

Influence of the northern Yellow Sea Cold Water Mass on picoplankton distribution around the Zhangzi Island, northern Yellow Sea

ZHAO Li^{1,2}, ZHAO Yanchu^{1,2,3}, DONG Yi^{1,2}, ZHAO Yuan^{1,2*}, ZHANG Wuchang^{1,2}, XU Jianhong¹, YU Ying⁴, ZHANG Guangtao⁵, XIAO Tian^{1,2}

¹ CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

² Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

³ University of Chinese Academy of Sciences, Beijing 100864, China

⁴ Tianjin Bohai Sea Fisheries Research Institute, Tianjin 300457, China

⁵ Jiaozhou Bay Marine Ecosystem Research Station, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

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Abstract

Picoplankton distribution around the Zhangzi Island (northern Yellow Sea) was investigated by monthly observation from July 2009 to June 2010. Three picoplankton populations were discriminated by flow cytometry, namely *Synechococcus*, picoeukaryotes and heterotrophic prokaryotes. In summer (from July to September), the edge of the northern Yellow Sea Cold Water Mass (NYSCWM) resulting from water column stratification was observed. In the NYSCWM, picoplankton (including *Synechococcus*, picoeukaryotes and heterotrophic prokaryotes) distributed synchronically with extremely high abundance in the thermocline (20 m) in July and August (especially in August), whereas in the bottom zone of the NYSCWM (below 30 m), picoplankton abundance was quite low. *Synechococcus*, picoeukaryotes and heterotrophic prokaryotes showed similar response to the NYSCWM, indicating they had similar regulating mechanism under the influence of NYSCWM. Whereas in the non-NYSCWM, *Synechococcus*, picoeukaryotes and heterotrophic prokaryotes exhibited different distribution patterns, suggesting they had different controlling mechanisms. Statistical analysis indicated that temperature, nutrients (NO_3^- and PO_4^{3-}) and ciliate were important factors in regulating picoplankton distribution. The results in this study suggested that the physical event NYSCWM, had strong influence on picoplankton distribution around the Zhangzi Island in the northern Yellow Sea.

Key words: picoplankton, northern Yellow Sea Cold Water Mass, thermocline, Zhangzi Island

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1 Introduction

Since the discovery of cyanobacteria *Synechococcus* (Johnson and Sieburth, 1979; Waterbury et al., 1979) in the late 1970s, a number of studies have widely documented the ecological importance of picoplankton ($\leq 2 \mu\text{m}$, including *Synechococcus*, *Prochlorococcus*, picoeukaryotes and heterotrophic prokaryotes) in various marine environments, from the eutrophic estuaries to the oligotrophic oceans (Platt et al., 1983; Agawin et al., 2003; Pan et al., 2007; Lin et al., 2010). In the coastal waters, picoplankton is generally subjected to higher pressure on environmental variability (i.e., water-mass and tidal front influences) than open oceans (Calvo-Díaz and Morán, 2006; Mitbavkar et al., 2009). For instance in the Uchiumi Bay (Japan), abundance and composition of picophytoplankton was strongly influenced by the phys-

ical events of “Kyucho” (warm surface water) and bottom intrusion (Katano et al., 2005). In the South Australian continental shelf waters, picophytoplankton distribution was affected by local physical event (downwelling and dense water outflowing) (Van Dongen-Vogels et al., 2011).

The Yellow Sea is a semi-enclosed marginal sea in the Pacific, which connects to the East China Sea in the southern part and Bohai Sea in the northern part. It is quite shallow with an average depth of about 44 m. One of the most striking hydrological phenomenon is the existence of the Yellow Sea Cold Water Mass (YSCWM) during summer period. The YSCWM forms locally during the previous winter owing to the surface cooling and strong vertical mixing. In summer, the surface water temperature increased, a cold water mass is retained in the bottom zone and

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*Corresponding author, E-mail: yuanzhao@qdio.ac.cn

forms the YSCWM. It is characterized with low temperature showing a remarkable variation (5–12°C) and a rather constant salinity (31.5–32.5) (He et al., 1959; Su and Huang, 1995). The YSCWM can be recognized as northern and southern parts, with dissimilar characteristics. The northern YSCWM (NYSCWM) locates at the bottom layer within a small area of 37°–38.9°N and 121.3°–124°E, with low temperature ($T < 8^\circ\text{C}$) and salinity ($S < 32.5$) in the core. The southern YSCWM (SYSCWM) locates within a larger area of 34°–37°N and 121°–126°E, with relatively higher temperature ($T < 9^\circ\text{C}$) and salinity ($S > 32.5$) and more intensive thermocline in the core (Zhang et al., 2008; Bao et al., 2009; Yao et al., 2012; Xin et al., 2015).

The YSCWM has an important effect on marine ecosystems (Hur et al., 1999; Kang and Kim, 2008). The impact of the SYSCWM on picoplankton distribution has been reported by Li et al. (2006) and Zhao et al. (2011), showing low picoplankton abundance inside the SYSCWM. With dissimilar characteristics from SYSCWM, NYSCWM could have a different impact on picoplankton distribution. There has been only few reports about picoplankton variation in the northern Yellow Sea, mentioning that picoplankton abundance decreased sharply in the NYSCWM in summer (Bai et al., 2012). However, it is still unclear how the NYSCWM influenced picoplankton dynamics by changing stratification and nutrient supply. In this manuscript, we investigated the spatial and temporal dynamics of picoplankton around the Zhangzi Island in the northern Yellow Sea from July 2009 to June 2010. It is by far the first report tracking picoplankton variation during the formation to vanishment process of NYSCWM. Our objectives include: (1) clarifying the effect of NYSCWM on picoplankton distribution during summer period; (2) illustrating how NYSCWM influenced picoplankton dynamics around the Zhangzi Island in the northern Yellow Sea.

2 Materials and methods

2.1 Study area and sampling strategy

Thirteen stations around the Zhangzi Island in the northern Yellow Sea were investigated monthly from July 2009 to June 2010, except January and May 2010 (Fig. 1). According to previous studies (Zhang et al., 2008; Bao et al., 2009), Transect 6 (i.e.,

Sta. A6-B6-C6) was set as the NYSCWM influenced area. At each station, seawater was collected with a rosette multi-sampler equipped with 5 L Niskin bottles. The maximum depth was 55 m and the water samples were collected every 10 m from the surface to 2 m above the bottom, defining vertical profiles by up to five points.

2.2 Physical, chemical and biological measurements

Water temperature and salinity were determined with an AAQ 1183-1F CTD (conductivity, temperature, depth; Alec Electronics Co., Japan). Seawater subsamples (500 mL) for nutrient concentration analysis were filtered through Whatman GF/F filters and kept at -20°C until analysis in the laboratory. The concentrations of NO_3^- and PO_4^{3-} were determined using a QuAatroSFA Analyzer (Bran-Lubbe Co., Germany). The precision estimated with repeated measurements of selected samples was smaller than 3% in this study (Yin et al., 2013).

For chlorophyll *a* (Chl *a*) concentration analysis, another 500 mL subsamples were filtered on-board onto Whatman GF/F filters and kept at -20°C until analysis in the laboratory. Chl *a* was extracted with 90% acetone at -20°C in darkness for 24 h. The Chl *a* concentrations were determined using a Turner Designs Model 7200 fluorometer that was calibrated with pure Chl *a* from Sigma.

Subsamples (1 L) for ciliate enumeration were fixed with 1% acid Lugol's iodine solution and stored in a cool and dark place until analysis. Each sample was settled for at least 24 h. The supernatant was siphoned out and 150 mL sample was left. The concentrated sample (20–25 mL) was settled in an Utermöhl counting chamber for 24 h and enumerated using an inverted microscope (Olympus IX51) at 100× or 200× (Yu et al., 2013).

2.3 Picoplankton enumeration

Seawater subsamples (4 mL) for picoplankton enumeration were fixed on-board with paraformaldehyde (final concentration 1%) immediately, kept at room temperature for 15 min, and then freeze-trapped in liquid nitrogen until laboratory analysis within two months after the cruise.

A FACSVantage SE (Becton Dickinson, USA) flow cytometer equipped with a water-cooled Argon laser (488 nm, 1 W, Coher-

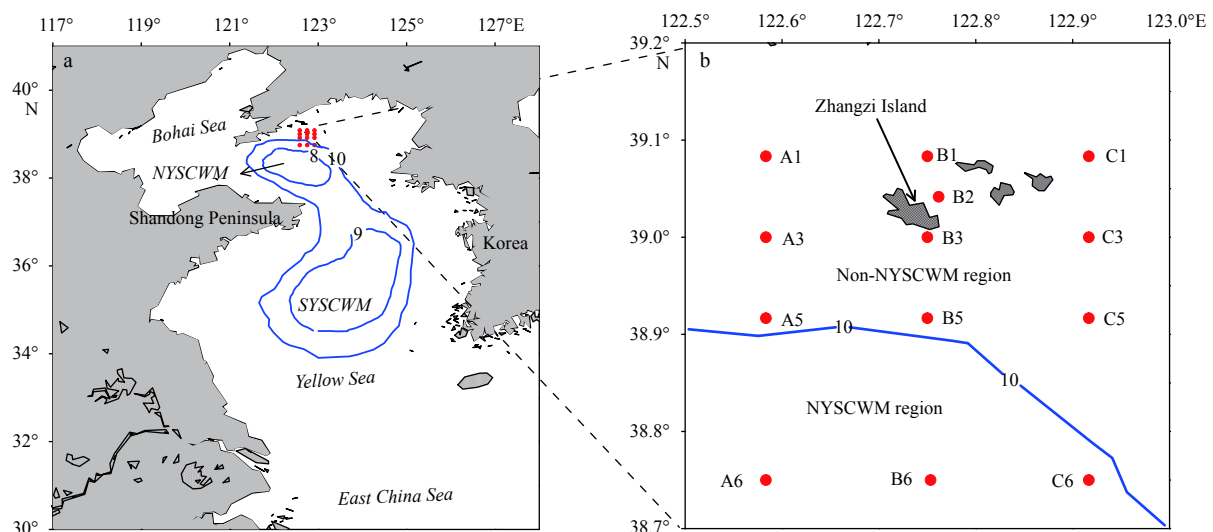


Fig. 1. Sampling stations around the Zhangzi Island in the northern Yellow Sea. The blue lines were the bottom temperature ($^\circ\text{C}$) isobaths showing the range of Yellow Sea Cold Water Mass in summer (Zhang et al., 2008; Bao et al., 2009).

ent, USA) was used for picoplankton analysis at the single cell level. Five optical signals were recorded: forward scatter (FSC, related to cell size), side scatter (SSC, related to cell structure and shape), green fluorescence ((530±15) nm), orange fluorescence ((585±21) nm, related to phycoerythrin) and red fluorescence ((695±20) nm, related to Chl *a*).

Before analysis, seawater samples were thawed at room temperature in dark (about 20 min). For each sample, 2 µm fluorescent beads (Polysciences) were used as internal standard for the instrument set-up. When analyzing autotrophic picoplankton, red fluorescence was set as the trigger to eliminate signals from heterotrophic prokaryotes and inorganic particles. Two major autotrophic picoplankton groups: *Synechococcus* (SYN) and picoeukaryotes (PEUK) were resolved based on the signals of side scatter, orange and red fluorescences. *Prochlorococcus* was not detected in this study.

For the determination of heterotrophic prokaryote (HP) abundance, seawater samples were diluted five folds with TE buffer (Tris-EDTA, 100 mmol/L Tris-Cl, 10 mmol/L EDTA, pH=8.0, Sigma), and then stained with the nucleic acid dye SYBR Green I (molecular probes) (final dilution 10⁻⁴, v/v). Samples were stained and kept in the dark at room temperature for 20 min, then analyzed with flow cytometry for 30 s. Heterotrophic prokaryote cell-groups were resolved on the basis of their green fluorescence ((530±15) nm) and scatter properties.

Flow cytometric data were collected and analyzed with CellQuest software (Version 3.3, Becton Dickinson). Since each 4 mL subsample for flow cytometry analysis was taken from 1 L of homogeneous seawater of a Niskin bottle, the derived cell abundance are representative of that initial volume.

2.4 Standard of thermocline

According to Zou et al. (2001), when water depth less than 200 m, the minimum standards for thermocline and halocline are $I_t = \Delta T / \Delta Z$ (strength of thermocline) = 0.2°C/m and $I_s = \Delta S / \Delta Z$ (strength of halocline) = 0.1 m⁻¹, respectively.

2.5 Data analysis

Seasonal variations of the data were drawn with the software Origin (Version 8.5, OriginLab Corporation). The contour plot graphs were generated using the software Surfer (Version 11, Golden Software). Statistical analysis was conducted using SPSS (Version 19, IBM SPSS Statistics). Spearman rank-order correlation analysis was used to estimate potential relationships between picoplankton abundance and environmental variables (temperature, salinity, NO₃⁻, PO₄³⁻, Chl *a* concentration and ciliate abundance). Picoplankton and ciliate abundances used for statistical analysis were log-transformed to satisfy a roughly normal distribution.

3 Results

3.1 Physicochemical conditions

In this study, we use the water temperature lower than 12°C in summer as definition of the NYSCWM. The bottom distribution of temperature and salinity in our study revealed the existence of NYSCWM in July, August and September, with temperature lower than 12°C (Fig. 2). The NYSCWM was most prosperous in July, covering 2/3 of the survey area, then diminished in August and September. In October, bottom temperature was higher than 12°C, indicating the vanishing of NYSCWM. Since Transect 6 was mostly covered by the NYSCWM in summer, it was con-

sidered as NYSCWM region and the rest stations as non-NY-SCWM region (Fig. 1). NO₃⁻ and PO₄³⁻ concentrations in the bottom waters were higher in the NYSCWM than in the non-NY-SCWM regions.

3.2 Spatial-temporal variation of picoplankton abundance in the NYSCWM region

Along Transect 6, water column stratification was observed from June to October. In July and August, pronounced thermocline was found between 10 m and 20 m depth with $I_t > 1^\circ\text{C}/\text{m}$. Halocline also existed in July, showing $I_s > 0.1 \text{ m}^{-1}$ between 10 m and 20 m depth. In September, thermocline was less intensive ($I_t > 0.6^\circ\text{C}/\text{m}$) and occurred between 20 m and 30 m depth (Figs 3a and b). In June and July, Chl *a* concentrations were higher below 10 m depth whereas in August, September and October, higher concentrations of Chl *a* were observed in the upper 20 m layer. From June to October, nutrient NO₃⁻ increased gradually with depth and ciliate was more abundant in the upper 20 m layer (Fig. 3a).

In June, September and October, picoplankton was more abundant in the upper layer and decreased gradually with depth (Fig. 3a). In July and August, SYN, PEUK and HP abundances had same vertical pattern with maximum abundances in the thermocline (20 m) and minimum abundances in the bottom zone of the NYSCWM (below 30 m) (Figs 3a and c). Picoplankton abundance in August was much higher than that in July in the thermocline region. In July, the maximum abundances of SYN, PEUK and HP were 47.04×10³ cells/mL (20 m, Sta. B6), 15.84×10³ cells/mL (20 m, Sta. C6) and 6.37×10⁵ cells/mL (10 m, Sta. C6), respectively. In August, the maximum abundances of SYN and PEUK (421.28×10³ cells/mL and 30.15×10³ cells/mL, respectively) were found in the thermocline (20 m) of Sta. A6. SYN and PEUK abundances in the thermocline was 1 or 2 order of magnitude higher than the rest of the water column. The maximum abundance of HP (18.08×10⁵ cells/mL) was observed in the thermocline (20 m) of Sta. B6. HP abundance in the thermocline was one order of magnitude higher than the rest of the water column (Figs 3a and c).

From June to October, water column stratification intensified from Stas B1 to B6 (Fig. 4). The NYSCWM was observed below 30 m depth of Sta. B6 in July, August and September. In June, higher concentrations of Chl *a* and nutrient NO₃⁻ were both observed at the bottom of Sta. B6. Ciliate was more abundant at the surface of Stas B1 and B5. SYN was more abundant in the upper 20 m of Sta. B6. PEUK showed maximum abundance in the upper 10 m of Sta. B1. HP abundance varied less obvious than SYN and PEUK, with higher abundance in the middle layer (10–30 m) of Sta. B3. In July, the NYSCWM was observed below 30 m of Sta. B5 and 20 m of Sta. B6. Higher concentration of Chl *a* was found at 20 m of Sta. B3. Nutrient NO₃⁻ exhibited higher concentrations inside the NY-SCWM. Ciliate was more abundant at 10 m of Stas B2 and B6. SYN was more abundant in the central part of Transect B and the maximum abundance (86.26×10³ cells/mL) was observed at 20 m of Sta. B5. PEUK exhibited similar pattern as SYN with higher abundances in the middle layer of Transect B. HP were more abundant in the non-NYSCWM region. In August, bottom temperature was higher than 11°C, implying the less obvious NY-SCWM than in July. Chl *a* exhibited much higher concentrations (>4 µg/L) at the surface of Stas B1, B2 and B3. In contrast, nutrient NO₃⁻ showed higher concentrations at the bottom of Stas B5 and B6. Higher abundance of ciliate was observed at the surface of Stas B1 and B5. SYN and PEUK had extremely high abund-

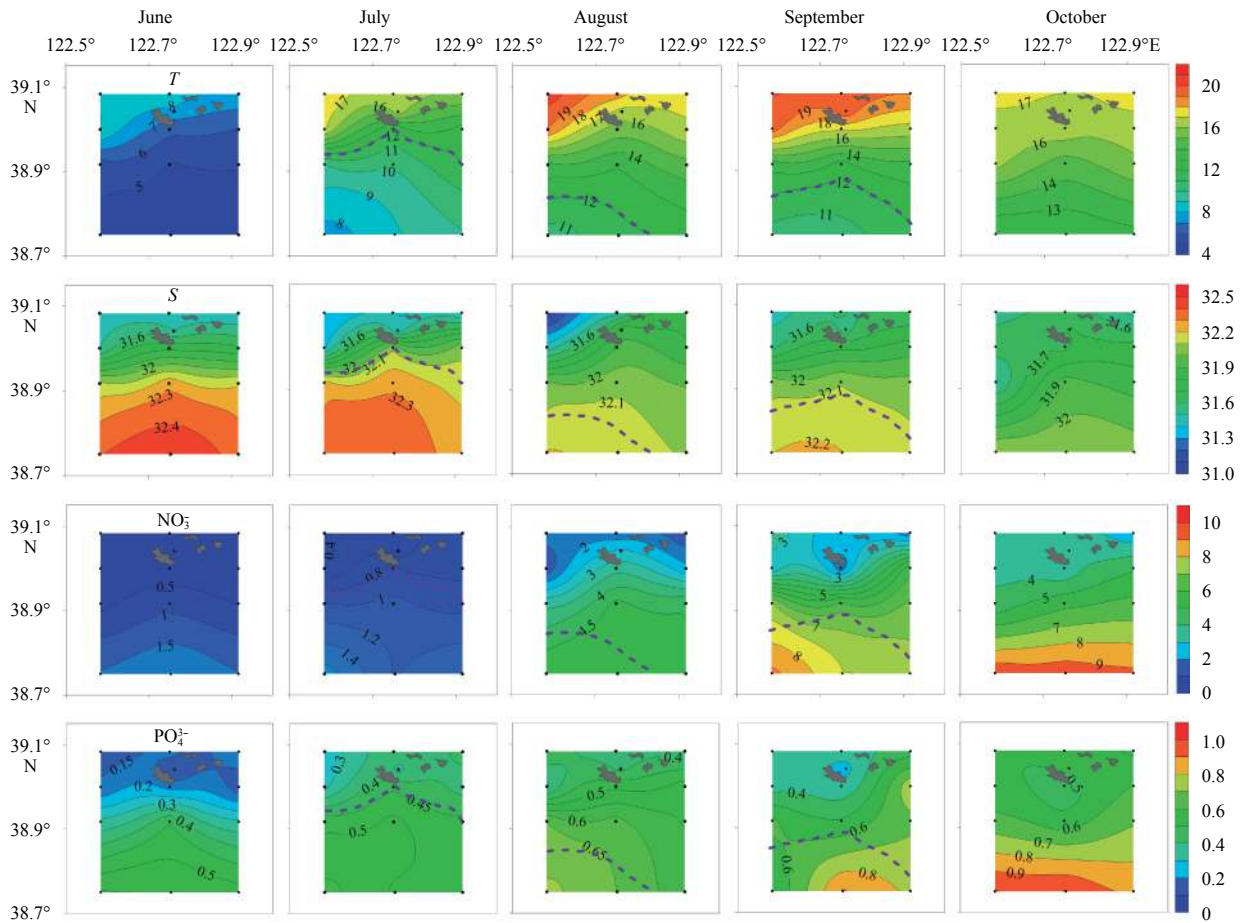


Fig. 2. Bottom distribution of temperature (T , °C), salinity (S) and nutrient concentrations (NO_3^- , PO_4^{3-} ; $\mu\text{mol/L}$) from June to October, showing the existence-fading process of NYSCWM. The dashed line was $T=12^\circ\text{C}$ showing the boundary of the NYSCWM.

ances at Sta. B6 (20 m , 355.48×10^3 cells/mL and 28.53×10^3 cells/mL, respectively). HP were more abundant above 20 m depth. The maximum abundance (18.08×10^5 cells/mL) was also observed at 20 m of Sta. B6. In September and October, picoplankton showed similar distribution pattern as temperature and salinity, which was more abundant above 30 m depth and decreased gradually below 30 m (Fig. 4).

3.3 Seasonal comparison of picoplankton abundance between non-NYSCWM and NYSCWM stations

The coastal Sta. B2 was not influenced by the NYSCWM, in contrast with Sta. B6 in the open-water (Fig. 4). At Sta. B2, the vertical distributions of temperature and salinity presented well-mixed water columns almost the whole year. A weak stratification occurred in August and September. A sharp decrease in salinity was observed in the upper 20 m in June, July and August (Fig. 5). Chl a concentration reached its maximum value (10 m , $4.93\text{ }\mu\text{g/L}$) in August. During the rest of the year, Chl a concentrations were rather low ($<2\text{ }\mu\text{g/L}$). Nutrients NO_3^- exhibited lower concentrations from March to June and higher concentrations from October to February. Ciliate exhibited higher abundances in September and October, and lower abundances in the rest of the year. The distribution pattern of SYN was synchronized with temperature. SYN abundance sustained extremely low level ($<0.2 \times 10^3$ cells/mL) from March to June, and then reached the maximum abundance (63.06×10^3 cells/mL) at the surface in September when temperature was the highest. PEUK presented a

different distribution pattern, exhibiting higher abundances in June and October and lower abundances in March and April. HP showed higher abundances in August, September and November and lower abundances in December, February and April.

At Sta. B6, a well-mixed water column was observed from November to April. Thereafter, water column stratification built up showing the thermocline at about 20 m depth from July to September. Due to the water column stratification in summer, the bottom NYSCWM was observed with temperature lower than 12°C (Figs 2 and 5). Chl a concentration reached a high value ($6.53\text{ }\mu\text{g/L}$) at the bottom in March, but remained low ($<0.5\text{ }\mu\text{g/L}$) from surface to bottom during the rest of the year, especially in the NYSCWM region (Chl $a < 0.1\text{ }\mu\text{g/L}$). Nutrient NO_3^- showed lower concentrations ($\text{NO}_3^- < 0.5\text{ }\mu\text{mol/L}$) in the upper 20 m from April to September and higher concentrations in the bottom zone of the NYSCWM. The maximum concentration of NO_3^- ($9.62\text{ }\mu\text{mol/L}$) were observed at the bottom in October. Ciliate showed higher abundances in April (10 m , 25.05×10^3 ind./L) and July (10 m , 20.52×10^3 ind./L). It was less abundant in the NYSCWM ($<5 \times 10^3$ ind./L). SYN, PEUK and HP had similar distribution pattern with extremely high abundances (355.48×10^3 cells/mL, 28.53×10^3 cells/mL and 18.08×10^5 cells/mL, respectively) at 20 m depth in August where the thermocline and halocline were well established. From October to June, SYN and PEUK sustained low abundances, especially in March and April. In the bottom zone of the NYSCWM, picoplankton abundance was quite low (Fig. 5).

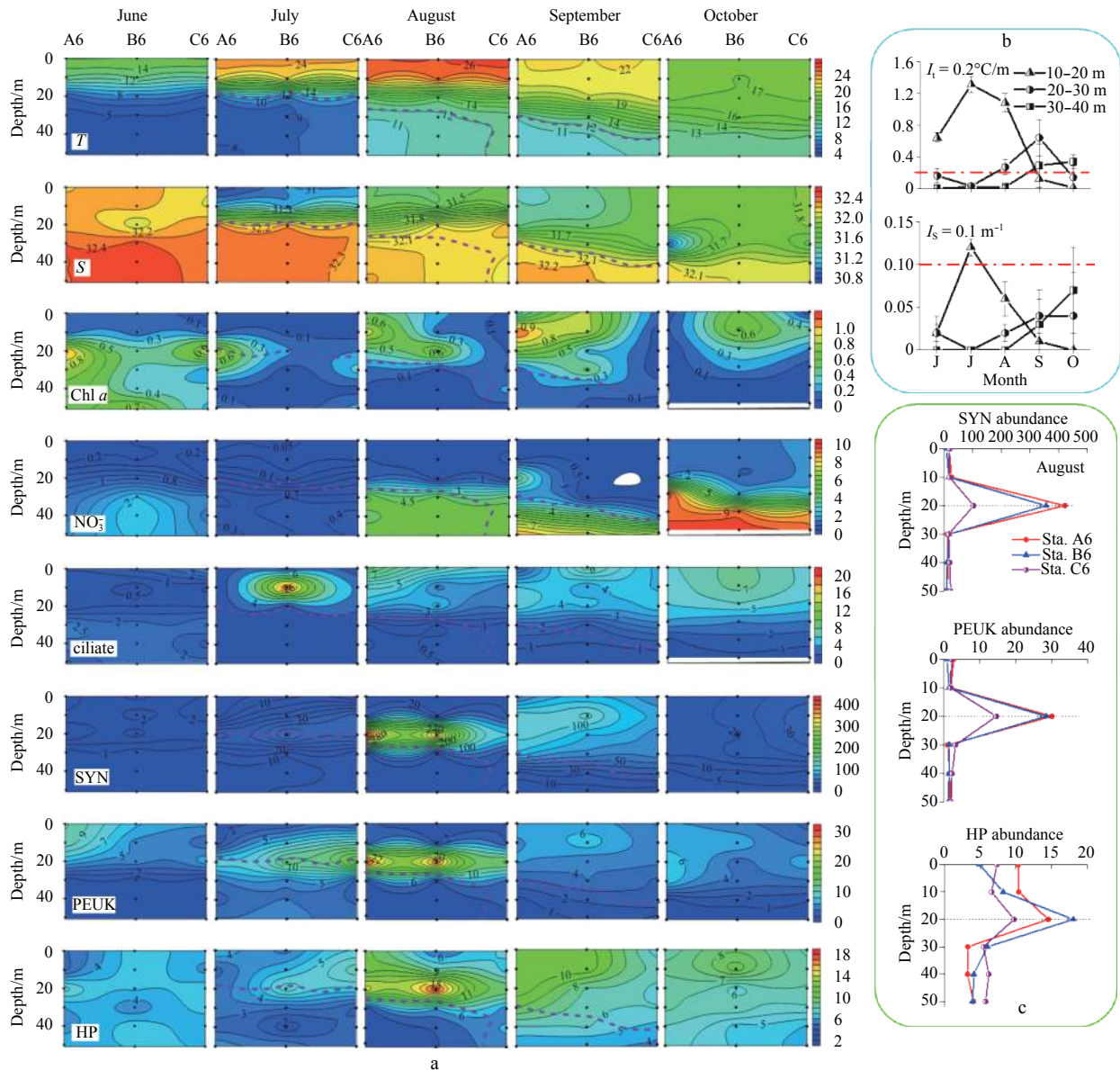


Fig. 3. Vertical distribution of temperature (T , $^\circ\text{C}$), salinity (S), Chl a ($\mu\text{g/L}$), nutrient concentrations (NO_3^- , $\mu\text{mol/L}$), ciliate abundances ($\times 10^3$ ind./L), *Synechococcus* (SYN, $\times 10^3$ cells/mL), picoeukaryotes (PEUK, $\times 10^3$ cells/mL) and heterotrophic prokaryotes abundances (HP, $\times 10^5$ cells/mL) along Transect 6 from June to October (the dashed line was $T=12^\circ\text{C}$ showing the boundary of the NYSCWM) (a); the strength of thermocline (I_t , $^\circ\text{C/m}$) and halocline (I_s , m^{-1}) from June to October (b); and the vertical distribution of *Synechococcus* (SYN, $\times 10^3$ cells/mL), picoeukaryotes (PEUK, $\times 10^3$ cells/mL) and heterotrophic prokaryotes (HP, $\times 10^5$ cells/mL) abundances at Stas A6, B6 and C6 in August (c).

3.4 Seasonal variation of picoplankton water column integrated abundance between non-NYSCWM and NYSCWM regions

The water column integrated abundance of SYN showed similar seasonal pattern in the non-NYSCWM (Transects 1, 3 and 5) and NYSCWM regions (Transect 6), except in summer. The maximum abundance in the NYSCWM was observed in August, one month earlier than that in the non-NYSCWM area. In August, SYN in the NYSCWM region ($(3.56 \pm 1.59) \times 10^{12}$ cells/ m^2) was almost three times higher than that in the non-NYSCWM region ($(0.97 \pm 0.31) \times 10^{12}$ cells/ m^2) (Fig. 6a).

Similar to SYN, PEUK in the NYSCWM exhibited highest water column integrated abundance ($(3.15 \pm 0.06) \times 10^{11}$ cells/ m^2) in August. In the non-NYSCWM, the maximum abundance ($(2.71 \pm 10.7) \times 10^{11}$ cells/ m^2) occurred in June, two month earlier

than that in the NYSCWM. During the rest of the year, PEUK was slightly higher in the NYSCWM region than the non-NYSCWM region (Fig. 6b).

Unlike the autotrophic picoplankton, HP integrated abundance exhibited similar distribution patterns between the non-NYSCWM and NYSCWM regions, showing major peaks ($(3.78 \pm 0.31) \times 10^{13}$ cells/ m^2 and $(3.41 \pm 0.48) \times 10^{13}$ cells/ m^2 , respectively) in August and minor peaks in March. HP in the NYSCWM were slightly higher than in the non-NYSCWM during the rest of the year except July (Fig. 6c).

3.5 Relationship between picoplankton abundance and environmental variables

Relationships between picoplankton abundance and envir-

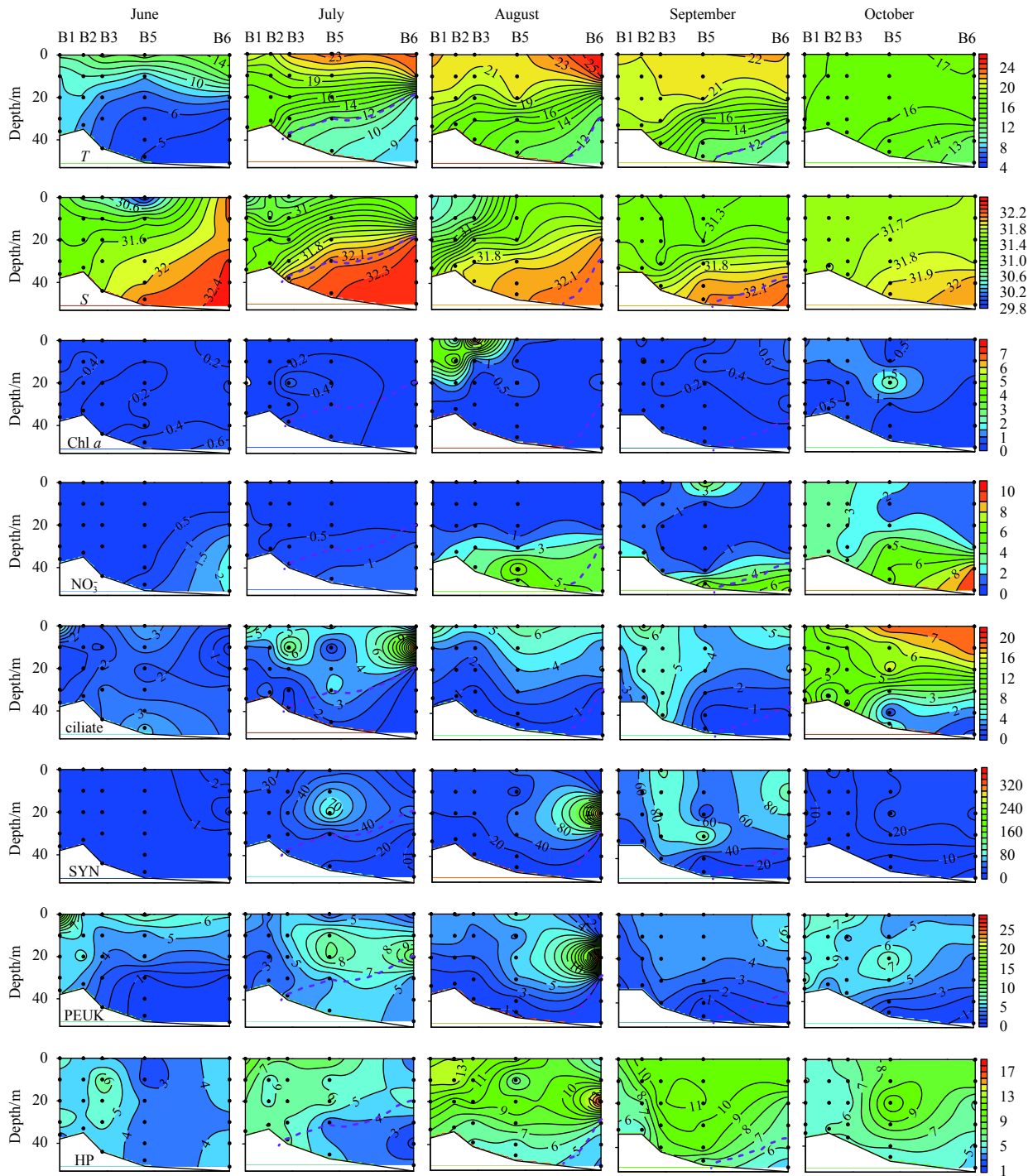


Fig. 4. Vertical distribution of temperature (T , $^{\circ}\text{C}$), salinity (S), $\text{Chl } a$ ($\mu\text{g/L}$), nutrient concentrations (NO_3^- , $\mu\text{mol/L}$), ciliate abundances ($\times 10^3$ ind./L), *Synechococcus* (SYN, $\times 10^3$ cells/mL), picoeukaryotes (PEUK, $\times 10^3$ cells/mL) and heterotrophic prokaryote abundances (HP, $\times 10^5$ cells/mL) along Transect B from June to October. The dashed line was $T=12^{\circ}\text{C}$ showing the boundary of the NYSCWM.

onmental variables in the NYSCWM (Transect 6) in summer (July–September) were performed using Spearman correlation analysis (Table 1). In the NYSCWM region in summer, abundances of SYN, PEUK and HP were positively correlated with each other. They were also positively correlated with $\text{Chl } a$ and ciliate, and negatively correlated with nutrients NO_3^- and PO_4^{3-} . Besides, HP abundances were also positively correlated with temperature and negatively correlated with salinity.

4 Discussion

In previous studies, the contours of 10°C were taken as the boundary of NYSCWM (Zhang et al., 2008; Xin et al., 2015). In this study, it is reasonable to use the relatively broad definition of the NYSCWM with water temperature lower than 12°C since (1) the YSCWM is characterized with low temperature showing a remarkable variation ($5\text{--}12^{\circ}\text{C}$) (He et al., 1959; Su and Huang, 1995), (2) the temperature of NYSCWM has been reported in-

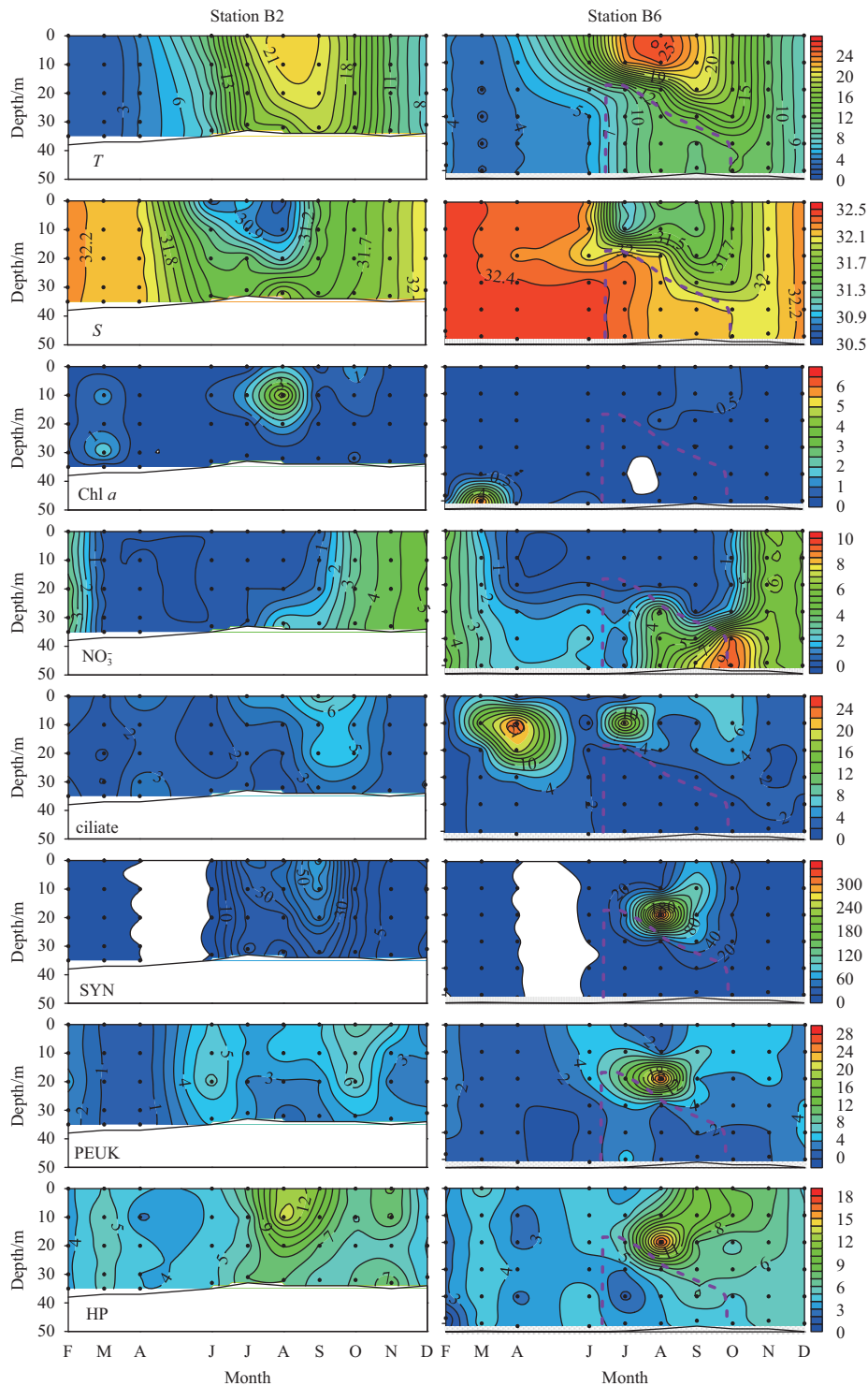


Fig. 5. Seasonal changes in the vertical distribution of temperature (T , °C), salinity (S), Chl a ($\mu\text{g/L}$), nutrient concentrations (NO_3^- , $\mu\text{mol/L}$), ciliate abundances ($\times 10^3$ ind./L), *Synechococcus* (SYN, $\times 10^3$ cells/mL), picoeukaryotes (PEUK, $\times 10^3$ cells/mL) and heterotrophic prokaryotes (HP, $\times 10^5$ cells/mL) at Sta. B2 (non-NYSCWM) and Sta. B6 (NYSCWM).

creasing slightly from 1976 to 1999 (Li et al., 2015), and (3) the Zhangzi Island locates on the edge of the NYSCWM.

4.1 Impact of NYSCWM on physical, chemical and biological conditions

The YSCWM is a typically seasonal water mass in the Yellow Sea. It forms locally in winter owing to the surface cooling and

strong vertical mixing. In spring, when surface water temperature increases, the cold water is retained in the bottom zone. In summer, this cold water mass flows southward under the thermocline. Extremely high intensity of the YSCWM occurs between July and August. In late autumn and winter, the YSCWM disappear because of the strong vertical mixing (Wang et al., 2000; Li et al., 2006).

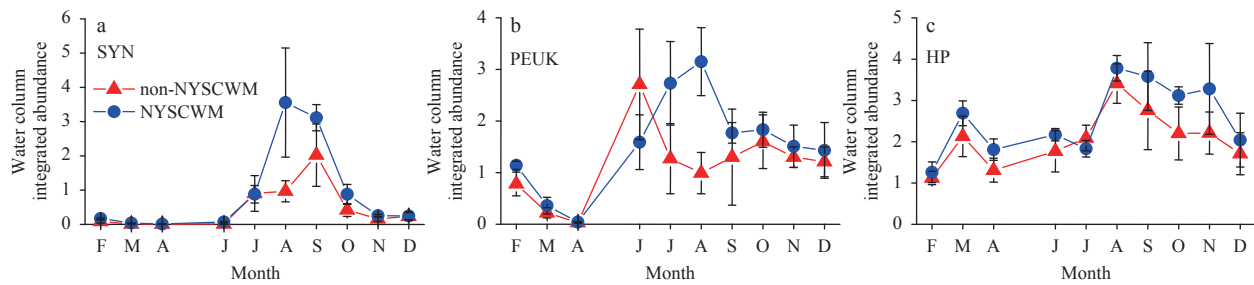


Fig. 6. Seasonal comparison of water column integrated abundances of *Synechococcus* (SYN, $\times 10^{12}$ cells/m²) (a), picoeukaryotes (PEUK, $\times 10^{11}$ cells/m²) (b) and heterotrophic prokaryotes (HP, $\times 10^{13}$ cells/m²) (c) in the non-NYSCWM and NYSCWM regions around the Zhangzi Island in the northern Yellow Sea.

Table 1. Spearman's rank correlation coefficient between environmental and biological variables and picoplankton abundances along Transect 6 in July, August and September

| Spearman's rho | Lg PEUK | Lg HP | <i>T</i> | <i>S</i> | NO ₃ ⁻ | PO ₄ ³⁻ | Chl <i>a</i> | Lg ciliate |
|----------------|---------|---------|----------|----------|------------------------------|-------------------------------|--------------|------------|
| Lg SYN | 0.684** | 0.757** | | | -0.411** | -0.630** | 0.468** | 0.664** |
| Lg PEUK | | 0.342* | | | -0.528** | -0.445** | 0.287* | 0.368** |
| Lg HP | | | 0.589** | -0.532** | -0.346* | -0.664** | 0.411** | 0.656** |

Note: ** Correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed). *T* represents temperature, *S* salinity, SYN *Synechococcus*, PEUK picoeukaryotes, and HP heterotrophic prokaryotes.

In this study, the NYSCWM was observed in summer (July–September) with temperature lower than 12°C. Pronounced thermocline and halocline were observed between 10 and 30 layer, especially in July and August (Figs 2 and 3). Chlorophyll *a* showed high concentration in the upper layer and low concentration in the bottom zone. The maximum chlorophyll *a* concentration occurred above the thermocline layer (10–20 m), perhaps resulting from the coaction of thermocline and intensive photosynthesis due to subsurface chlorophyll maximum in the same layer. In the bottom zone, chlorophyll *a* was the lowest because of the low temperature (<12°C) and insufficient light (Fig. 3).

Nitrogen was considered as the proximal limiting factor for primary production (Davey et al., 2008). In the NYSCWM, nutrient NO₃⁻ was almost depleted (<0.5 μmol/L) by phytoplankton above the thermocline, but enriched in the bottom zone of the NYSCWM through organism decomposition. The existence of stratification prevented the exchange of nitrate from the bottom to the surface.

4.2 Impact of NYSCWM on picoplankton distribution

Our study is by far the first report tracking picoplankton variation during the formation to vanishment process of NYSCWM. The impact of YSCWM on picoplankton distribution has been reported by Li et al. (2006) and Zhao et al. (2011) in the southern Yellow Sea and Bai et al. (2012) in the northern Yellow Sea in summer. Li et al. (2006) reported the minimum biomass of SYN and HP in the SYSCWM. Zhao et al. (2011) observed relatively low abundance of picoplankton at the surface and bottom (in the SYSCWM). Bai et al. (2012) found low abundance of SYN and PEUK in the NYSCWM, whereas no significant variations of HP were observed between NYSCWM and non-NYSCWM waters.

In this study, pronounced thermocline was observed in July and August. Picoplankton abundance was much higher in the thermocline (20 m). Especially in August, extremely high abundances of all three picoplankton groups (SYN, PEUK and HP) were observed (Figs 3–5). Whereas in the bottom zone of the NYSCWM, minimum abundance of picoplankton was detected (Fig. 3c). Extremely high abundance in the thermocline was not common in previous studies (Li et al., 2006; Zhao et al., 2011; Bai et

al., 2012). How NYSCWM influenced on picoplankton dynamics was still unclear, it is necessary to address the regulating factors on picoplankton distribution.

Temperature is considered an important factor regulating picoplankton variations (Agawin et al., 2000). In colder water ($T < 14^\circ\text{C}$), temperature was the dominant factor in SYN distribution (Li, 1998). In this study, temperature in the thermocline was higher than 14°C, indicating temperature was not the limiting factor. Whereas in the bottom zone of the NYSCWM, temperature was lower than 12°C, turning out temperature was the dominant factor on picoplankton distribution. Besides temperature, nutrient supply is another important factor influencing picoplankton distribution (Agawin et al., 2000; Moisan et al., 2010). Although the stratification prevented the exchange of nutrient from the bottom to the surface, in the thermocline layer nutrient was sufficient to supply the growth of picoplankton (Fig. 3). In the bottom zone of the NYCWM, even though nutrient was sufficient, the effect of low temperature seemed to be more important than nutrient supply. Ciliate might be another important controlling factor on picoplankton distribution. Ciliate and heterotrophic nanoflagellates have been recognized as the primary consumers of picoplankton (Tsai et al., 2008; Guo et al., 2014). In the present study, ciliate was less abundant in the thermocline, indicating lower pressure of predating on picoplankton variations. In the bottom zone of the NYSCWM, minimum abundance of ciliate was observed, suggesting ciliate was not important in controlling picoplankton distribution.

In the thermocline, with warmer temperature, sufficient nutrient supply and lower pressure of predating, the environment of thermocline was suitable for the growth of picoplankton, which explains why such high abundance of picoplankton occurring in the thermocline. In the bottom zone of the NYSCWM, in spite of sufficient nutrient supply and low predator pressure, low temperature ($T < 12^\circ\text{C}$) was the dominant factor on picoplankton distribution.

4.3 Seasonal comparisons between non-NYSCWM and NYSCWM

Around the Zhangzi Island, stations not influenced by the NYSCWM (namely non-NYSCWM) showed a clear seasonality in

picoplankton abundance (Fig. 5). SYN, PEUK and HP exhibited different distribution patterns, which was very common in previous studies. Seasonal dynamics of SYN abundance in this study was similar to previous reports in the coastal waters of Japan (Shimada et al., 1995; Katano et al., 2005), coastal waters of Mediterranean (Agawin et al., 1998; Bec et al., 2005; Charles et al., 2005), Bay of Biscay (Calvo-Díaz and Morán, 2006; Morán, 2007), Bedford Basin (Li, 1998), western English Channel (Tarran and Bruun, 2015) and Levantine Basin shelf waters (Uysal and Köksalan, 2006) (Table 2). A strong relationship between temperature and SYN was observed in this study, suggesting the importance of temperature in regulating SYN distribution (Fig. 7a), which has been conformed in previous studies (Agawin et al., 1998; Moisan et al., 2010). The seasonality of PEUK abundance was more complicated than SYN. PEUK reached maximum abundance in June and low abundances were observed in March

and April (Fig. 7b). Our result agreed with previous observations in the southern Yellow Sea (Zhao et al., 2011) and Iwanai Bay (Miyazono et al., 1992) (Table 2). Negative relationship between PO_4^{3-} and PEUK was observed, indicating the importance of nutrient supply on PEUK distribution (Fig. 7b). HP showed major peak in August and minor peak in March (Fig. 7c). Seasonal variations of HP and Chl *a* overlapped (Fig. 7c), implying that HP relied on the released substrate by phytoplankton (Garneau et al., 2008). In the non-NYSCWM, SYN, PEUK and HP distributed diversely and they were influenced by different factors, indicating they may have different controlling mechanisms.

In the NYSCWM, the thermocline provided a suitable environment (warm water, sufficient nutrient supply and low predating pressure) for the growth of picoplankton, SYN, PEUK and HP exhibited similar distribution patterns. It seemed they had similar controlling mechanism under the influence of NYSCWM.

Table 2. Previous studies on seasonal variations of *Synechococcus* (SYN, $\times 10^3$ cells/mL), picoeukaryotes (PEUK, $\times 10^3$ cells/mL) and heterotrophic prokaryotes abundance (HP, $\times 10^5$ cells/mL) reported in coastal waters

| Study area | Study period | SYN/ 10^3 cells·mL ⁻¹ | | PEUK/ 10^3 cells·mL ⁻¹ | | HP/ 10^5 cells·mL ⁻¹ | | Method | Reference |
|---------------------------------|----------------|------------------------------------|--------------|-------------------------------------|--------------|-----------------------------------|--------------|--------|-----------------------------|
| | | Min | Max | Min | Max | Min | Max | | |
| Iwanai Bay (Hokkaido, Japan) | 1989.4–1990.3 | 0.14 (Dec.–Mar.) | 190 (Jun.) | 0.04 (Dec.–Mar.) | 4.3 (May) | | | EFM | Miyazono et al. (1992) |
| Mediterranean coastal lagoon | 1999.2–2000.1 | <0.3 (Nov.–Apr.) | 8.2 (Jul.) | 5.2 (Sep.) | 90.8 (Apr.) | | | FCM | Bec et al. (2005) |
| NW Mediterranean shallow bay | 2000.8–2001.7 | <2 (Dec.–Apr.) | 95 (Sep.) | <1 (Dec.) | 21 (Jan.) | | | FCM | Charles et al. (2005) |
| Bay of Biscay | 2003.1–2003.12 | <1 (Apr.) | 106 (Jul.) | 1.6 (Aug.) | 32.6 (Jul.) | | | FCM | Morán (2007) |
| Western English Channel | 2007–2013 | 0.1 (May) | 120 (Sep.) | 0.2 (Aug.) | 80 (Apr.) | | | FCM | Tarran and Bruun (2015) |
| Suruga Bay, Japan | 1992.5–1993.10 | <1 (Apr.) | 220 (Sep.) | | | | | FCM | Shimada et al. (1995) |
| Bay of Blane (NW Mediterranean) | 1995.1–1997.1 | 0.5 (Feb.) | 70 (Aug.) | | | | | EFM | Agawin et al. (1998) |
| Bay of Biscay | 2002.4–2003.4 | <0.1 (Mar.–Apr.) | 149 (Sep.) | | | | | FCM | Calvo-Díaz and Morán (2006) |
| Levantine Basin shelf waters | 1998.1–1999.1 | 3 (Jan.) | 160 (Sep.) | | | | | EFM | Uysal and Köksalan (2006) |
| Uchiumi Bay, Japan | 2002.3–2002.10 | | 154.9 (Aug.) | <10 (Jun., Aug., Sep.) | 93.9 (May) | | | FCM | Katano et al. (2005) |
| Bay of Marseille | 1997.9–1999.10 | | 70 (Mar.) | | 30 (Jan.) | | | FCM | Grégori et al. (2001) |
| Bedford Basin | 1991.1–1998.1 | <0.01 (Jun.) | 445 (Sep.) | | | 3.2 (Feb.) | 74.5 (Jun.) | EFM | Li (1998) |
| Northern Gulf of Bothnia | 1989.3–1990.4 | | | | | <2 (Feb.) | 25 (July) | EFM | Wikner and Hagström (1991) |
| NW Mediterranean Franklin Bay | 1999.1–2000.1 | | | | | 1.4 (Jan.) | 11 (April) | EFM | Lemée et al. (2002) |
| Franklin Bay | 2003.11–2004.8 | | | | | 1 (Feb.) | 17 (Jul.) | EFM | Garneau et al. (2008) |
| Blanes Bay (NW Mediterranean) | 2005.5–2007.4 | | | | | 4.6 (Aug.) | 16 (May) | EFM | Boras et al. (2009) |
| This study | 2009.7–2010.6 | 0.08 (Mar.) | 421 (Aug.) | 0.25 (Mar.) | 30.15 (Aug.) | 1.83 (Feb.) | 18.08 (Aug.) | FCM | |

Note: SYN represents *Synechococcus*, PEUK picoeukaryotes, HP heterotrophic prokaryotes, EFM epifluorescence microscopy, and FCM flow cytometry.

5 Conclusions

Around the Zhangzi Island in the northern Yellow Sea, the bottom water mass-NYSCWM had strong influence on picoplankton distribution, showing low abundance in the bottom zone of the NYSCWM and extremely high abundance in the thermocline, which was not exactly the same as the influence of the SYSCWM on picoplankton distribution. How NYSCWM influenced picoplankton dynamics by changing thermocline layer and nutrient supply has been illustrated in this study. Since the

survey region locates at the edge of the NYSCWM, it is necessary to investigate picoplankton distribution in the cores of NYSCWM and SYSCWM regions to better understand and compare the influencing strength of NYSCWM and SYSCWM on picoplankton distribution in the future.

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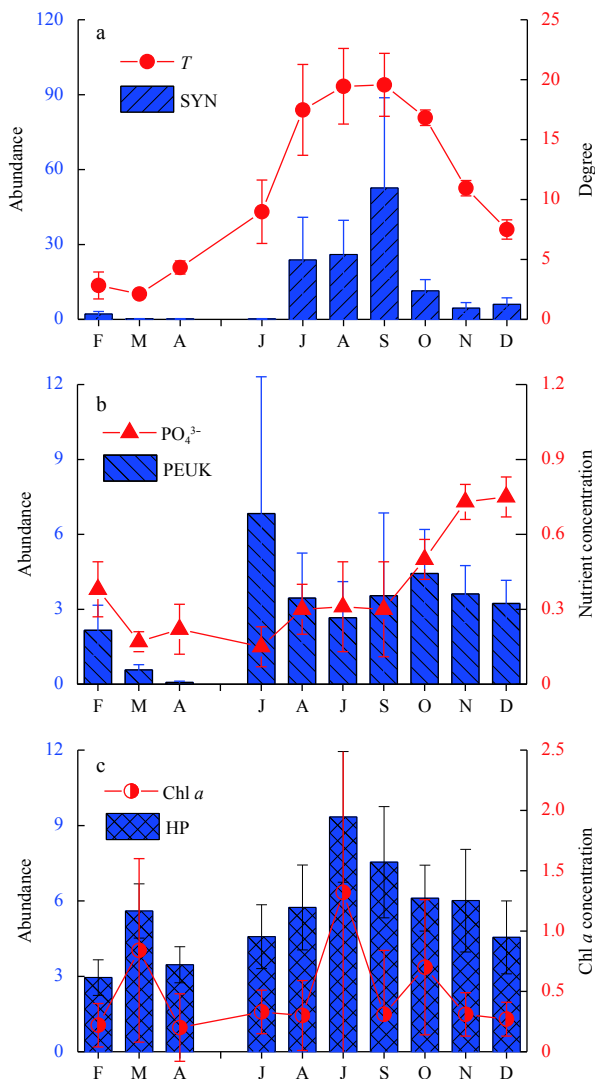


Fig. 7. Seasonal variations of average temperature (T , °C) vs. *Synechococcus* abundance (SYN, $\times 10^3$ cells/mL) (a), PO_4^{3-} ($\mu\text{mol/L}$) vs. picoeukaryotes abundance (PEUK, $\times 10^3$ cells/mL) (b), Chl a ($\mu\text{g/L}$) vs. heterotrophic prokaryote abundances (HP, $\times 10^5$ cells/mL) (c) in the non-NYSCWM region around the Zhangji Island in the northern Yellow Sea.

field sampling.

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