



Dynamics of autotrophic picoplankton and heterotrophic bacteria in the East China Sea

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Abstract

Dynamics of *Synechococcus*, *Prochlorococcus*, picoeukaryotes and heterotrophic bacteria in the East China Sea, a marginal sea of the Northwest Pacific, were investigated by flow cytometry in winter 1997 and summer 1998. Temporally, *Prochlorococcus* were always more abundant in the summer than in the winter, the same was true to *Synechococcus* except for the oceanic region. In contrast, picoeukaryotes were more abundant in the winter than in the summer. Heterotrophic bacteria were the least variable component among the four groups of picoplankton. Spatially, *Prochlorococcus* were extremely variable in the sea. They were largely confined to the warm water current areas and absent in the coastal areas in the winter, but present at most locations in the shelf water in the summer. *Synechococcus* were more abundant in the coastal areas than in the open waters in the summer but inverse in the winter. Compared with other picoplankters, picoeukaryotes showed more responses to water fronts on the shelf. In surface water, the lower boundary temperature for *Prochlorococcus* to present was around 15.6 °C in the winter but 26.4 °C in the summer, while it could also be found in the stratified deep water where temperature was as low as 14.3 °C in the summer. The higher boundary concentrations of total inorganic nitrogen for *Prochlorococcus* were about 6.5 μmol L⁻¹ in the winter and about 3.0 μmol L⁻¹ in the summer in the surface layer. The lower boundary salinities for *Prochlorococcus* were 33.5 psu in the winter and 29.1 psu in the summer. The key limiting factor for the coastward distribution of *Prochlorococcus* in the East China Sea were considered to be the movements of the Kuroshio Current and the Taiwan Warm Water Currents year around, temperature in winter and freshwater input in summer. *Synechococcus* correlated to temperature positively and nitrogen negatively in the winter when the high phycourobilin (PUB) to phycoerythrobilin (PEB) ratio strain dominated, and were independent from temperature but were associated with nutrients in the

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summer when the low PUB to PEB ratio strain dominated. Heterotrophic bacteria were not significantly affected by temperature but showed associations with nutrients in the summer. The big seasonal difference in the abundance of picoeukaryotes was most likely due to species succession.

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Keywords: *Prochlorococcus*; *Synechococcus*; Picoeukaryotes; Bacteria; Seasonal variation; East china sea

1. Introduction

Microorganisms have been known to be significant components of marine ecosystems since the discovery of abundant tiny prokaryotes (Hobbie et al., 1977) and formalization of the concept of the microbial loop (Azam et al., 1983). The discovery of *Prochlorococcus*, widely distributed bacterial-sized, divinyl chlorophyll containing photoautotrophs, have added significantly to the understanding of functions of marine ecosystems (Chisholm et al., 1988), which have displaced *Synechococcus* as the most abundant marine autotrophs known. Flow cytometry has been instrumental in quantifying picoplankton (Olson et al., 1988). At least four groups of picoplankton may be identified in stained samples: *Prochlorococcus*, *Synechococcus*, picoeukaryotes and heterotrophic bacteria (Marie et al., 1997). These organisms have been studied extensively in oceanic waters of the Pacific (Campbell and Vaulot, 1993; Blanchot and Rodier, 1996; Campbell et al., 1994, 1997), Atlantic (Olson et al., 1990; Buck et al., 1996), Mediterranean Sea (Vaulot et al., 1990), and Arabian Sea (Campbell et al., 1998). On the other hand, few studies dealt with coastal and shelf waters where ecological conditions are much more variable seasonally and spatially, and thus more efforts are needed for a better understanding of the dynamics and regulation mechanisms of picoplankton in such ecosystems. The East China Sea is one of the largest continental shelf seas in the world. It has nutrient-replete waters in the coastal area and oligotrophic oceanic waters in the outer region, and diverse hydrographical and physicochemical conditions. It is thus an ideal field for ecological studies on temporal and spatial dynamics of biota. However, little is known about picoplankton in the East China Sea. We have previously reported the existence of *Prochloro-*

coccus in the East China sea (Jiao et al., 1998) and revealed that hydrographic conditions are important for their distribution in summer (Jiao et al., 2002), but seasonal variations of picoplankton and the regulating factors remain unclear. Questions including whether or not temperature is critical for the seasonal difference in the distribution of *Prochlorococcus* toward the coast? Does nutrient level matter with *Prochlorococcus* in the coastal water? What are the dynamics of *Prochlorococcus* in comparison with other picoplankton groups? In this paper, we compared the summer data and the winter data on the four groups of picoplankton, *Prochlorococcus*, *Synechococcus*, picoeukaryotes and heterotrophic bacteria, and discussed the possible mechanisms controlling their dynamics.

2. Materials and methods

2.1. Description of the study area

The East China Sea is a marginal sea of the Northwest Pacific Ocean. The Yangtze River estuary in the west, the Yellow Sea cold water mass in the north, the Taiwan warm Currents in the south and the Kuroshio Current (from the Western Equatorial Pacific) in the east give great impacts on the sea (Fig. 1, Fig. 2A,a). There are roughly three water systems in the East China Sea: The coastal water system affected strongly by the fresh water input from the Yangtze River is characterized by low salinity of below 31 psu (Gong et al., 1996); The Kuroshio Current water system located in the outer, deep water region of the sea is featured by high temperature and high salinity (Miao and Yu, 1991); and the transitional (mixing) water system located between the two where currents and water masses mix and interact, makes up the majority of the continental shelf

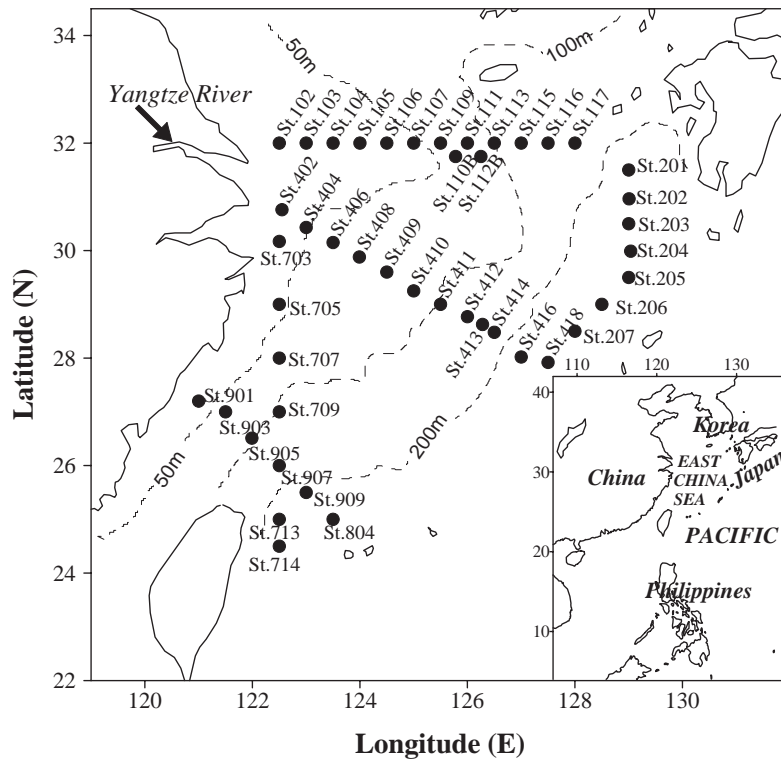


Fig. 1. Location of the East China Sea and the investigation stations. Transects (1, 2, 4, 7 and 9) are indicated by the first digit of the sampling station numbers.

water. Thirty-four stations along three transects (1, 2 and 4) and forty-two stations along five transects (1, 2, 4, 7 and 9) were investigated in January, 1997 and in July, 1998, respectively (Fig. 1). Transect 1 (32°N , 122.2°E – 32°N , 128°E) was located in the northern part of the sea from the Yangtze River estuary to the northeastern region of the sea near Japan. Transect 2 (28°N , 127.5°E – 31.5°N , 129°E) was situated on the Kuroshio Current along the western edge of Pacific Ocean. Transect 4 (127.5°E , 28°N – 122.3°E , 31°N) was set from the Yangtze River estuary through the central and southeast edge of the sea. Transect 7 was from the Yangtze River estuary to the northeast of the Taiwan Island (24.5°N , 125.5°E) along longitude 125.5°E , and transect 9 (27.2°N , 121°E – 24.75°N , 123°E) was set from the southern coast to southeast end of the sea (Fig. 1). During our winter cruise, due to the shrink of the freshwater plume, the coastal

water, defined as with salinity of below 31 psu (Gong et al., 1996), was not covered by our fixed investigation stations. Therefore, only shelf mixing water and Kuroshio water were discriminated and discussed for the winter data.

2.2. Sample collection and flow cytometry measurement

Water samples were collected at 0, 10, 20, 30, 50, 75, 100 and 150 m depths using Niskin bottles (Oceanic Co., USA) depending on the water depth. Samples for flow cytometry analysis were fixed with glutaraldehyde (final concentration: 1%, Vulot et al., 1989), quick-frozen in liquid nitrogen and stored in a freezer (-20°C) on board and replaced in liquid nitrogen in the lab for later analysis. The 1997 samples were analyzed with a FACScan flow cytometer (Becton-Dickinson) modified for high sensitivity (Dusenberry and

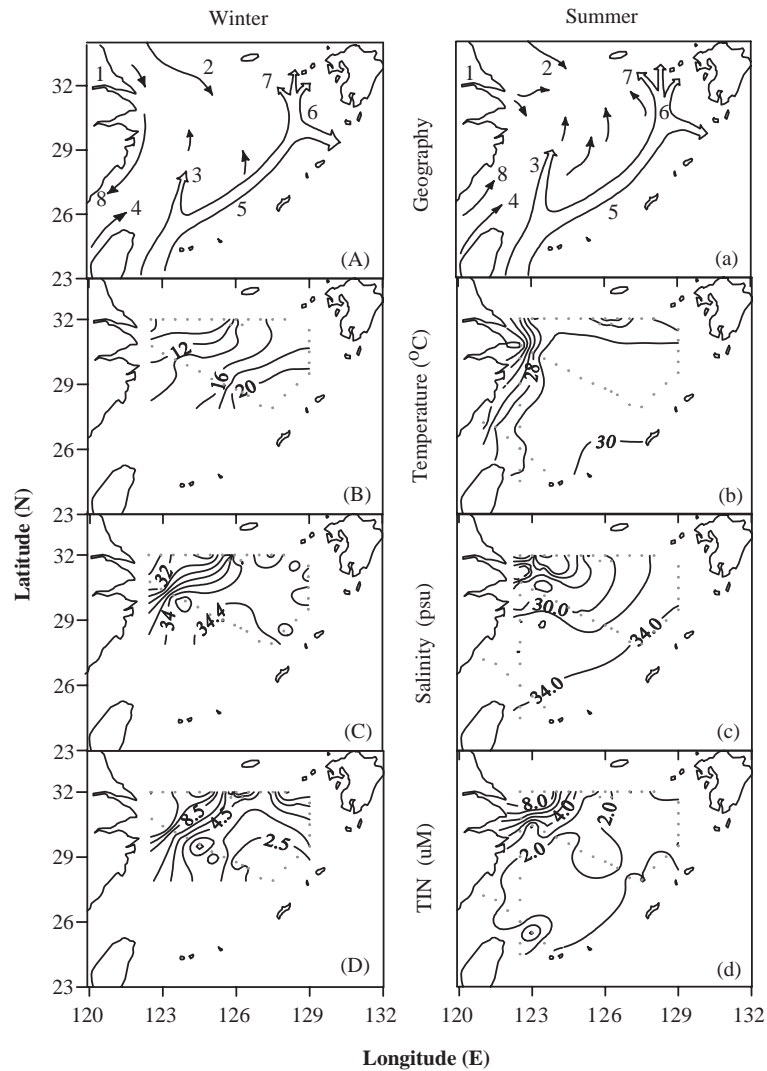


Fig. 2. Distribution of water masses/currents (A, a) (1, Yangtze River; 2, Yellow Sea cold water current; 3, Taiwan Warm Current; 4, Taiwan Warm Current west branch; 5, Kuroshio Current; 6 Tsushima Warm Current; 7, Yellow Sea Warm Current; 8 coastal current according to Su, 1989 with modification based on physical data from the present cruises), surface temperature (B, b; unit: °C), salinity (C, c; unit: psu), and TIN (D, d; unit: $\mu\text{mol L}^{-1}$) in February 1997 (left) and July 1998 (right).

Frankel, 1994), and equipped with a Harvard Apparatus SI 100 quantitative pump injector. The 1998 samples were run on a FACSCalibur flow cytometer (Becton-Dickinson), equipped with an external quantitative sample injector (Harvard Apparatus PHD 2000). Procedures were as described by Olson et al. (1990). The three autotrophs were distinguished according to their positions in plots of red fluorescence (FL3) vs.

90°-angle light scatter (SSC), and orange fluorescence (FL2) vs. SSC. Picoeukaryotes were identified by their large size and red fluorescence. SYBR green I (Molecular Probes) was employed as the nucleic acid stain (Marie et al., 1997) for bacteria identification in plots of FL3 vs. green fluorescence (FL1). Samples for flow cytometry enumeration of autotrophs were run separately from those for heterotrophic bacteria. Flow

cytometry data were collected in list mode, and analyzed with CytoWin 4.1 software (<http://www.sb-roscoff.fr/Phyto/cyto.html>). Data for nutrients, temperature, salinity, and transparency were from the Chinese JGOFS project reports of the same cruises.

3. Results

3.1. Physical and chemical conditions

The Kuroshio Current water system was characterized by relatively high temperature and low nutrients (Table 1). Conversely low salinity, low temperature and high nutrients identified the coastal water. In the summer, salinity varied from less than 20 psu in the coastal area to 34.4 psu in the Kuroshio area. In the winter, however, salinity ranged from 31.4 to 34.8 psu and typical coastal waters were largely out of our investigation stations (Table 1). Surface water temperature ranged from 30 °C in the oceanic water to 25 °C in the coastal water in the summer and from 22 °C in the Kuroshio area to 6 °C in the northwest near coast area in the winter (Fig. 2B,b; Table 1). Nutrient levels were distinctly higher in the winter than in the summer. In the summer (Fig. 2d; Table 1), total inorganic nitrogen (TIN) were usually higher than 3 $\mu\text{mol L}^{-1}$, and up to 12.7 $\mu\text{mol L}^{-1}$ in the coastal water, from 0.7 to 3.6 $\mu\text{mol L}^{-1}$ in the shelf mixing water, and from 1.7 to 5 $\mu\text{mol L}^{-1}$ in the Kuroshio water; $\text{PO}_4\text{-P}$ were usually higher than 0.3 $\mu\text{mol L}^{-1}$ and up to 0.9 $\mu\text{mol L}^{-1}$ in the

coastal water, from 0.13 to 0.41 $\mu\text{mol L}^{-1}$ in the shelf mixing water, and from 0.13 to 0.29 $\mu\text{mol L}^{-1}$ in the Kuroshio water. In the winter (Fig. 2D; Table 1), TIN varied from 1.5 to 15.2 $\mu\text{mol L}^{-1}$ and from 1.7 to 5 $\mu\text{mol L}^{-1}$ in the shelf mixing water and the Kuroshio water, respectively; $\text{PO}_4\text{-P}$ varied from 0.27 to 0.66 $\mu\text{mol L}^{-1}$ and from 0.31 to 0.49 $\mu\text{mol L}^{-1}$ in the shelf mixing water and the Kuroshio water, respectively. During the winter, water columns were usually well mixed within the sampling depths and nitraclines existed around 50 m only in the deep-water areas. In the summer, most of the investigation sites were stratified and nitraclines usually developed (Fig. 3).

3.2. Distribution of picoplankton in winter

Synechococcus were ubiquitous in the East China Sea, and was most abundant in the south-eastern region around the Kuroshio Current ($>2 \times 10^4$ cells mL^{-1}) in the winter. The populations decreased toward the shore and the lowest abundance ($<2 \times 10^3$ cells mL^{-1}) occurred in the northwest near-shore stations 105 and 106 where both salinity and temperature were low due to cold water from the Yellow Sea (Fig. 4A).

Prochlorococcus were present at stations 115 (32°N, 127°E), 412 (28.75°N, 126°E) and eastward in the sea (Fig. 4B, Fig. 5 Pro). The depth-weighted average cell abundance ranged from 1×10^3 in the shelf mixing water to 5×10^4 cells mL^{-1} in the Kuroshio water. Populations were maximal (5.6×10^4 cells mL^{-1}) at 30 m of St. 418. From St. 202 (31°N, 129°E) and 413 (28.63°N,

Table 1
Cell abundance and environmental variables in different water systems of the East China Sea in summer 1998 and winter 1997

Season	Summer			Winter	
Region	Coastal	Transitional	Kuroshio	Transitional	Kuroshio
T(°C)	27.8 (24.6–29.3)	28.0 (24.9–29.7)	29.2 (27.9–29.7)	13.6 (6.0–17.1)	20.4 (16.8–21.9)
Salinity (psu)	28.1 (19.3–30.7)	32.6 (31.5–34.0)	34.0 (33.7–34.4)	32.7 (31.4–34.3)	34.4 (33.8–34.8)
TIN (μM)	4.6 (1.1–12.7)	1.8 (0.7–3.6)	1.4 (0.7–2.1)	7.1 (1.5–15.2)	3.0 (1.7–5.4)
PO_4^- (μM)	0.32 (0.20–0.90)	0.26 (0.13–0.41)	0.21 (0.13–0.29)	0.36 (0.27–0.66)	0.38 (0.31–0.49)
Syn (10^4 cells mL^{-1})	3.1 (0.1–8.5)	1.2 (0.1–5.6)	0.2 (0.1–0.4)	0.15 (0.03–0.45)	0.83 (0.17–2.22)
Pro (10^4 cells mL^{-1})	0.9 (0–4.6)	2.2 (0–5.8)	4.7 (2.3–9.8)	0.05 (0–0.60)	2.2 (0.16–5.0)
Euk (10^4 cells mL^{-1})	0.05 (0.01–0.14)	0.04 (0.02–0.12)	0.02 (0.01–0.03)	0.45 (0.14–1.08)	0.58 (0.40–0.84)
Bact (10^4 cells mL^{-1})	73.0 (26.5–172.4)	41.9 (23.2–72.2)	20.6 (17.6–28.0)	44.7 (35.6–67.9)	42.9 (28.2–79.0)

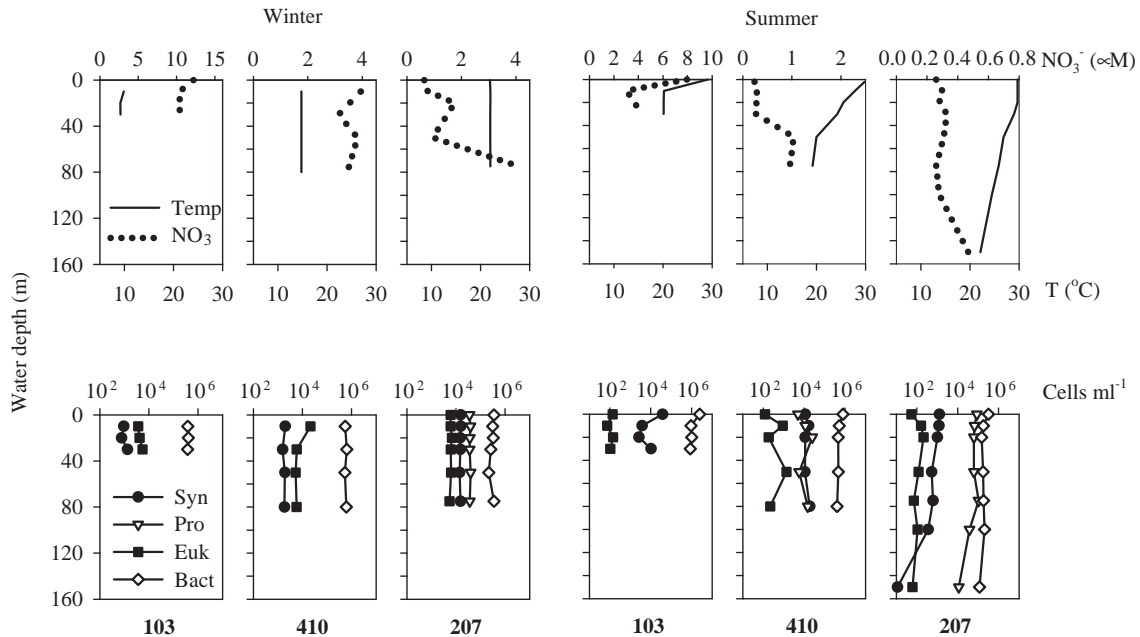


Fig. 3. Profiles of temperature (solid lines) and nitrate (dotted lines) and the four picoplankton groups (Syn: *Synechococcus*; Pro: *Prochlorococcus*; Euk: picoeukaryotes; Bact: Heterotrophic bacteria) at typical stations.

126.25°E) to the southeast, *Prochlorococcus* abundance began to exceed that of other autotrophs. In this area the abundance of *Prochlorococcus* was 2–3 times higher than that of *Synechococcus*, and 5–7 times that of picoeukaryotes. *Prochlorococcus* were generally more abundant in the Kuroshio Current and adjacent regions where temperatures were high but nutrients were low. Picoeukaryotes were widespread in the East China Sea (Fig. 4C). In the northern part, they dominated the autotrophic abundance throughout Tr.1. The same was true for Tr. 4 through west of St.412 where *Prochlorococcus* began to present eastward. In the oceanic water along the Kuroshio Current (Tr. 2), *Prochlorococcus* and *Synechococcus* populations were greater than that of picoeukaryotes. However, as one proceeded north, the abundance of the two prokaryotes decreased rapidly while that of picoeukaryotes remained about 6×10^3 cells mL⁻¹ throughout Tr. 2 (Fig. 5). Bacterial populations were typically 4 to 6×10^5 cells mL⁻¹, even when autotrophs were relatively rare (Fig. 4D).

3.3. Distribution of picoplankton in summer

In the summer, the distribution pattern of *Synechococcus* was opposite that observed in the winter. They were more abundant in the coastal areas than in the shelf mixing water and Kuroshio water. They were most abundant around St.105, St.106, and St. 406 near the Yangtze River estuary where the depth-weighted average cell abundance reached a magnitude of 10^4 cells mL⁻¹. In the east and south parts of the sea, *Synechococcus* were evenly distributed at a low abundance of about 10^3 cells mL⁻¹ (Fig. 4a). *Prochlorococcus* were present in almost all the stations investigated, except for the very northwest part and the Yangtze River estuary (Fig. 4b). In general, *Prochlorococcus* abundance decreased from the Kuroshio Current oceanic water to the northwest coastal water. In contrast to the winter distribution pattern, the summer coastward invasion of *Prochlorococcus* extended further to St.113 (32°N, 126.5°E) at Tr.1 and beyond St.408 (29.88°N, 124°E) at Tr.4 (Fig. 5). The abundance of *Prochlorococcus* at St.

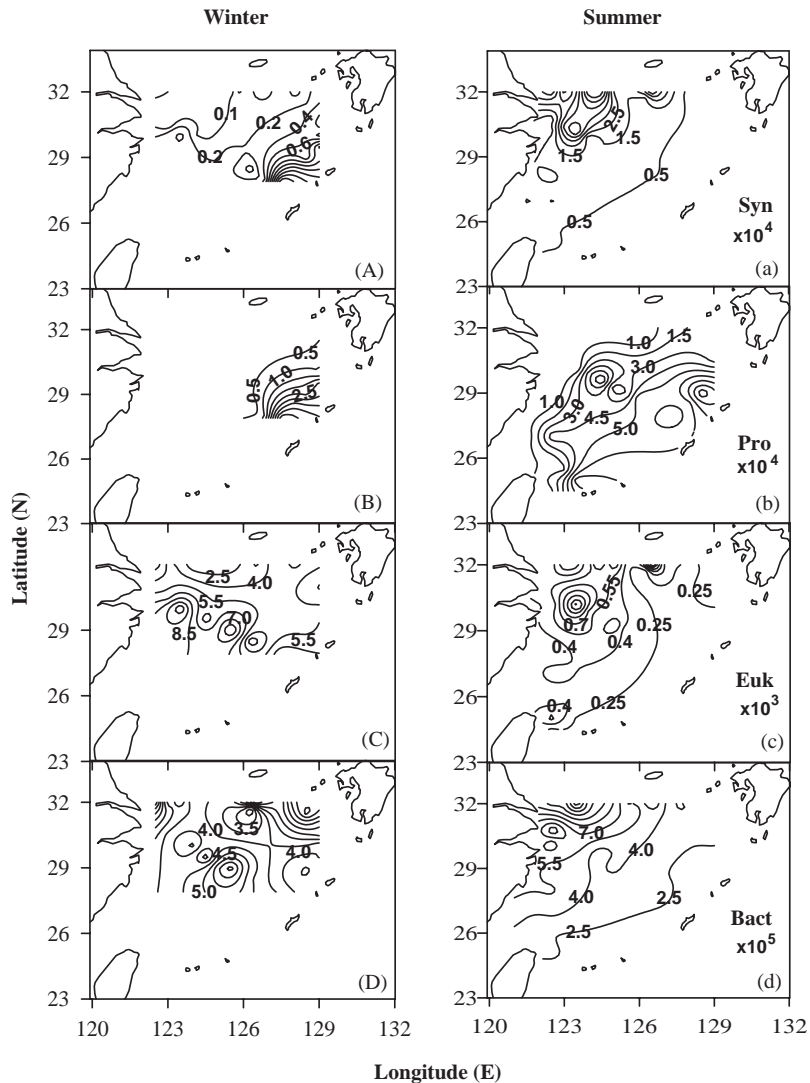


Fig. 4. Distribution of the depth-weighted average cell abundance (cells mL⁻¹) of *Synechococcus* (A, a), *Prochlorococcus* (B, b), picoeukaryotes (C, c) and heterotrophic bacteria (D, d) in the winter (left) and the summer (right).

408 and St. 409 reached 7.2×10^4 cells mL⁻¹, which was very close to the maximal abundance recorded at the Kuroshio Current (St.206: 9.8×10^4 cells mL⁻¹, St.804: 7.6×10^4 cells mL⁻¹), and exceeded that of the adjacent stations by a significant amount. The distribution pattern of picoeukaryotes was similar to that of *Synechococcus* (Fig. 4c), it was most abundant around St.402 and St.111 where depth-weighted average cell abundance was at the level of 10^3 cells mL⁻¹. Near

the Kuroshio Current, depth-weighted average cell abundance of picoeukaryotes dropped to the level of 10^2 cells mL⁻¹. This southeastward decreasing trend was also found in the distribution of bacteria (Fig. 4d). The high abundance zone of bacteria (St.104, 1.7×10^6 cells mL⁻¹) appeared to be associated with the Yellow Sea and the Yangtze River estuary. The minimum concentration of bacteria occurred within the Kuroshio Current. Notably, there was a low abundance zone of

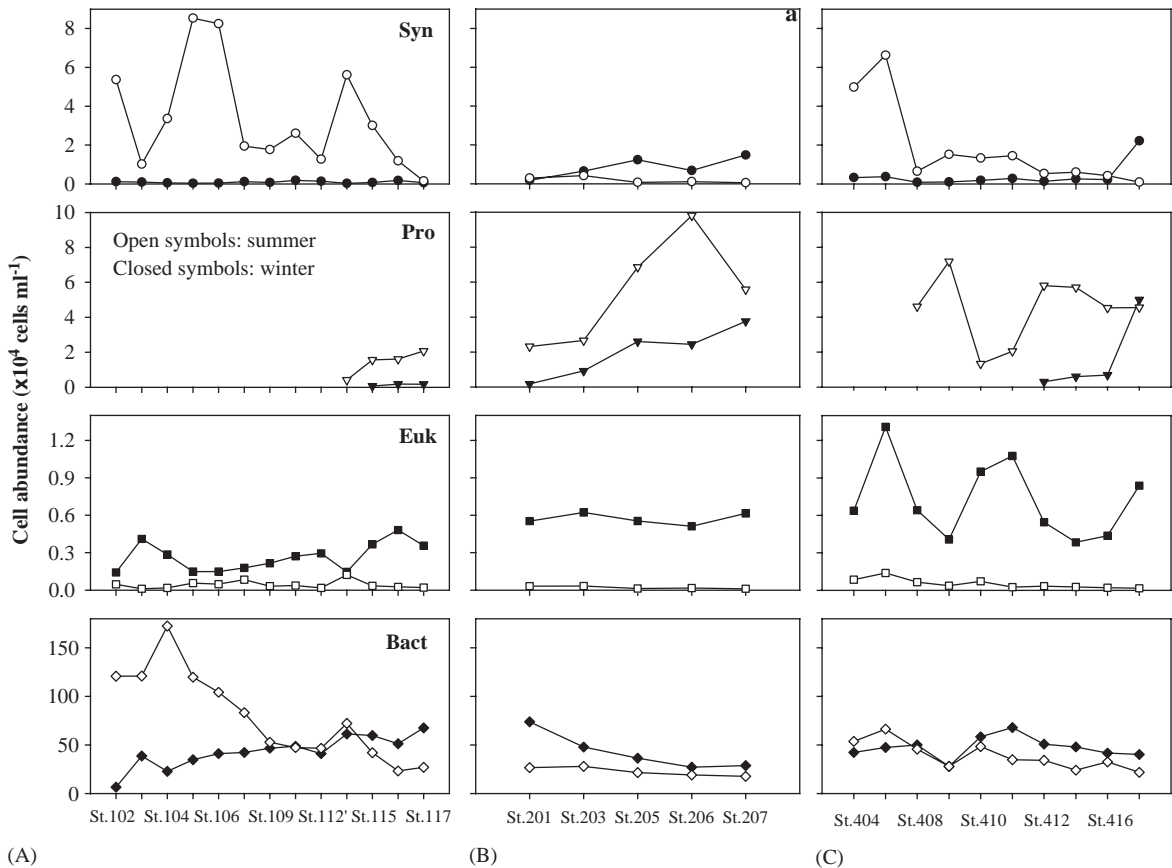


Fig. 5. Seasonal variation of depth-weighted average cell abundance of picoplankton along transect 1 (A), 2(B), 4(C). Open symbols: summer; Closed symbols: winter. Syn: *Synechococcus*; Pro: *Prochlorococcus*; Euk: picoeukaryotes; Bact: heterotrophic bacteria).

bacteria of 3.5×10^5 cells mL^{-1} around St.409 where the highest abundance of *Prochlorococcus* was recorded.

3.4. Seasonal variation

Synechococcus were generally more abundant in the summer than in the winter. Along Tr. 1 (Fig. 5A, Syn), for example, its abundance ranged from 8.5×10^4 cells mL^{-1} in the summer to below 2×10^3 cells mL^{-1} in the winter. The most extreme seasonal variation in *Synechococcus* abundance occurred at St.105 where the populations in the summer were 200-fold those in the winter. *Prochlorococcus* were generally absent in the region, but present in the east part of the transect where summer populations (up to 2×10^4

cells mL^{-1} on average) were 7–23 times higher than in the winter (Fig. 5A, Pro). Picoeukaryotes populations along Tr.1, increased from an average of 4.3×10^2 cells mL^{-1} in the summer to 2.1×10^3 cells mL^{-1} in the winter (Fig. 5A, Euk). Bacterial populations were 6-fold higher in the summer (1.2×10^6 cells mL^{-1}) than in the winter in the near shore area from station 102–107 but similar or even lower in the summer than in the winter in the off shore area (from 3.1×10^5 in summer to 5.9×10^5 cells mL^{-1} in winter).

Along transect 4, *Synechococcus* were more abundant in the summer (0.43 – 6.63×10^4 cells mL^{-1}) than in the winter (average of 0.22×10^4 cells mL^{-1}), except for station 418 in the Kuroshio Current. The variability between the seasons ranged from 2-to 18-fold with an average

of 8.5-fold. The largest seasonal variations occurred near the coast (St.406, Fig. 5C, Syn). With respect to geographical distribution, the abundance of *Prochlorococcus* varied more abruptly than that of *Synechococcus* (Fig. 5C, Pro). For instance, *Prochlorococcus* were undetectable from St. 410 coastward in the winter but its concentration increased to 7×10^4 cells mL⁻¹ in the summer at the same station. The averaged abundances of *Prochlorococcus* along this transect were 4.5×10^4 cells mL⁻¹ in the summer vs. 1.8×10^3 cells mL⁻¹ in the winter. Picoeukaryotes cell numbers were consistently higher in the winter, and increased 5–70-fold from 5.6×10^2 cells mL⁻¹ in the summer to 7.1×10^3 cells mL⁻¹ in the winter (Fig. 5C, Euk). Seasonal difference in bacterial abundance was the smallest among the four groups of microorganisms (Fig. 5C, Bact).

Along transect 2, cell abundances of *Synechococcus*, bacteria and picoeukaryotes were all higher in the winter than in the summer (Fig. 5B). This was not the case for *Prochlorococcus*, which were more abundant in the summer. Along the Kuroshio Current, seasonal differences in *Synechococcus* abundance were smaller but variation trends were opposite to each other in the two seasons (Fig. 5B, Syn). In contrast, the cell abundance of *Prochlorococcus* basically decreased from south to north along the Kuroshio Current in both seasons. Cell abundance of picoeukaryotes varied little geographically but the seasonal difference was consistently huge throughout the whole transect. In the opposite of *Prochlorococcus*, bacterial abundance decreased along the Kuroshio from north to south in both seasons.

Geographically, distinct patterns can be seen in all the picoplankton populations. For *Synechococcus*, there was a gradient in abundance which was increasing from the northwest (estuarine area) to the southeast (oceanic warm water) in the winter but decreasing in the same direction in the summer (Fig. 4A,a). In contrast, abundance of *Prochlorococcus* were always higher in the southeastern region with a further coastward distribution in the summer than in the winter (Fig. 4B,b). Picoeukaryotes and heterotrophic bacteria showed geographical distribution patterns just in the reflection of that of *Prochlorococcus*, i.e., more

abundant in the northwestern region than the southeastern region of the sea with a stronger trend in the summer and more fluctuations in the winter (Fig. 4C,c,D,d).

The depth profiles of these four groups can be distinguished between the two seasons. Due to stratification, picoplankton profiles are more curved and distinct peaks could be recognized in the summer. In the winter, however, the upper water columns were mixed very well, and picoplankton profiles were less variable (Fig. 3).

4. Discussion

Comparing the distribution patterns of picoplankton and hydrological and physico-chemical parameters, similarities can be seen between the biological and environmental variables (Table 1, Figs. 2 and 4). The difference in the intrusion extent of the warm current, Kuroshio Current into the continental shelf between the two seasons obviously caused differences in the distribution of temperature, salinity, nutrients and so on, and the later consequently resulted in differences in biological responses between the two seasons.

Temperature is reported to be crucial to *Prochlorococcus* (Olson et al., 1990; Moore et al., 1995). In this study, significant correlations between the abundance of *Prochlorococcus* and temperature were found in both the summer ($r = 0.49$, $n = 141$, $p < 0.01$) and the winter ($r = 0.84$, $n = 57$, $p < 0.01$) (Fig. 6 Pro). *Prochlorococcus* were more abundant and less temperature-dependent in the summer. In the surface water, the lowest temperature for *Prochlorococcus* to present in the winter was 15.6 °C (Fig. 6 Pro) which was consistent with the reported critical temperature (15 °C) for *Prochlorococcus* to grow (Olson et al., 1990; Buck et al., 1996), suggesting that the winter distribution of *Prochlorococcus* in the marginal sea was limited by temperature. In contrast, the lower boundary temperature for *Prochlorococcus* in the summer was 26.4 °C, much higher than 15 °C. While *Prochlorococcus* could also be found in the stratified deep water where temperature was as low as 14.3 °C in the summer (Fig. 6 Pro). Therefore, summer coastward distribution of *Prochlorococcus*

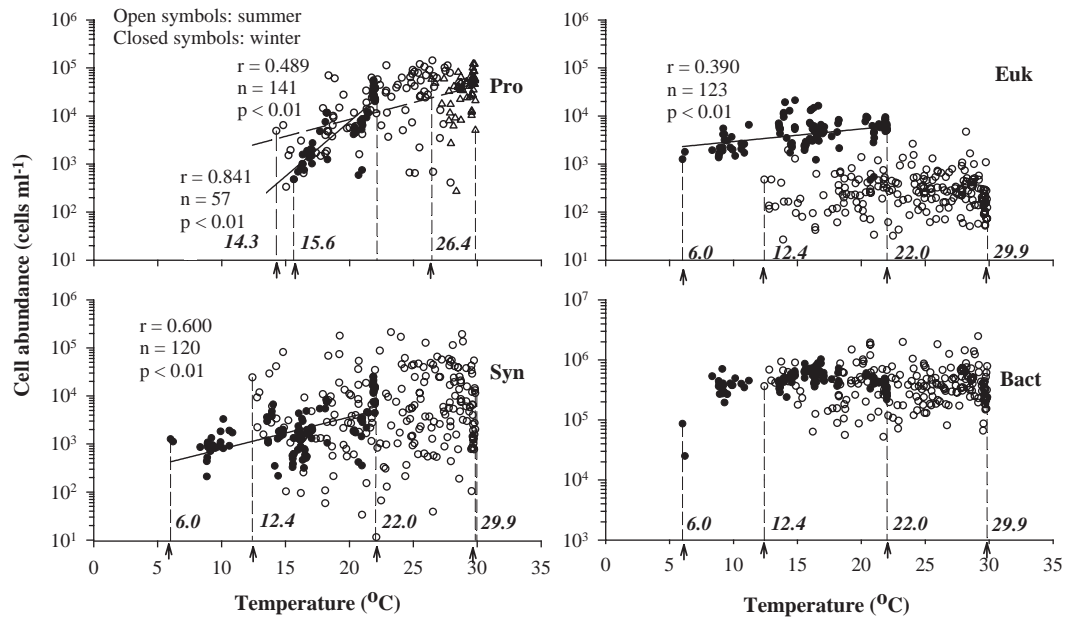


Fig. 6. Seasonal patterns of picoplankton as a function of water temperature. (Triangle symbols are surface water samples; the temperature ranged from 6.0 to 22.0 °C in the winter and 12.4–30 °C in summer. Numbers at arrows in the top panel were boundary temperatures for *Prochlorococcus*, see text).

in the marginal sea was not limited by water temperature but other factors. Nutrients have been suggested to play a role in the distribution of *Prochlorococcus* (Blanchot et al., 1992; Campbell and Vault, 1993). By plotting abundance of *Prochlorococcus* vs. nutrients, inverse correlations between the two were found in both the winter and the summer (Fig. 7 Pro). In terms of the nutrient boundaries, *Prochlorococcus* disappeared when surface water TIN was greater than $3 \mu\text{mol L}^{-1}$ on the way intruding coastward in both the seasons. On the other hand, *Prochlorococcus* were also found when surface water TIN was more than $6 \mu\text{mol L}^{-1}$ in the northeastern region of the sea where a branch of the Kuroshio Current intruding into the nutrient rich water of the Yellow Sea (Fig. 2C, and Fig. 7 Pro). Furthermore, *Prochlorococcus* were even present under TIN of up to $14.6 \mu\text{mol L}^{-1}$ in the deep water. These concentrations are within the reported nutrient tolerance range of *Prochlorococcus* (Chavez et al., 1991; Vault and Partensky, 1992). However, half of the TIN in the study area was contributed by nitrate ($12.5 \mu\text{mol L}^{-1}$ in winter and $2.1 \mu\text{mol L}^{-1}$ in sum-

mer) that is not really utilized by *Prochlorococcus* (Moore et al., 2002; Dufresne et al., 2003). Therefore, for the coastward distribution of *Prochlorococcus*, nutrients are not necessarily critical either, just like what Partensky et al. (1999a) pointed out that the role of nutrients in regulating *Prochlorococcus* is very tricky to evaluate. Salinity, as a variable in the marginal sea, showed a different boundary values for *Prochlorococcus* in the two seasons, 33.5 psu in the winter and 29.1 psu in the summer. And a significant correlation between *Prochlorococcus* and salinity in surface layer in the summer ($r = 0.58$) was recorded. Although *Prochlorococcus* could be found in river mouth with salinity as low as 1.2 psu (Vault et al., 1990), the correlation between *Prochlorococcus* and salinity here in the summer suggesting salinity indicated comprehensive impacts of the Yangtze River input on the sea, such as, high heavy metal concentrations by river inputs may inhibit the growth of *Prochlorococcus* (Chisholm et al., 1992). Similar correlations between *Prochlorococcus* and salinity are also observed in other boundary margin areas (Crosbie

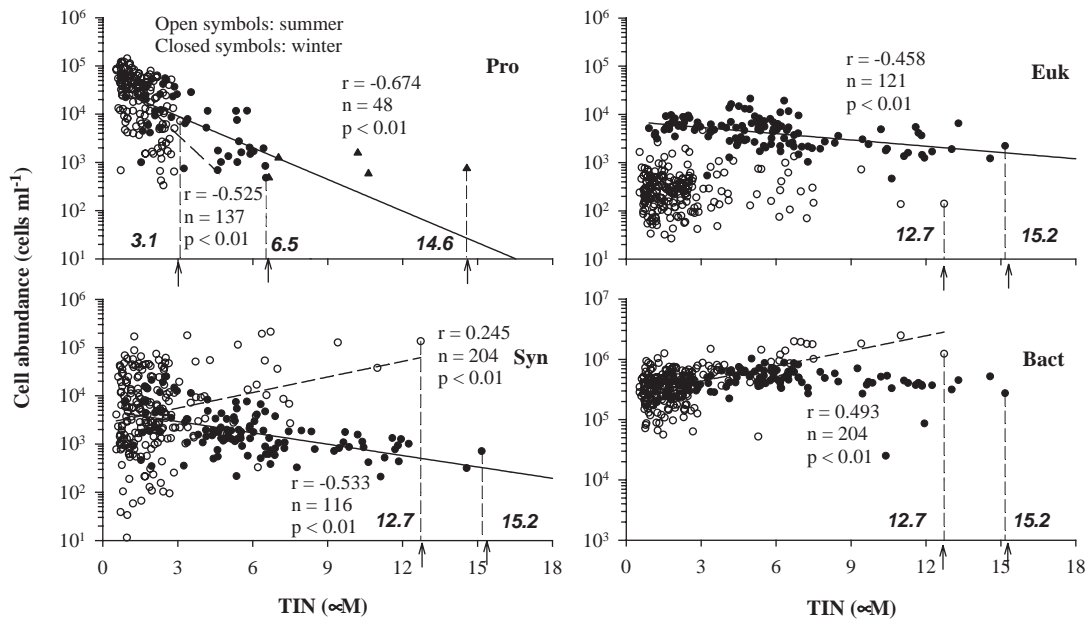


Fig. 7. Seasonal patterns of picoplankton as a function of nutrients (12.7 and 15.2 $\mu\text{mol L}^{-1}$ are the highest observations in summer and winter respectively. 6.5 and 3.1 $\mu\text{mol L}^{-1}$ at arrows in the top panel are the nitrogen thresholds for *Prochlorococcus* in the surface water in winter and summer respectively. Triangles are winter samples from deep water with high nutrients).

and Furnas, 2001; Calvo-íDaz et al., 2004). Therefore, it appeared that, in addition to the influences of warm water currents (Jiao et al., 2002), the coastward distribution of *Prochlorococcus* in the East China Sea was significantly regulated by temperature in winter and by freshwater input and related factors in summer.

In open oceans, there are usually two clusters of *Prochlorococcus* (typical representatives: SS120 and MED4) can be found (Ferris and Palenik, 1998; West et al., 2001). We could not discriminate any clusters by flow cytometry in all of the samples from all water depths in the East China Sea. Exploration with 16S rDNA analysis of a sample from the depth of 50% surface light density of the St.413 showed that only one cluster, the high light genotype II (HL II) was present in the East China Sea (data not shown). This is consistent with the molecular observations in the South China Sea which is another marginal sea of the Northwest Pacific located in the south of the East China Sea that HL II type of *Prochlorococcus* was the only population present at both surface and bottom of the euphotic zone (Ma et al., 2004). This is also

consistent with that a *Prochlorococcus* strain from the Suruga Bay, Japan, was shown by the 16S rRNA sequence (1147 bp) to be very similar to that of the GP2 strain (99.6% homology) which belongs to High Light-Adapted Clade (Shimada et al., 1995; Partensky et al., 1999b).

Compared with *Prochlorococcus*, *Synechococcus* are reported to be more nutrient-dependent and usually more abundant in winter when mixing enhances the availability of nutrients in the euphotic zone (Olson et al., 1990; Blanchot et al., 1992; Campbell and Vaultot, 1993; Campbell et al., 1997; Michele et al., 2001). In the case of the marginal sea in this study, there seemed to be a conflict with the above conclusions obtained from the oceans. That is, *Synechococcus* were actually more abundant in the summer when nutrients were less abundant than in the winter. This pattern is consistent with a previous microscopic observation on *Synechococcus* in the same sea (Chiang et al., 2002). Thus, there must be some other controlling mechanisms behind the phenomena. Further statistical analysis showed significant correlations between *Synechococcus* and temperature existing

in the winter, indicating that temperature could be a limiting factor in the cold season as also suggested by Chiang et al., 2002. In summer, however, temperature was no longer limiting (Fig. 6 Syn), *Synechococcus* thrived in the northwestern region where temperature was relative low but nutrients were replete (Fig. 4A,a). Looking at the seasonal differences in the abundance of *Synechococcus* in the Kuroshio water (oceanic water) where winter average temperature (20.4°C) was not limiting, one would find that the winter abundance were about 4-fold the summer abundance. Such difference is quite the same seen at ALOHA in the central Pacific (Campbell et al., 1997) and at BATS in the Sargasso Sea (Michele et al., 2001). Since the winter nutrient levels were about 2-fold the summer ones in the Kuroshio water due to winter mixing (Table 1), the higher winter abundance of *Synechococcus* was obviously due to high availability of nutrient in the winter. This explains the above apparent conflicts, and shows the difference in seasonal dynamics of *Synechococcus* between marginal seas and the oceans.

From the observed distributions, it appeared that the summer-dominant population is more nutrient-dependent (positive trend with increasing TIN, Fig. 7 Syn) and temperature-independent when temperature is relatively high (Fig. 6 Syn). The winter-dominant population is more temperature-dependent (Fig. 6 Syn) and better suited to successful competition at lower nutrient levels (Fig. 7 Syn). The former resembled the low phycoerythrobilin (PUB) to phycoerythrobilin (PEB) ratio strain that liked coastal water and the later acted as the high PUB/PEB ratio strain that liked oceanic water (Wood et al., 1985; Olson et al., 1988; Campbell et al., 1998). Although we could not successfully discriminate the two *Synechococcus* strains in the summer samples, we did observe them in the winter samples (Fig. 8A). The low and high PUB/PEB ratio strains were predominant in the coastal and open waters, respectively (Fig. 8B). In the continental shelf water, the two strains co-existed but they vertically differentiated along water depth. The two strains shared the upper layer of the euphotic zone, but in the bottom layer of the euphotic zone, the low

PUB/PEB ratio strain disappeared (Fig. 8 upper pannel).

Compared with other picoplankters, picoeukaryotes were most variable between the two seasons (Fig. 6 Euk, Fig. 7 Euk). The abrupt seasonal variability was most likely to be caused by seasonal species succession. Recently, molecular studies have brought to light the great diversity of picoeukaryotes (Díez et al., 2001), and diverse composition provide the basis for species succession. Meanwhile, nutrient structure changes between the two seasons also add fuel to the fire for the succession. Nitrogen was relatively limiting in the summer, whereas phosphorus was relatively limiting in the winter (Table 1). Analysis of pigment components by HPLC did show remarkable species succession of phytoplankton in the East China Sea that diatoms are the most abundant taxa followed by chlorophytes, cryptophytes, chrysophytes and prymnesiophytes in the cold season, but in summer, diatom dominance was observed only at station nearest to the coast and become less abundant sharply toward the mid and off shelf (Furuya et al., 2003). We speculate that the high winter abundance of picoeukaryotes, as assemblages being composed of different species, was most probably contributed by diatoms which like low temperature. This was confirmed by later cruises (2001–2002) to the same area (Li, 2004).

There were no correlations between temperature and heterotrophic bacteria either in the winter or in the summer. Bacteria showed a positive correlation with TIN in the summer but not in the winter. The seasonal variation in bacterial abundance was the smallest among all the microorganisms investigated. It was only slightly affected by nutrients in the summer (Fig. 7 Bact).

Compared to other marginal seas such as the northwestern Indian Ocean (Veldhuis et al., 1997) and the Gulf of Aqaba in the Red Sea (Lindell and Post, 1995), a striking difference is that the abundance of picoeukaryotes in the East China Sea was relatively high in winter and very low in summer, on the contrary, *Synechococcus* in the outer region of the estuarine area were extremely abundant in summer. This “more *Synechococcus*, less picoeukaryotes” pattern characterized the East

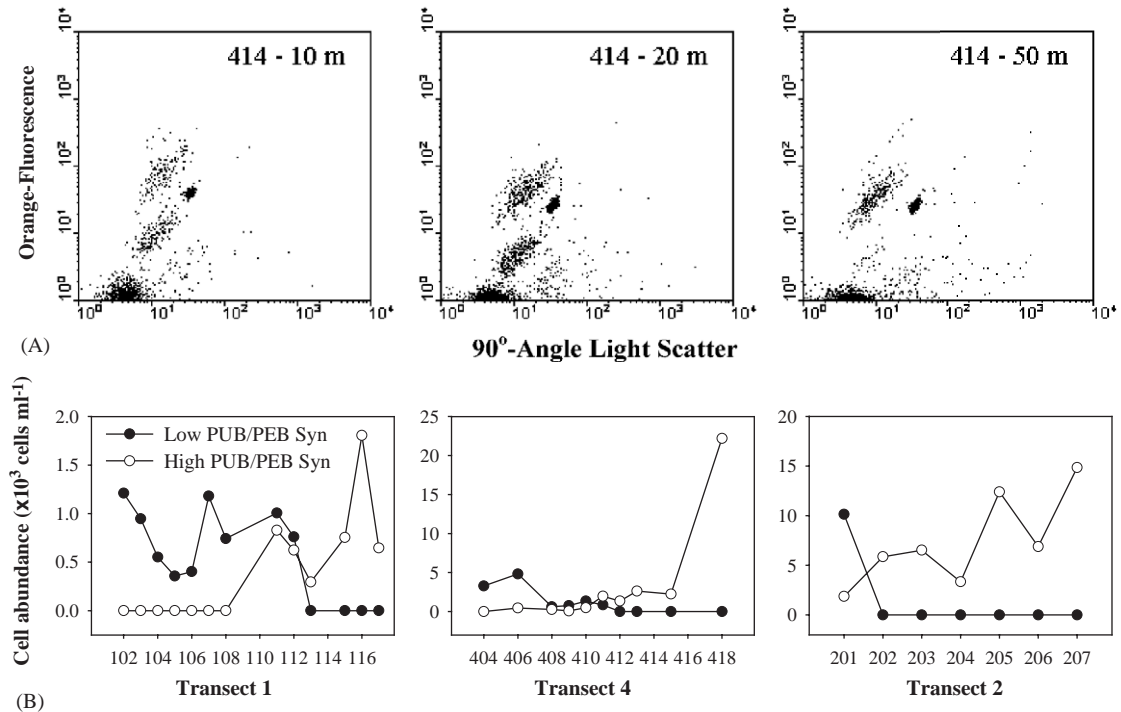


Fig. 8. Distribution of the low and high PUB/PEB ratio *Synechococcus* strains in winter. A. Flow cytograms of the two strains at different water depths of the continental shelf waters. B. Distribution of the two strains along different transects.

China Sea. Between *Prochlorococcus* and *Synechococcus*, the abundance ratio was similar to those in Arabian Sea near shore and nitrate replete regions (Campbell et al., 1998) and the Mediterranean Sea (Vaulot et al., 1990), but much less than those in the oligotrophic oceans, by 50–200-fold (Buck et al., 1996; Landry et al., 1996; Campbell et al., 1997).

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Further reading

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