a strong activity of tidal mixing, surface water of the Yellow Sea is rich in nutrients. Together with seasonal warming and the seasonal onset of higher light availability, those conditions would trigger the spring phytoplankton bloom.

The picoplankton distribution during spring phytoplankton bloom was investigated in several marine environments and different patterns were observed. On the one hand, an abrupt abundance decrease for *Synechococcus* and/or picoeukaryote followed by their abundance increase was observed during phytoplankton blooms in mesocosm and field investigations (Cunliffe et al., 2009; Larsen et al., 2004; Martinez-Martinez et al., 2006;

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Šimek et al., 1995). On the other hand, autotrophic picoplankton (*Synechococcus* and picoeukaryote) could maintain a "bloomer growth strategy with higher abundance and biomass during bloom than non-bloom conditions" (Mackey et al., 2009). The dissolved organic carbon (DOC) released from phytoplankton is an important carbon source for heterotrophic bacteria in the ocean (Azamet al., 1983). It was commonly observed that heterotrophic bacteria had higher abundance and biomass during bloom than non-bloom conditions (Martinez-Martinez et al., 2006; Larsen et al., 2004; Hyun and Kim, 2003).

Researches focused on the distribution of picoplankton during Yellow Sea spring bloom are very limited (Cho et al., 1994; Hyun and Kim, 2003). In this paper, we report investigations on picoplankton distribution in the Yellow Sea conducted during 2007 and 2009 spring blooms.

2. Materials and methods

2.1. Cruise description

In 2007 and 2009, two survey cruises were conducted in the central Yellow Sea (33.5–37.5°N, 121–124.5°E) to observe the spring bloom. On the basis of 9 years historical data (1998–2006), seawater chlorophyll *a* concentration >4 mg m^{-3} was set as the bloom criterion.

In 2007, a cruise was carried on from 30 March to 23 April. Seawater samples were collected from 32 stations (25 main-cruise stations and 7 anchor-stations) (Fig. 1). The ship track and station locations are displayed in Fig. 2. The cruise started from Transect A (Fig. 2A). When Transect G was completed, remote sensing detected high chl *a* concentration south of the study area which initiated the bloom tracking from St. F4 (Fig. 2B). Some stations were visited repeatedly as follows: A6-a→BM1-a→C4-a→D3-a→C4-b→BM1-b→A6-b→BM1-c→C4-c→D3-b (-a, -b, -c after the St.-name meaning the 1st, 2nd, 3rd time the vessel visited the stations respectively). After the bloom faded away, cruises went on as shown in Fig. 2C.

The main cruise survey conducted in 2009, initially set up non-bloom stations as reference. Then, two blooms were detected by remote sensing at St. B20 and St. B23 (Fig. 3). A drogue was released at each station in order to track the phytoplankton bloom development in a Lagrangian mode. The blooms were monitored for a period of 102 h (03:00 4 April–9:00 8 April) from St. B20 and 126 h (03:00 9 April–9:00 14 April) from St. B23. The B20 drogue drifted to southwest about 20 km from its initial position and the B23 drogue remained within a 7 km distance to its initial position (Fig. 4). Seawater samples were collected every 3 h during both blooms.

2.2. Sampling strategy

At each station, seawater samples were collected with 5 L Niskin bottles on a "Sea-Bird" CTD (Conductivity/Temperature/Depth) rosette sampler. In 2007, seawater samples were mainly collected from five depths (surface, 10 m, 30 m, 50 m and bottom).

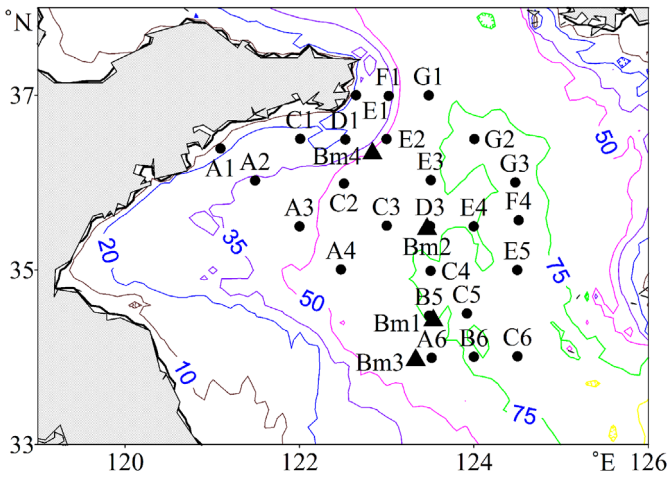

Fig. 1. Location of the sampling stations of the Yellow Sea Spring Bloom Cruise in 2007. (●: Main cruise stations, ▲: Anchor stations and contour-lines: isobath of the study area).

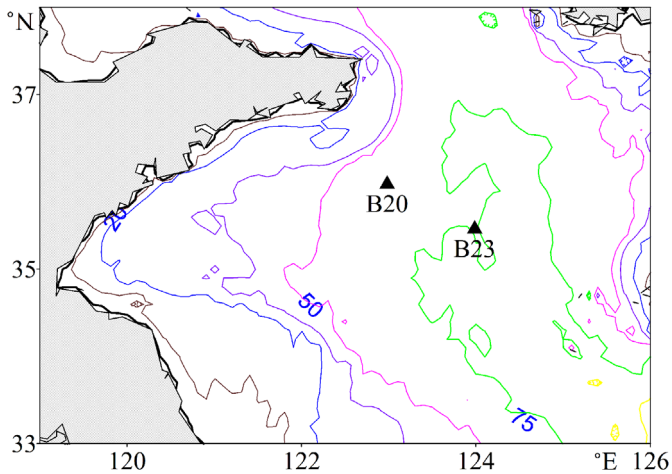

Fig. 3. Location of the bloom tracking stations in 2009 (contour-lines: isobath of the study area).

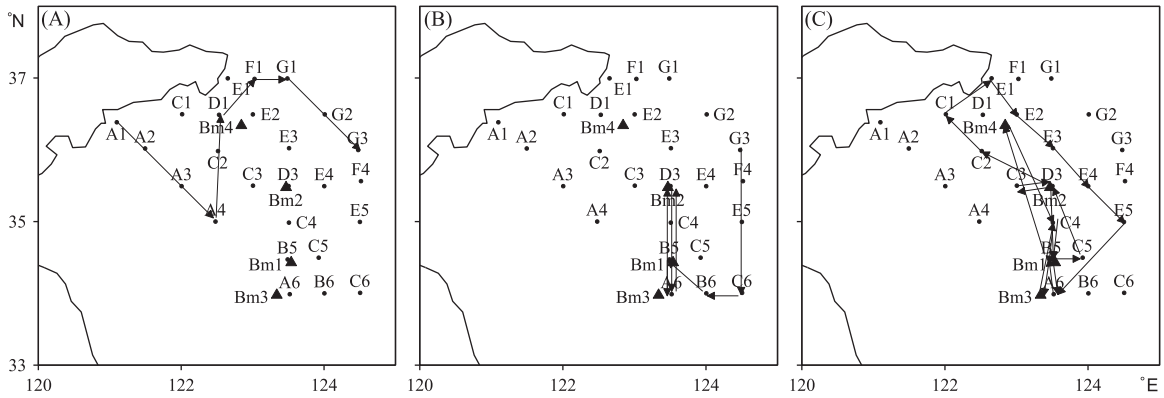

Fig. 2. Observation routes in 2007 (a) pre-bloom period; (b) bloom period and (c) post-bloom period.

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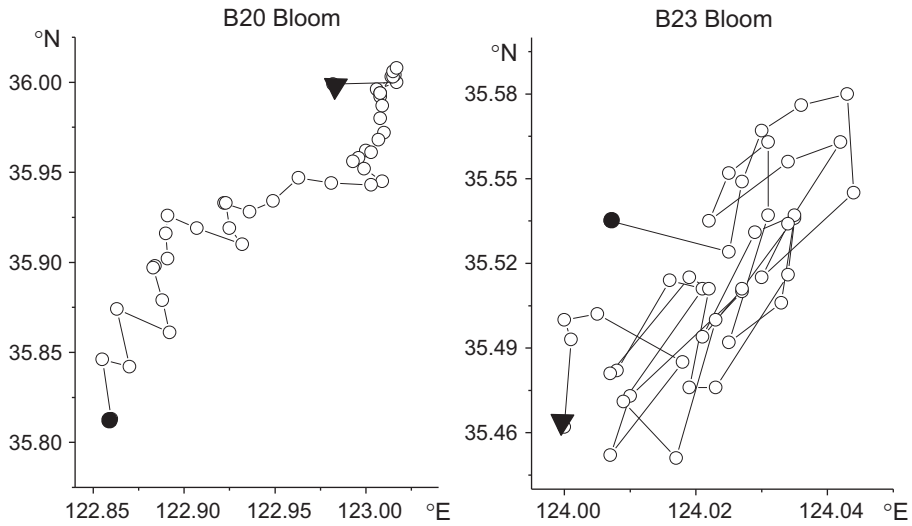

Fig. 4. Tracking routes of two phytoplankton blooms in 2009 (▲: beginning spot and ●: finishing spot).

At some stations, samples were also collected from maximum chl *a* depth (MCD) (3, 12 or 15 m depths). In 2009, one chl *a in situ* fluorescence detector equipped the sampler to determine the MCD, and the following depths were sampled: surface, MCD, 30 m, 50 m and bottom. Subsamples (5 cm^3) were fixed with paraformaldehyde (final concentration 1%) immediately after collection and then freeze-trapped in liquid nitrogen until analysis by flow cytometry in the laboratory (Marie, 2000a). Water temperature and salinity data were collected with the SEB-25 Conductivity/Temperature/Depth profilers (CTD) (Seabird Electronics).

2.3. Flow cytometry

Single cell analyses of picoplankton were run with a FACSVantage SE flow cytometer (Becton Dickinson) equipped with a water-cooled Argon laser (488 nm, 1 W, Coherent). Protocols were adapted from Marie et al. (2000a, 2000b). Fluorescent beads (2 μm, Polysciences) were used as the internal standard for the enumeration of picoplankton cells (Olson et al., 1993). During all analyses, the laser power was kept constant at 80 mW. Flow cytometric data were collected and analyzed with the CellQuest software (Version 3.3, Becton Dickinson).

For *Synechococcus* and picoeukaryote analysis, 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 fluorescence to discard signals from heterotrophic organisms and inorganic particles. *Synechococcus* and picoeukaryote were distinguished on the basis of their scatter and fluorescence signals.

For heterotrophic bacteria analysis, 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 bacteria cell-groups were resolved on the basis of their green (530 ± 15 nm) fluorescence signal.

2.4. Chlorophyll a

For determining chl *a* concentrations, 500 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* concentrations were determined by the acidification method (Strickland and Parsons, 1972) using a Turner Design (Model Trilogy 040) fluorometer, which was calibrated with pure chl *a* (Sigma).

2.5. Biomass

For *Synechococcus* and picoeukaryote, the equivalent spherical diameter of cells was estimated from the calibration curve of FSC against particle diameter derived from the measurements of 0.5, 1.0, 2.0, 3.0 and 6.0 μm polystyrene beads (Polysciences) (Zhao et al., 2011). Cell carbon content was derived from biovolume by using a predictive regression equation: $C(pg) = 0.433 \times (Biovolume)^{0.863}$ (Verity et al., 1992). For heterotrophic bacteria cells, a conversion factor of 20 fg C $cell^{-1}$ was used (Lee and Fuhrman, 1987). For each cell-group, carbon biomass per unit volume was then determined by multiplying the cell carbon content by the related cell abundance.

Total phytoplankton biomass per unit volume was estimated from chl *a* concentration assuming a constant *C*:chl *a* ratio of 50 (mg:mg) (Krempin and Sullivan, 1981).

The depth averaged biomasses(DAB) per unit volume of the three groups pf picoplankton and total phytoplankton were obtained by calculating the average concentration for each profile, i.e. to divide the integrated biomass between surface and bottom by the depth of the profile.

2.6. Statistical analysis

A 2-tailed *t*-test was conducted to compare the picoplankton abundance between pre-bloom and bloom period using SAS version 9.1 (SAS Institute Inc, Cary, North Carolina, USA). A linear model was applied with SPSS (version 7.5, SPSS Inc., Chicago, IL, USA) to obtain a correlation coefficient and a *p* value between picoplankton abundance and biological and hydrologic parameters.

3. Results

3.1. Bloom in 2007

3.1.1. Environmental parameters

The vertical profiles of seawater parameters are showed in Fig. 5. Stations are displayed according to their sampling sequence.

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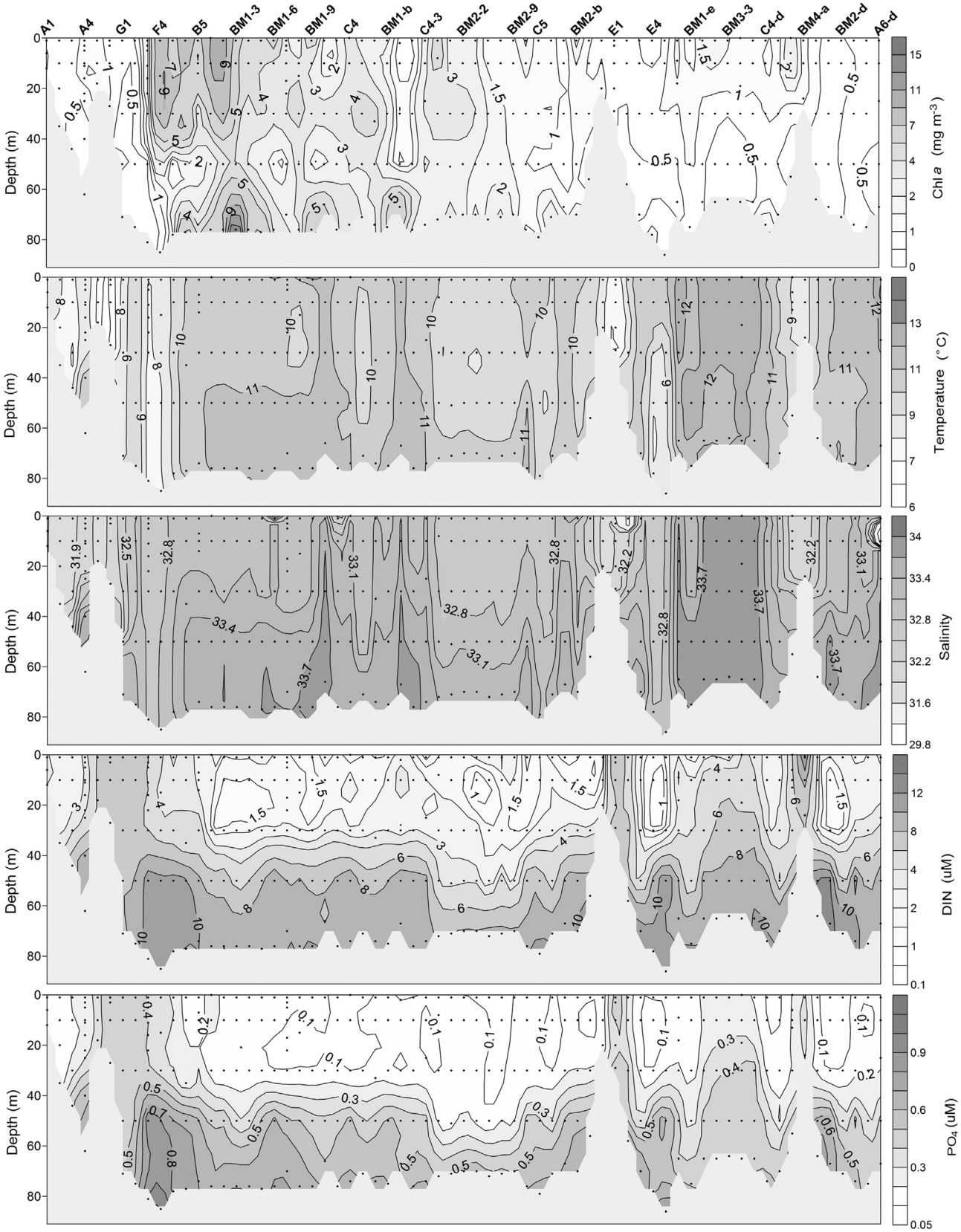

Fig. 5. Vertical distribution of environmental variables in 2007.

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Table 1
Water chlorophyll *a* concentration (mg m^{-3}), temperature (°C), salinity (‰), DIN (μM) and PO_4^{3-}(μM) during three blooms. Reported data are average values together with (min–max).

		Pre-bloom	Bloom period	Post-bloom
Chlorophyll *a* (mg m^{-3})	Bloom 2007	1.09 (0.09–6.08)	3.99 (0.50–41.36)	0.92 (0.21–3.16)
Temperature (°C)		8.29 (5.97–11.07)	10.24 (7.79–12.46)	10.54 (6.46–13.26)
Salinity (‰)		32.42 (31.57–33.88)	33.16 (31.42–33.87)	33.15 (29.92–33.96)
DIN (μM)		5.87 (1.92–11.42)	4.55 (0.22–12.28)	5.41 (0.40–13.38)
PO_4^{3-} (μM)		0.38 (0.11–0.94)	0.29 (0.06–1.04)	0.30 (0.06–0.86)
Chlorophyll *a* (mg m^{-3})	Bloom B20	0.96 (0.35–1.84)	1.61 (0.22–6.39)	0.91 (0.25–2.82)
Temperature (°C)		8.68 (7.72–9.20)	8.83 (7.22–9.51)	9.19 (8.30–9.92)
Salinity (‰)		32.87 (32.27–33.15)	32.88 (30.41–33.30)	32.98 (32.23–33.30)
DIN (μM)		7.06 (2.63–10.47)	5.38 (0.41–10.48)	6.60 (0.49–10.13)
PO_4^{3-} (μM)		0.44 (0.17–0.59)	0.36 (0.07–0.65)	0.41 (0.06–0.62)
Chlorophyll *a* (mg m^{-3})	Bloom B23	1.10 (0.45–2.20)	2.35 (0.27–29.54)	1.65 (0.25–9.62)
Temperature (°C)		7.46 (7.34–8.00)	7.81 (7.28–12.78)	7.95 (7.29–12.45)
Salinity (‰)		32.73 (32.68–32.78)	32.71 (32.60–32.76)	32.69 (32.61–32.72)
DIN (μM)		7.27 (5.66–8.22)	4.99 (0.29–11.05)	5.37 (0.32–8.62)
PO_4^{3-} (μM)		0.52 (0.45–0.59)	0.37 (0.06–0.60)	0.44 (0.09–0.63)

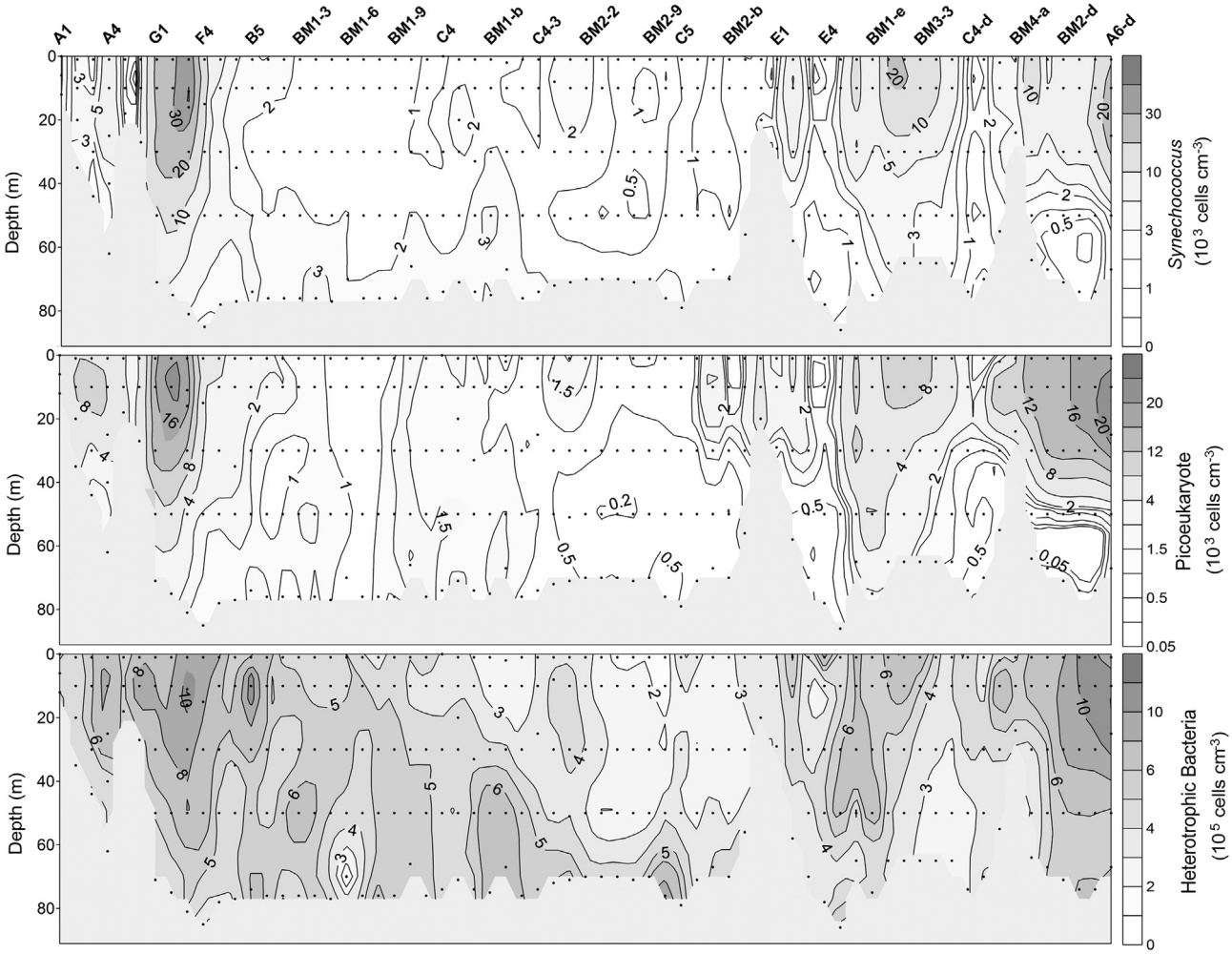

Fig. 6. Time course of the vertical distribution of picoplankton abundances (*Synechococcus* and picoeukaryote: × 10^3 cells cm^{-3} and heterotrophic bacteria: × 10^5 cells cm^{-3}) in 2007.

From St. A1 (20:20, 31 March) to St. G3 (09:00, 4 April), chl *a* concentration was relatively low (< 3 mg m^{-3}, except at St. G3, 3 m depth, 6.08 mg m^{-3}). Data collected during this period were set up as a reference pre-bloom situation. At St. F4 (12:50, 4 April), chl *a* concentration was > 10 mg m^{-3} (surface, 10 and 15 m depths). From St. F4 to anchor station BM1 (5–6 April), the chl *a* concentration remained high (> 5 mg m^{-3}). Except for high chl *a* concentration at surface and subsurface, high chl *a* concentrations were also observed occasionally in deeper levels. For example, the highest chl *a* concentration during the 2007 Bloom was 18.72 mg m^{-3}, observed near the bottom (76 m) at St. BM1-3 (21:00, 5 April). From anchor station BM2 (9 April), chl *a*

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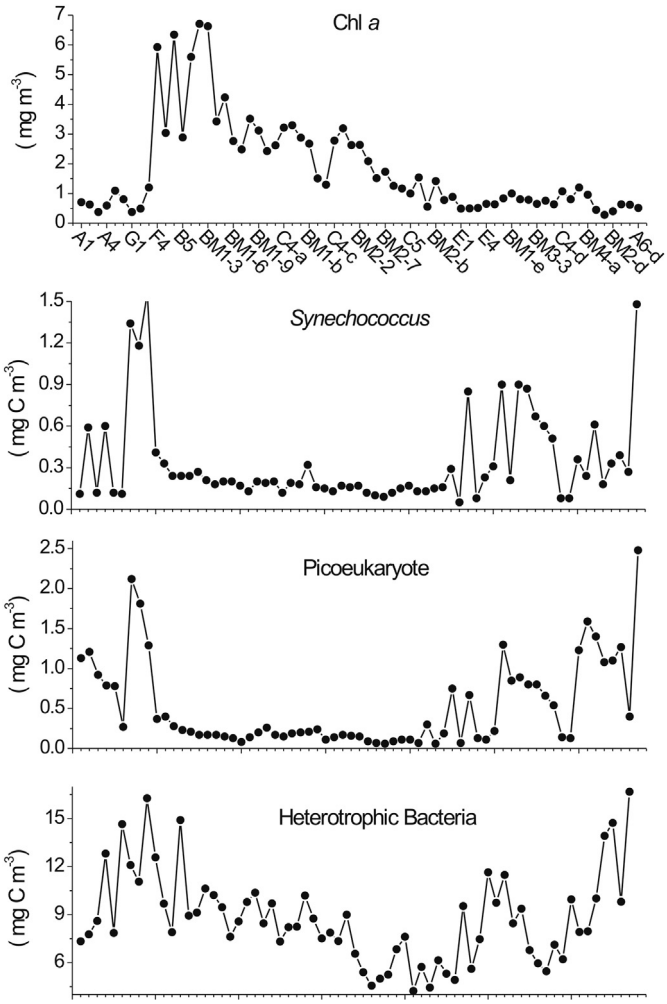

Fig. 7. Depth averaged chl *a* concentration (mg m^{-3}) and picoplankton biomasses (mg C m^{-3}) in 2007.

concentration began to drop. The period of St. F4 to St. BM2-9 was considered as the 2007 bloom period. From St. BM1-d (18:50, 9 April) to the last observation station St. A6-d (14:30, 23 April), chl *a* concentration remained low, and this period was considered as post-bloom. Diatoms *Skeletonema dohrnii*, *Thalassiosira decipiens* and *Thalassiosira pacifica* were dominant phytoplankton species in Bloom 2007.

During the 2007 Bloom, temperature and salinity were relatively homogeneous along the water column (Fig. 5). The bloom-period average temperature (10.24 °C, calculated from all temperature measurements) was higher than that of pre-bloom (8.29 °C), and lower than that of post-bloom (10.54 °C) (Table 1). The bloom-period average salinity (33.16‰) was slightly higher than that of pre-bloom (32.42‰) and post-bloom (33.15‰). Nutrient (DIN and PO_4^{3-}) concentrations in the upper 40 m sharply decreased during the bloom, and slightly recovered in post-bloom (Table 1). During the whole cruise, nutrient concentrations remained relatively constant below 40 m depth (Fig. 5).

3.1.2. Picoplankton distribution

Synechococcus and picoeukaryote abundances exhibited similar trends during the 2007 Bloom (Fig. 6). Estimated average cell sizes for *Synechococcus* and picoeukaryote were 0.76 ± 0.08 and 0.88 ± 0.08 μm, respectively, leading to the average cellular carbon contents of 126 ± 44 and 184 ± 43 fg C cell^{-1} for *Synechococcus* and picoeukaryote. During pre-bloom, *Synechococcus* and picoeukaryote were abundant in the water column, with average abundances of 10.17 and 7.93×10^3 cells cm^{-3} respectively. When the bloom occurred at St. F4, the abundances and depth averaged biomass per unit volume of both *Synechococcus* and picoeukaryote dropped sharply, and remained very low during the whole bloom period (Figs. 6 and 7, Table 2). The abundances of *Synechococcus* and picoeukaryote (1.90 and 1.23×10^3 cells cm^{-3} in average, respectively) were significantly lower during bloom than during pre-bloom (*t*-test, $p < 0.01$, respectively). After bloom, the abundances of *Synechococcus* and picoeukaryote showed a recovering trend, with average abundances of 4.26 and 4.80×10^3 cells cm^{-3}, respectively. During bloom, the depth averaged biomass of *Synechococcus* and

Table 2
Picoplankton abundance (*Synechococcus* and picoeukaryote: 10^3 cells cm^{-3}, heterotrophic bacteria: 10^5 cells cm^{-3}), depth averaged biomasses (mg Cm^{-2}) and their contributions to total phytoplankton biomass per unit volume during three blooms.

Reported data are average values together with (min–max). SAIB-*Synechococcus* depth averaged biomass; PAIB-picoeukaryote depth averaged biomass; HBAIB-heterotrophic depth averaged biomass; PhytoAIB-phytoplankton depth averaged biomass per unit volume.

		Pre-bloom	Bloom period	Post-bloom
Synechococcus (10^3 cells cm^{-3})		10.17 (0.55–45.10)	1.90 (0.35–7.49)	4.26 (0.22–29.95)
Picoeukaryote (10^3 cells cm^{-3})		7.93 (1.12–21.00)	1.23 (0.17–4.97)	4.80 (0.16–24.82)
Heterotrophic bacteria (10^5 cells cm^{-3})		6.37 (3.48–12.26)	4.57 (0.87–14.32)	4.64 (0.77–12.30)
SAIB (mg C m^{-3})		0.64 (0.11–1.56)	0.19 (0.09–0.41)	0.39 (0.05–1.48)
PAIB (mg C m^{-3})	Bloom	1.15 (0.27–2.12)	0.18 (0.06–0.40)	0.67 (0.06–2.48)
HBAIB (mg C m^{-3})	2007	10.94 (7.33–16.28)	8.59 (4.56–14.91)	8.31 (4.22–16.69)
PhytoAIB (mg C m^{-3})		35.08 (18.97–59.82)	162.74 (63.03–334.86)	52.08 (13.93–406.16)
SAIB/PhytoAIB (%)		2.19 (0.23–7.03)	0.13 (0.06–0.25)	1.14 (0.15–5.66)
PAIB/PhytoAIB (%)		4.13 (0.67–11.11)	0.12 (0.05–0.32)	2.12 (0.08–9.47)
HBAIB/PhytoAIB (%)		35.42 (14.26–63.23)	6.16 (2.49–11.60)	25.04 (5.49–71.93)
Synechococcus (10^3 cells cm^{-3})		8.04 (1.89–10.81)	4.76 (1.05–44.83)	9.90 (3.03–31.39)
picoeukaryote (10^3 cells cm^{-3})		7.80 (2.59–11.10)	5.63 (1.79–17.93)	8.02 (2.25–22.39)
Heterotrophic bacteria (10^5 cells cm^{-3})		5.60 (3.65–8.43)	7.42 (3.80–12.58)	6.19 (5.02–9.33)
SAIB (mg C m^{-3})		0.77 (0.56–0.98)	0.62 (0.36–3.12)	0.45 (0.30–0.61)
PAIB (mg C m^{-3})	Bloom	1.50 (1.00–2.00)	1.04 (0.70–1.47)	0.91 (0.65–1.14)
HBAIB (mg C m^{-3})	B20	9.76 (8.06–11.45)	13.11 (10.82–16.27)	13.23 (10.72–15.40)
PhytoAIB (mg C m^{-3})		38.91 (37.57–40.24)	69.36 (47.23–94.58)	41.70 (32.48–55.53)
SAIB/PhytoAIB (%)		1.96 (1.48–2.44)	0.89 (0.45–3.57)	1.10 (0.81–1.57)
PAIB/PhytoAIB (%)		3.82 (2.65–4.98)	1.55 (0.92–2.33)	2.22 (1.74–2.90)
HBAIB/PhytoAIB (%)		24.95 (21.45–28.46)	19.53 (13.22–28.04)	32.68 (26.11–45.41)
Synechococcus (10^3 cells cm^{-3})		5.77 (2.21–15.59)	5.58 (1.87–24.46)	6.31 (2.79–20.90)
picoeukaryote (10^3 cells cm^{-3})		6.72 (2.46–14.21)	5.45 (1.51–16.55)	4.31 (1.52–12.39)
Heterotrophic bacteria (10^5 cells cm^{-3})		3.96 (2.12–6.88)	6.83 (0.77–19.01)	6.82 (4.36–11.24)

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Table 2 (*continued*)

		Pre-bloom	Bloom period	Post-bloom
SAIB (mg C m^{-3})		0.59 (0.44–0.75)	0.71 (0.45–1.30)	0.88 (0.68–1.09)
PAIB (mg C m^{-3})	Bloom	1.23 (0.72–1.82)	1.03 (0.68–1.65)	0.81 (0.68–1.06)
HBAIB (mg C m^{-3})	B23	7.11 (4.79–11.75)	11.80 (8.35–19.00)	12.03 (11.13–13.38)
PhytoAIB (mg C m^{-3})		47.18 (42.71–51.79)	103.13 (45.49–299.28)	54.72 (30.23–91.97)
SAIB/PhytoAIB (%)		1.26 (0.86–1.50)	0.77 (0.38–1.61)	1.78 (1.18–2.27)
PAIB/PhytoAIB (%)		2.63 (1.41–3.52)	1.12 (0.55–2.45)	1.7 (0.88–2.24)
HBAIB/PhytoAIB (%)		14.96 (9.34–22.69)	12.77 (5.13–25.30)	26.31 (12.86–36.82)

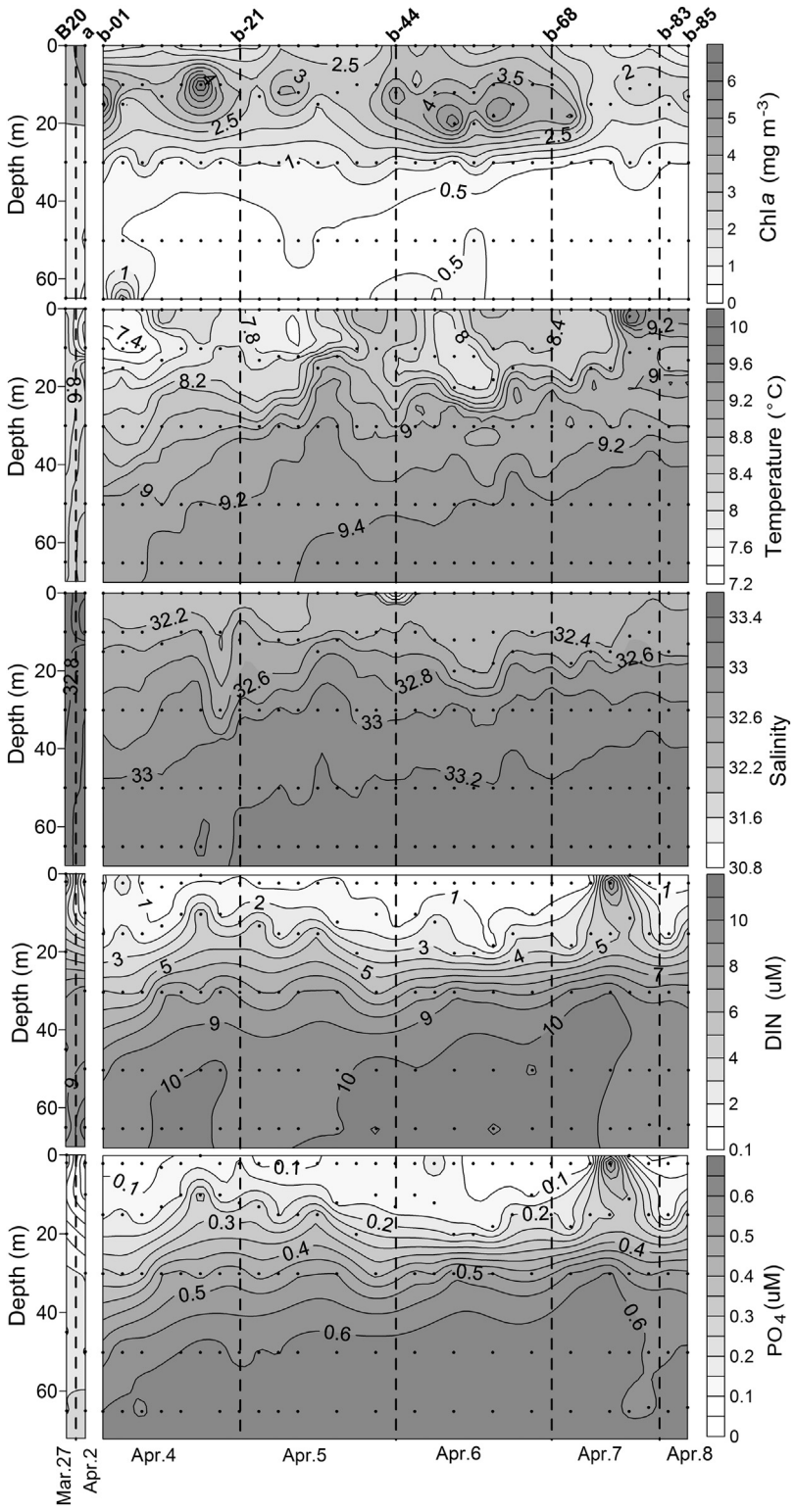

Fig. 8. Vertical distribution of environmental variables during Bloom B20 in 2009.

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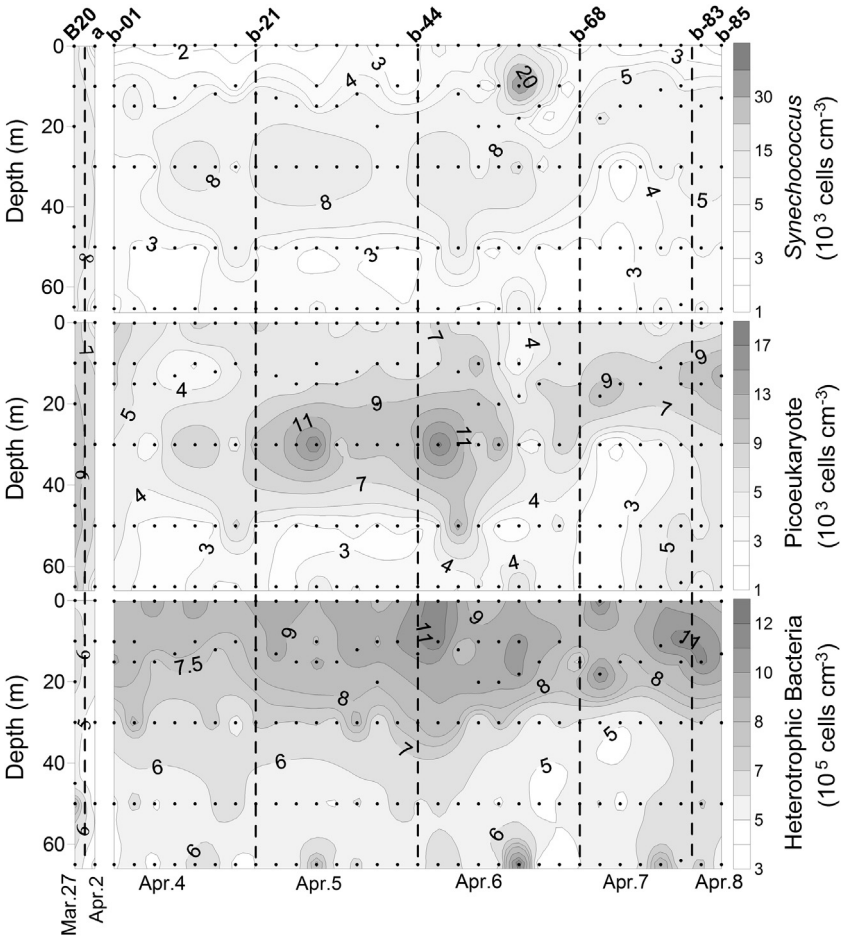

Fig. 9. Time course of the vertical distribution of picoplankton abundances (*Synechococcus* and picoeukaryote: $\times 10^3$ cells cm^{-3} and heterotrophic bacteria: $\times 10^5$ cells cm^{-3}) during Bloom B20 in 2009.

picoeukaryote were 0.19 and 0.18 mg C m^{-3} respectively. They only contributed 0.13% and 0.12% respectively to the total depth averaged phytoplankton biomass per unit volume(162.74 mg C m^{-3}), while their contributions were 2.19% and 4.13% respectively during pre-bloom when total depth averaged phytoplankton biomass per unit volume was 35.08 mg C m^{-3} (Table 2).

At the beginning of bloom period, the abundance of heterotrophic bacteria slightly dropped (Figs. 6 and 7). Compared to pre- and post-bloom, the bloom-heterotrophic bacteria abundance was significantly lower (4.57×10^5 cells cm^{-3}) (t-test, $p < 0.01$). But due to deeper water depth, the highest depth averaged bacteria biomass was observed during the bloom period (8.59 mg C m^{-3}), which was different from *Synechococcus* and picoeukaryote biomass variation. The depth averaged biomass of heterotrophic bacteria could amount to 6.16% of total phytoplankton biomass per unit volume during the Bloom 2007 (Table 2).

No correlation was found between abundances of *Synechococcus*, picoeukaryote and heterotrophic bacteria, and temperature and salinity ($p > 0.05$ for all).

No *Prochlorococcus* was detected in the study area.

3.2. Bloom of B20 in 2009

3.2.1. Environmental parameters

Station B20 was visited on 27 March and 2 April, and on both occasions, chl *a* concentration was < 2 mg m^{-3}. Data collected on these two visits were assigned to a pre-bloom situation to serve as a reference for the bloom process. At St. B20b-01 (03:00, 4 April), high chl *a* concentration (5.39 mg m^{-3}, MCD, 15 m depth) was detected, and the bloom tracking was initiated. As revealed by changes in chl *a* concentration, the tracking period from St. B20b-01 to St. B20b-71 (03:00 4 April–3:00 7 April) corresponded with the peak-to-decline phase of a spring algal bloom (Fig. 8). During Bloom B20, the maximum chl *a* depth was about 10–15 m (Fig. 8), and the highest chl *a* concentration (6.39 mg m^{-3}) was found at St. B20b-14 (18:00, 4 April, 10 m depth). Diatoms *Detonula pumila* and *Guinardia delicatula* were the dominant phytoplankton species at Bloom B20.

Temperature and salinity increased with depth under both pre-bloom and bloom situations at St. B20 (Fig. 8). No obvious thermocline was observed. Temperature had a gradual increase toward the end of observation (pre-bloom 8.68 °C, bloom 8.83 °C, post-bloom 9.19 °C). Nutrients were lower during bloom than pre-bloom (Table 1). In comparison with the water column below 20 m depth, nutrient concentrations in the upper 20 m remained very low through the 5 days bloom tracking (Fig. 8).

3.2.2. Picoplankton variation

Under the pre-bloom conditions, the vertical distribution of picoplankton was rather homogeneous (Fig. 9) with average abundances of 8.04×10^3 cells cm^{-3} for *Synechococcus*, 7.80×10^3 cells cm^{-3} for picoeukaryote and 5.60×10^5 cells cm^{-3} for heterotrophic bacteria. Estimated average cell sizes for *Synechococcus* and picoeukaryote were 0.77 ± 0.07 and 0.91 ± 0.06 μm, respectively, leading to the average cellular carbon contents of 130 ± 33 and 196 ± 37 fg C cell^{-1} for *Synechococcus* and picoeukaryote.

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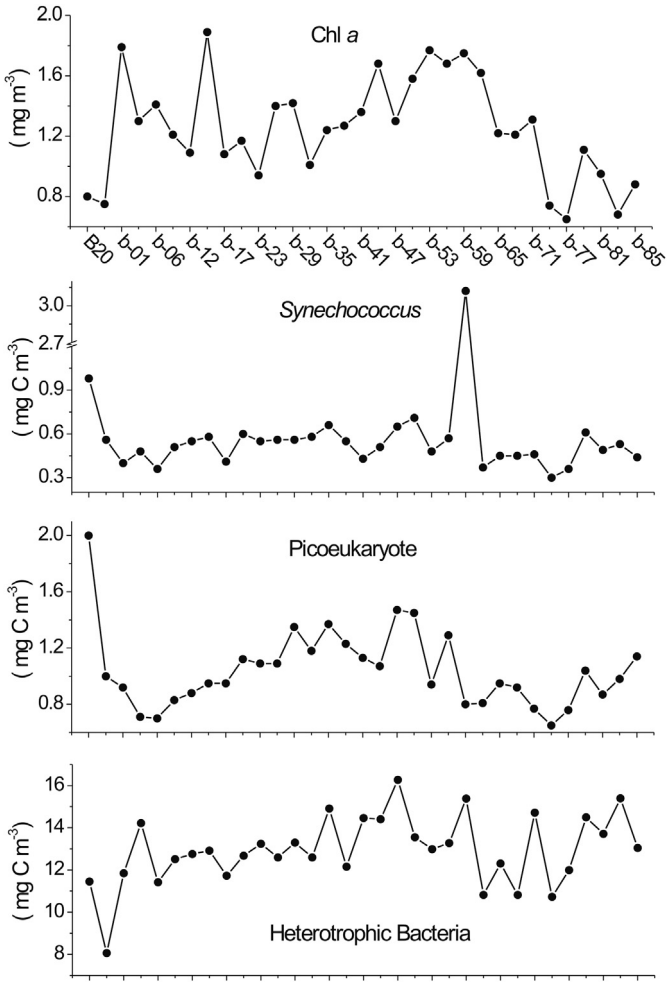

Fig. 10. Depth averaged chl *a* concentration (mg m^{-3}) and picoplankton biomasses (mg C m^{-3}) during Bloom B20 in 2009.

During the bloom period, the vertical distributions of *Synechococcus* and picoeukaryote varied similarly. At 03:00, 4 April when the bloom tracking started, *Synechococcus* and picoeukaryote abundances were much lower than during the pre-bloom (Fig. 9). During the bloom period, the average *Synechococcus* abundance was 4.76×10^3 cells cm^{-3}, about 41% lower than its pre-bloom abundance. The abundance and depth integrated biomass *Synechococcus* was rather constant during the whole bloom tracking, except for peak abundance value (44.83×10^3 cells cm^{-3}) observed at St. B20b-59 (15:00, 6 April) (Figs. 9 and 10). The *Synechococcus* maximum abundance was observed at 30 m, below the depth of chl *a* maximum concentration (10–15 m, Fig. 8).

The average abundance of picoeukaryote was 5.63×10^3 cells cm^{-3} during bloom, about 28% lower than the pre-bloom value. The abundance and depth integrated biomass of picoeukaryote showed some fluctuations during bloom tracking. When the tracking started, picoeukaryote abundance and biomass decreased sharply (Figs. 8 and 9), then increased to reach maximum values of 17.93×10^3 cells cm^{-3} and 1.47 mg C m^{-3} respectively at St. B20b-47 (03:00, 6 April) (Fig. 10). After that, picoeukaryote abundance and biomass gradually decreased until post-bloom while maximum values remained observed at 30 m depth as for *Synechococcus*.

During the bloom period, the average abundance of heterotrophic bacteria was 7.42×10^5 cells cm^{-3}, a value about 32% higher than that during pre-bloom, in contrast with autotrophic picophytoplankton. Heterotrophic bacteria were the most abundant in the upper 10 m, above the maximum abundance depth (30 m) of picophytoplankton (Fig. 9). The abundance variation of heterotrophic bacteria during Bloom B20 was similar to that of picoeukaryote, with maximum abundance (11.99×10^5 cells cm^{-3}) and depth averaged biomass (16.27 mg C m^{-3}) at St. B20b-47 (03:00, 6 April).

The abundance of *Synechococcus* and picoeukaryote was significantly lower during B20 bloom than pre-bloom period (*t*-test, $p < 0.01$). In contrast, the abundance of heterotrophic bacteria during bloom was significantly higher than during pre-bloom (*t*-test, $p < 0.01$). picoeukaryote and heterotrophic bacteria abundances were negatively correlated with temperature and salinity.

During the B20 bloom period, the ratio (%) of picoplankton/phytoplankton biomass per unit volume was higher than during the Bloom 2007, with 0.89%, 1.55% and 19.53% for *Synechococcus*, picoeukaryote and heterotrophic bacteria, respectively (Table 2).

3.3. Bloom of B23 in 2009

3.3.1. Environmental parameters

St. B23 was visited four times before starting bloom tracking (St. B23, 26 March 19:00; St. B23a, 31 March 15:00; St. B23b, 2 April 7:30 and St. B23c, 3 April 17:50), and chl *a* concentration was < 2 mg m^{-3} (except at St. B23c, 10 m depth where it was 2.20 mg m^{-3}). The data collected during these visits were set as pre-bloom references. At 03:00 9 April, chl *a* concentration was 10.54 mg m^{-3} at 10 m depth, leading to the bloom tracking. Chl *a* concentration maximum was observed at about 20 m depth during Bloom B23, a distribution deeper than that observed during Bloom B20 (Fig. 11). On 11 April, at 21:00, a very high (29.54 mg m^{-3}) chl *a* concentration was, detected at 22 m depth. The B23 bloom mainly concerned dinoflagellates and diatoms with *Heterocapsa lanceolata*, *Prorocentrum minimum* and *Skeletonema dohrnii* as dominant species.

Under pre-bloom conditions, the water column was rather homogeneous at St. B23 (Fig. 11). However, during bloom tracking, a thermocline was observed at about 10 m depth, which was above the maximum chl *a* depth (Fig. 1). Temperature progressively increased until the end of the survey (Table 1, Fig. 11). During Bloom B23, the nutrient vertical distribution was similar to that during B20, with very low DIN and PO_4^{3-} concentrations in the upper 20 m during bloom tracking (Fig. 11).

3.3.2. Picoplankton variation

During Bloom B23, the abundance of the three picoplankton groups (*Synechococcus*, picoeukaryote and heterotrophic bacreria) had similar variation trends and were essentially present in the upper 25 m (Fig. 12), corresponding to the chl *a* distribution (Fig. 11).

Estimated average cell sizes for *Synechococcus* and picoeukaryote were 0.83 ± 0.05 and 0.98 ± 0.05 μm, respectively, leading to the average cellular carbon contents of 153 ± 23 and 234 ± 31 fg C cell^{-1}. The abundance and depth integrated biomass of *Synechococcus* and picoeukaryote were variable on top of an increasing trend through the bloom (Fig. 13). The average abundances of *Synechococcus* and picoeukaryote were 5.58 and 5.45×10^3 cells cm^{-3}, respectively. The average depth integrated biomasses for *Synechococcus* and picoeukaryote were 0.71 and 1.03 mg C m^{-3}, contributing 0.77% and 1.12% respectively to the overall phytoplankton biomass per unit volume. The abundance and depth-integrated biomass of heterotrophic bacteria were less fluctuating than those of picophytoplankton (Fig. 13). The highest values were observed at the bloom end-period (19.01×10^5 cells cm^{-3} and 19.00 mg C m^{-3}, St. B23d-92, 13 April 3:00,). During the bloom period, average heterotrophic bacteria depth-integrated biomass was 11.80 mg C m^{-3}, amounting to 12.77% of photosynthetic biomass per unit volume (Table 2).

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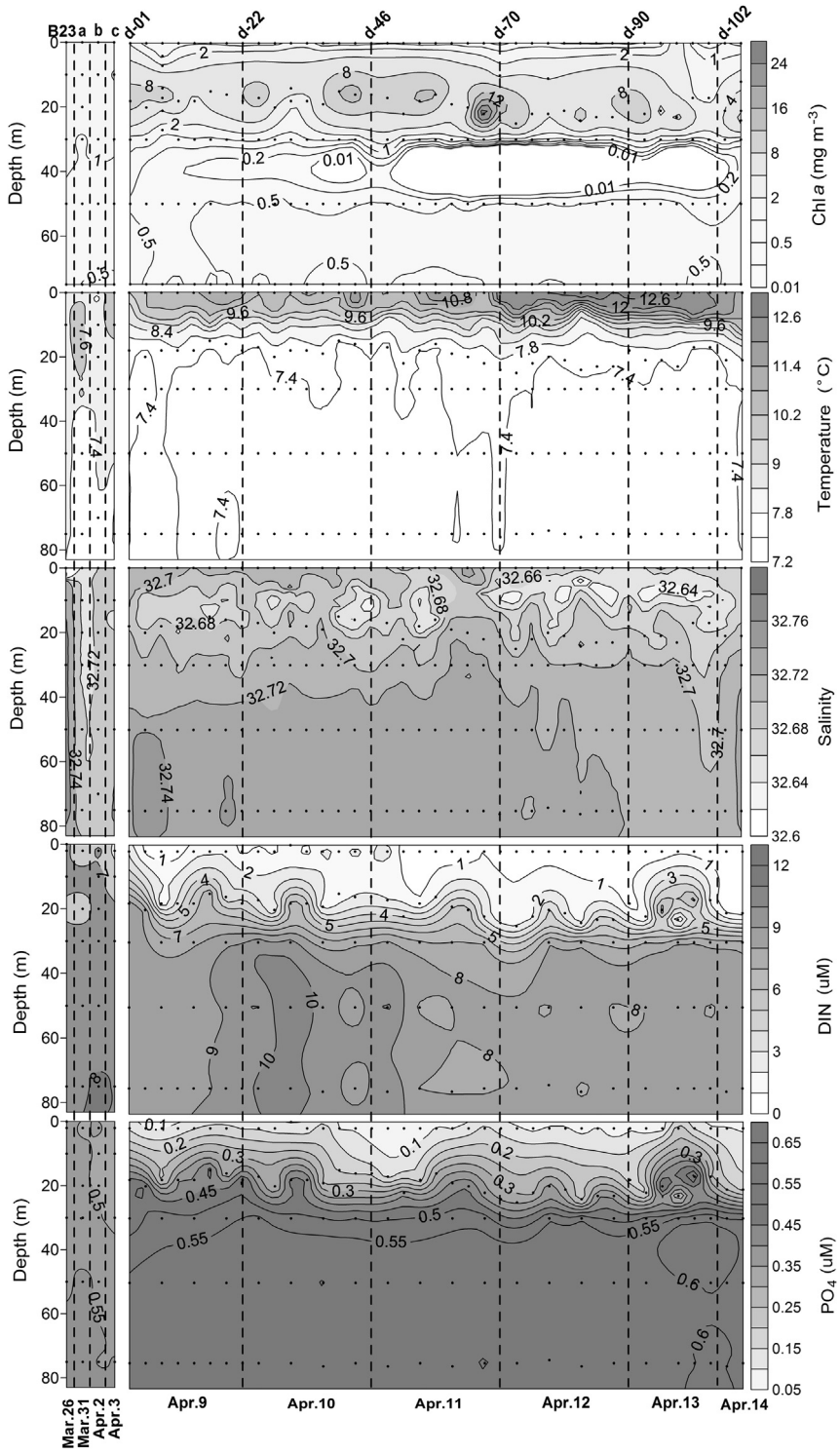

Fig. 11. Depth profiles of environmental parameters of Bloom B23 in 2009.

No significant difference was found between the *Synechococcus* and picoeukaryote abundances during pre-bloom and bloom periods (t-test, $p > 0.05$). In contrast, the heterotrophic bacteria abundance during the bloom period was significantly higher than during pre-bloom (t-test, $p < 0.01$). The abundances of the three picoplankton groups were all positively correlated with temperature ($r = 0.159$, 0.623 and 0.462 for *Synechococcus*, picoeukaryote and heterotrophic bacteria, respectively, $p < 0.05$ for all).

4. Discussion

The spring phytoplankton bloom could represent a pulsed source of organic carbon that is important to the ecosystem productivity and carbon flux into the world ocean. The bloom can amount to 1/3 of the annual phytoplankton production in some areas (Townsend et al., 1994). Despite the important role of marine picoplankton in the microbial food loop, the variation of picoplankton during spring

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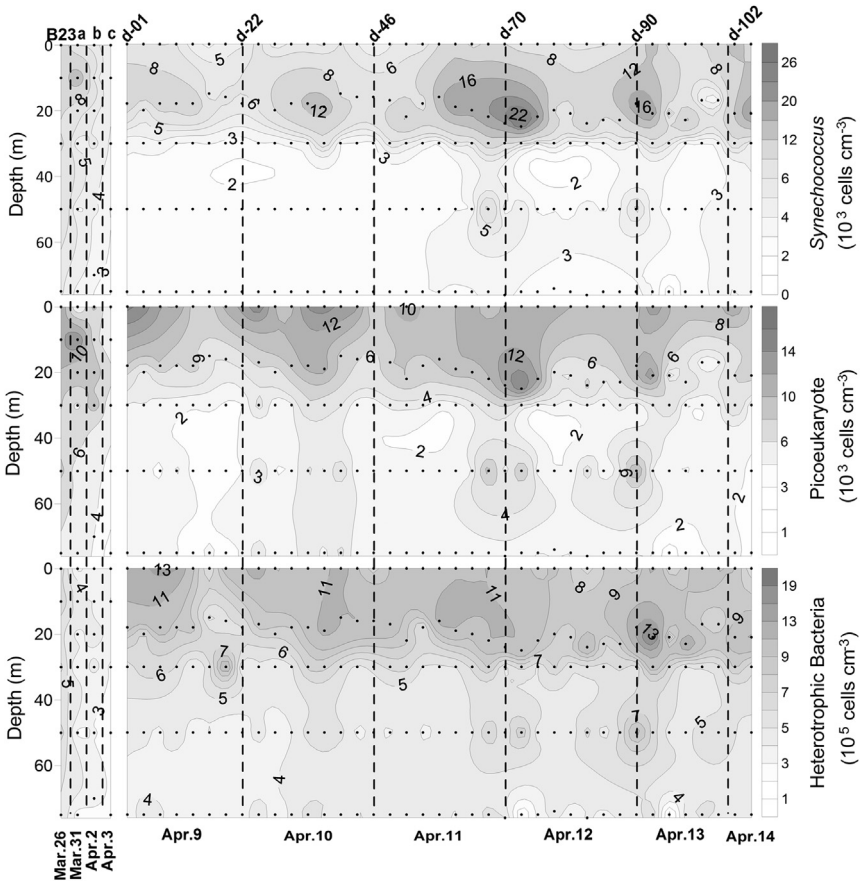

Fig. 12. Temporal changes in vertical distribution of picoplankton abundances (*Synechococcus* and picoeukaryote: $\times 10^3$ cells cm^{-3} and heterotrophic bacteria: $\times 10^5$ cells cm^{-3}) of Bloom B23 in 2009.

blooms has not been fully investigated. Many past researches are based on blooms induced in mesocosm experiments (Lewandowska and Sommer, 2010; Cunliffe et al., 2009; Saito et al., 2006; Larsen et al., 2001). Field observations of picoplankton abundance variation during natural blooms are important and yet limited. Nevertheless, many researches only focused on the heterotrophic component of picoplankton, i.e., heterotrophic bacteria, while the autotrophic picoplankton was neglected. In the present study we addressed both autotrophic and heterotrophic components of picoplankton in three phytoplankton field blooms investigated in 2007 and 2009. To our knowledge, this is the first report about autotrophic picoplankton in Yellow Sea spring bloom.

4.1. Autotrophic picoplankton

In our study, picophytoplankton abundance showed two different variation patterns. On the one hand, during two diatom blooms (Bloom 2007 and Bloom B20), *Synechococcus* and picoeukaryote abundances showed opposite trends to chl *a* variation. On the other hand, during the mixed dinoflagellate and diatom bloom (Bloom B23), both autotrophic picoplankton abundances showed a trend similar to that of chl *a* concentration.

An abrupt decrease, followed by an increase in *Synechococcus* and/or picoeukaryote abundance was observed during phytoplankton blooms in mesocosm and field studies (Cunliffe et al., 2009; Larsen et al., 2004; Martinez-Martinez et al., 2006; Šimek et al., 1995). Traditionally, large and small phytoplankton are viewed as having a critical growth dependence on inorganic nutrients. At low nutrient concentration, small phytoplankton cells can better uptake nutrients than large ones owing to their higher surface area to volume ratios (Morel et al., 1991; Chisholm, 1992). In our study, although the upper water column became nutrient depleted during the bloom periods (Figs. 5,8 and 11), the abundance decrease of *Synechococcus* and picoeukaryote was unlikely caused by nutrient limitation.

Temperature is an important factor affecting *Synechococcus* abundance (Li, 1998) and below 14 °C, *Synechococcus* abundance was shown to be directly related to temperature. However, in the survey of Bloom 2007 and Bloom B20, the *Synechococcus* abundance was not correlated with temperature, indicating a different forcing factor.

The low *Synechococcus* and picoeukaryote abundances during bloom periods could be attributed to protozoan gazing. The dominant role of heterotrophic nanoflagellates and ciliates as primary grazers of picoplankton in pelagic systems was well documented (Sherr and Sherr, 1988). During a summer phytoplankton abundance maximum in a eutrophic reservoir, high clearance rates of heterotrophic nanoflagellates and ciliates on picoplankton were observed (Šimek et al., 1995, 1997). In agreement, several on board experiments carried out by our co-workers during the spring bloom cruises also revealed high clearance rates. In an experiment during the Bloom 2007 cruise, after *Calanus sinicus* was added to graze the ciliates, *Synechococcus* abundance increased demonstrating a high grazing pressure of ciliates on *Synechococcus* (Zhang, unpublished data). Another grazing experiment was carried out during Bloom B20 and showed higher grazing pressure of heterotrophic nanoflagellates on *Synechococcus* during bloom than non-bloom situation (Table 3, data provided by Huang).

In contrast, during the mixed dinoflagellate and diatom bloom of Bloom B23, the abundance and depth integrated biomass *Synechococcus* and picoeukaryote was higher during bloom period

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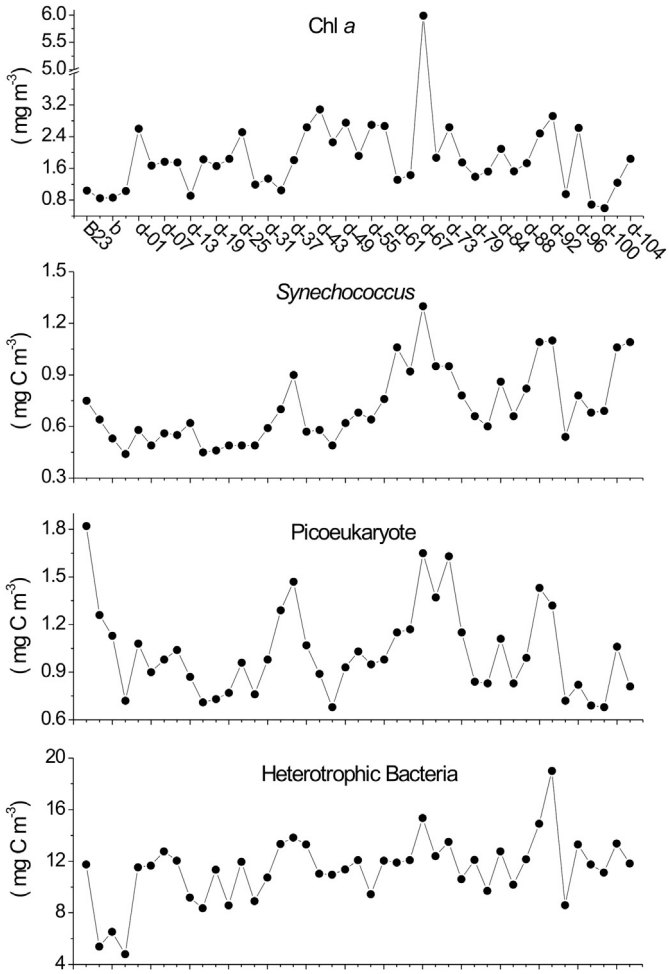

Fig. 13. Depth averaged chl *a* concentration (mg m^{-3}) and picoplankton biomasses (mg C m^{-3}) during Bloom B23 in 2009.

Table 3
Grazing of heterotrophic nanoflagellates (HNF) on *Synechococcus* during Bloom B20.

Bloom B20	HNF (10^3 cells cm^{-3})	*Synechococcus* (10^3 cells cm^{-3})	Grazing rate (cell HNF^{-1} h^{-1})	Grazing pressure (%)
Pre-bloom	0.79	9.8	0.23	16.50
Bloom	1.54	4.4	0.13	26.40

than pre-bloom, indicating a different control mechanism. Macro-ecological analysis demonstrated that picoplankton development was favored under stratification conditions, generally associated to low nutrient concentration and high temperature (Li, 2002). Unlike Bloom 2007 and Bloom B20, Bloom B23 occurred in stratified waters as supported by the vertical temperature distribution (Fig. 11). Thus stratification would explain the higher picoplankton abundance and biomass during bloom period in Bloom B23 than during the other blooms.

4.2. Heterotrophic bacteria

During three observed blooms, the variation of abundance and depth integrated biomass of heterotrophic bacteria followed that of chl *a* concentration, with highest values during bloom than pre- and post-bloom. Such pattern was largely reported in previous researches (Martinez-Martinez et al., 2006; Larsen et al., 2004; Hyun and Kim, 2003). Understandably, dissolved organic carbon (DOC) released by phytoplankton makes an important carbon source for the pelagic heterotrophic bacteria and drives their development while they are submitted to high grazing pressure from heterotrophic nanoflagellates and ciliates during bloom (Šimek et al., 1995, 1997). During a bloom in the Atlantic Ocean, it was estimated that about 1/3 of bacterial production was removed by grazers or other forms of mortality, the remaining 2/3 being accumulated as biomass (Ducklow et al., 1993). It is likely that the high DOC and grazing pressure co-affected the abundance of heterotrophic bacteria in Yellow Sea spring bloom.

5. Conclusion

We have investigated the picoplankton abundance variation during three spring phytoplankton blooms in the Yellow Sea. The results from field observation showed that *Synechococcus* and picoeukaryote abundances exhibited two different variation patterns during different blooms. However, in the three observed blooms, heterotrophic bacteria abundance and biomass presented similar increasing trends during the bloom periods. Ciliates and heterotrophic nanoflagellates grazing could be responsible for *Synechococcus* and picoeukaryote abundance and biomass decrease during bloom periods.

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