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Seasonal and spatial patterns of picophytoplankton growth, grazing and distribution in the East China Sea

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Abstract. Dynamics of picophytoplankton population distribution in the East China Sea (ECS), a marginal sea in the western North Pacific Ocean, were studied during two cruises in August 2009 (summer) and January 2010 (winter). Dilution experiments were conducted during the two cruises to investigate the growth and grazing among picophytoplantkon populations. Comparisons of phytoplankton growth (μ_0) and microzooplankton grazing rates (m) on seasonal (summer and winter), spatial (plume, transitional and Kuroshio regions) and vertical (surface and depth of chlorophyll maximum) scales were made. The three picophytoplankton populations occupied different ecological niches and showed different distribution patterns (especially in summer), which is, however, not coincident with their maximum growth rate. The distribution and population transition of picophytoplankton is therefore a result of the balance between growth and grazing mortality. Average growth rates (μ_0) for *Prochlorococcus* (Pro), *Synechococcus* (Syn) and picoeukaryotes (Peuk) were 0.36, 0.81 and 0.90 d^{-1} in summer, and 0.46, 0.58 and 0.56 d^{-1} in winter, respectively. Average grazing mortality rates (m) were 0.46, 0.63 and 0.68 d^{-1} in summer, and 0.25, 0.22 and 0.23 d^{-1} in winter for Pro, Syn and Peuk, respectively. The spatial pattern of both growth and grazing mortality rates showed decreasing trends from the inshore to offshore region, indicating a strong influence of the nutrient gradient induced by Yangtze River input. In summer, Pro, Syn and Peuk were dominant in Kuroshio, transitional and plume regions, respectively, while in winter all the three populations tended to thrive in the offshore regions, particularly for Pro and Syn. Vertically, picophyto-plankton exhibited the highest abundance at ~ 20 m in summer and at the surface in winter. Both growth rate and grazing mortality were higher at the surface than in the deep chlorophyll maximum (DCM) layer. On average, protist grazing consumed 84, 79 and 74 % and 45, 47 and 57 % of production for Pro, Syn and Peuk in summer and winter, respectively.

1 Introduction

Picophytoplankton ($< 5\,\mu m$) – including *Prochlorococcus* (Pro), *Synechococcus* (Syn), and picoeukaryotes (Peuk) – are the dominant components of primary producers in the oligotrophic oceanic waters due to their small size that enables them to have a high ability of nutrient utilization and carbon fixation (Raven, 1998). They are also the essential participants of the microbial food web, playing important roles in nutrient and carbon cycling (Azam et al., 1983; Raven, 1998). Many efforts have been made on studying the ecological, physiological and genetic characters of picophytoplankton in order to get a better understanding of their global distribution and biogeochemical significance (Partensky et al., 1999; Rocap et al., 2003; Zwirglmaier et al., 2008). Environmental factors – such as temperature, salinity, light and nutrient availability – are closely related to picophytoplankton distribution patterns (Flombaum et al., 2013). Seasonal and spatial shifts of different picophytoplankton populations that are adapted to different ecological niches have been reported in different oceanic regions (Olson et al., 1990; Li,

1995; Campbell et al., 1997; Agawin et al., 1998; Chen et al., 2011; Liu et al., 2002a).

Picophytoplankton have a relatively high growth rate and concomitant high mortality of protistan grazing due to their small size; consequently the majority of their fixed carbon is respired back to CO_2, although part of their carbon could reach higher trophic levels (Michaels and Silver, 1988; Legendre and Le Fèvre, 1995). In recent studies, the contribution of picophytoplankton to the global oceanic carbon flux is highlighted to be more important than previously recognized, which can be proportional to their net production through direct ingestion and defecation of gelatinous macrograzers or aggregate formation mediated by fecal pellet transport of mesozooplankton consuming microzooplankton (Richardson and Jackson, 2007; Stukel et al., 2013). The standing stocks of picophytoplankton are mainly regulated by two kinds of factors: one is the bottom-up controls of the ambient environment (e.g., nutrient, light, temperature); the other is the top-down pressure, including zooplankton grazing and viral lysis. As the major consumer of picophytoplankton, microzooplankton undertake the most important role in transferring carbon between picophytoplankton and higher trophic levels. We define microzooplankton here as $< 200\,\mu m$ grazers, which therefore include nanozooplankton (2–20 µm grazers). Previous studies have shown more efficient protist grazing control on picophytoplankton populations than on other phytoplankton groups (Gaul and Antia, 2001; Liu and Dagg, 2003). Furthermore, as revealed by previous nutrient manipulation experiments, protist grazers could respond quickly to picophytoplankton physiological changes induced by nutrient fluctuations and thus control picophytoplankton abundance through enhanced grazing (Landry et al., 2000; Worden and Binder, 2003). However, field studies on top-down mortality controls on picophytoplankton are few in the East China Sea (ECS).

The ECS is situated on the western edge of the western North Pacific, and covers one of the most extensive continental shelves in the world. As a transitional area, the ECS plays an important role in linking terrestrial and oceanic regions, and regulating the regional carbon budget through the "continental shelf pump" (Tsunogai et al. 1999; Liu et al., 2000). The physical environment of the ECS is highly variable, dictated by the distinct seasonality at mid-latitude and influenced by anthropogenic stresses from the adjacent landmass (Gong et al., 2003; Uematsu et al., 2010), as well as mixing among several principal water types. Tremendous amounts of nutrient-rich freshwater from the Yangtze River, one of the world's biggest rivers by discharge volumes, flows into the ECS, with an annual mean of about $3 \times 10^3\,m^3\,s^{-1}$ (Beardsley et al., 1985; Gong et al., 1996). The warm, saline and oligotrophic Kuroshio current flows from south to north along the continental slope and mixes with the shelf water (Chen 1996; Liu and Gan, 2012), creating a gradient of nutrients, as well as chlorophyll and primary production from nearshore to Kuroshio waters (Gong et al., 2000).

The diverse environmental conditions, especially the sharp cross-shelf gradient of physicochemical properties in the upper water column, make the ECS an interesting place to explore the temporal and spatial variabilities of biota. Although picophytoplankton have been the subject of research in the ECS in the past (Chiang et al., 2002; Chang et al., 2003; Jiao et al., 2005; Pan et al., 2005), most of these studies either focused on just one specific population or had limited spatial and temporal coverage. The integrated impact and relative strength of both bottom-up and top-down forcing that drives the picophytoplankton population dynamics remain poorly understood. In this study, we used the dilution technique (Landry and Hassett, 1982) and flow-cytometry-based measurement to estimate growth rate and grazing mortality associated with specific picoplankton populations in the dynamic shelf ecosystems of the ECS. Our goals were to determine the seasonal and spatial variations of the proportions of picophytoplankton populations that were consumed by microzooplankton so as to gain insight into the factors and processes that regulated picophytoplankton growth and mortality rates, and to better understand the complex trophic interaction in microbial food webs in the context of physical variability in the continental shelf system.

2　Methods

2.1　Cruise information

The study was conducted in the East China Sea during two "CHOICE-C" cruises of the R/V *Dongfanghong2* in summer (18–27 August 2009) and winter (14 December 2009–4 January 2010). Fifty and 67 stations along 7 transects were investigated during summer and winter cruise, respectively (Fig. 1). Characterized by distinct hydrographic features, stations can be roughly divided into three water systems: stations in the coastal water affected by Yangtze River input were defined as salinity of near or below 31 (Gong et al., 1996); stations of the Kuroshio warm current located in the outer and deep water region with relatively high temperature and high salinity were defined as salinity near or higher than 34 (Gong et al., 1996; Jiao et al., 2005); and stations situated between the two were defined as the transitional zone. Note that the hydrographical cut-offs were not the absolute standard to define the water systems. The location of stations was also taken into consideration when the hydrographical values were around the cut-offs. Temperature and salinity were determined by CTD probes (Sea-Bird's 911).

Nutrient samples were collected after filtering seawater through 0.45 µm acetate fiber membranes. The inorganic nutrients – including NO_3^-, NO_2^-, PO_4^{3-} and SiO_3^{2-} – were analyzed using the Technicon AA3 Auto-Analyzer (BRAN-LUEBBE) onboard (M. Dai et al., unpublished data). Chlorophyll *a* concentration was obtained using high-performance

Fig. 1. Location of investigated stations in **(A)** summer and **(B)** winter. Plume, transitional, and Kuroshio regions were divided by the grey dotted lines. The PN transect is labeled as a black line.

liquid chromatography (HPLC) following the modified method of Furuya et al. (1998).

For counting ciliates and dinoflagellates, water samples were collected into a 500 mL plastic amber bottle with acidic Lugol's solution (final concentration 5 %). The preserved samples were stored in the dark at room temperature and 100–500 mL was concentrated by settling for 24 h in glass cylinders. Ciliates and dinoflagellates were counted under an inverted microscope (200 ×, Leica Dmirb).

2.2 Flow cytometric analysis of picophytoplankton abundance

Picophytoplankton samples were collected at every station from 3–8 depths of the upper 150 m using Niskin bottles attached to a CTD rosette system for enumeration of population abundance. Seawater (1.8 mL) was fixed with 0.5 % (final concentration) seawater-buffered paraformaldehyde immediately after collection and stored at $-80\,^{\circ}\mathrm{C}$ before analysis. Abundances of autotrophic picophytoplankton (Pro, Syn and Peuk) were enumerated using a Becton-Dickson FACSCalibur flow cytometer under the High flow rate ($\sim 60\,\mu\mathrm{L}\,\mathrm{min}^{-1}$), delineated by the side scattering (SSC) and red/orange auto-fluorescences emitted by chlorophyll/phycoerythrin (Olson et al., 1993). The cytometric graph and differentiation of three populations was shown in Fig. S1. Generally, Pro could be identified on the cytometric plots without overlapping with noises in most cases. But in some cases when the red fluorescence of Pro was dim and overlapped with noises, we used the bright half

of the population histogram to obtain the total number of Pro ($\times 2$). Yellowish-green fluorescence beads (1 µm, Polysciences) were added as an internal standard to calibrate and normalize the fluorescence and light scattering signals. Biovolumes of Pro, Syn and Peuk were converted from normalized signals of SSC using empirically determined equations (Chen et al., 2011) and were then converted to carbon using the conversion factor of 0.28, 0.28 and 0.22 pg C µm^{-3}, respectively (Zubkov et al., 1998; Liu et al., 2007).

2.3 Dilution experimental setup and rate estimation

Dilution experiments were conducted at 12 stations in summer and 14 stations in winter at both the surface and the depth of the deep chlorophyll maximum (DCM) layer (note that at station DH02, DH04, and YZE in winter, the experiment was only conducted at the surface). Seawater samples were collected using an acid-washed plastic bucket or a Niskin bottle attached to a CTD rosette system.

Growth rate and grazing mortality of picophytoplankton populations were measured by the dilution technique (Landry and Hassett, 1982) following protocols of Landry et al. (2003). Duplicate sets of 1.2 L bottles were used to establish a nutrient-enriched dilution series consisting of 15, 27, 50, 73 and 100 % natural seawater (10 bottles in total). Measured amount of filtered seawater prepared by gravity flow through a 0.2 µm filter capsule (Pall Corporation) was added to the experimental bottles, following with gently filling the bottles with natural seawater prescreened through a 200 µm mesh. Inorganic

nutrients were added to all 10 bottles (final concentrations of $0.5\,\mu\text{mol}\,\text{L}^{-1}\,\text{NH}_4\text{Cl}$, $0.03\,\mu\text{mol}\,\text{L}^{-1}\,\text{KH}_2\text{PO}_4$, $1\,\text{nmol}\,\text{L}^{-1}\,\text{FeCl}_3$, and $0.1\,\text{nmol}\,\text{L}^{-1}\,\text{MnCl}_2$) to promote constant phytoplankton growth. Another two bottles filled with unfiltered natural seawater without nutrient amendment were run in parallel with nutrient-amended bottles to account for potential nutrient limitation. The bottles were incubated in an on-deck incubator for 24 h, with temperature controlled by running seawater and in situ light simulated by covering with a neutral density screen. Prior to each experiment, all incubation bottles, capsules, tubing and carboys were washed with 10 % HCl and rinsed thoroughly with distilled water and ambient seawater. The capsules were soaked in 10 % HCl for more than 2 h before the first use and were washed with 10 % HCl, distilled water and the ambient seawater between each experiment (Landry et al., 1995). Picophytoplankton samples for flow cytometric analysis were taken from initial seawater and all experimental bottles after incubation.

Picophytoplankton growth rate with nutrient amendment (μ_n) and grazing mortality (m) were calculated from the linear regression relationship between net growth rate (with nutrient) and dilution factor (the proportion of natural seawater in dilution treatment) (Landry et al., 2003). The instantaneous growth rate (μ_0, growth rate in water without nutrient addition) was determined by adding the grazing rate to the population net growth rate in bottles without nutrient addition (k_0). To assess the biomass production (P) and grazing loss (G) on picophytoplankton populations, the following formulas were used according to Landry et al. (2003):

$$P = \mu_0 \cdot C_m$$

$$G = m \cdot C_m$$

$$C_m = C_0\left[e^{(\mu_0-m)t} - 1\right] / (\mu_0 - m)\,t,$$

where C_m is the mean concentration of picophytoplankton carbon biomass during the incubations, C_0 is the initial concentration of picophytoplankton carbon biomass, and t is the incubation time (1 d). The percentage of production of each picophytoplankton population that was consumed by microzooplankton was calculated as m / μ_0.

2.4 Data analysis

Flow cytometric data were analyzed using WinMDI software 2.9 (Joseph Trotter, Scripps Research Institute, La Jolla, CA, USA). Contour plots were generated using Ocean Data View (Schlitzer, 2006). Spearman rank–order correlation analysis was used to detect the significant relationships between variables.

3 Results

3.1 Hydrographical condition in the ECS

Very different hydrographic features were obtained in summer and winter. The sea surface temperature ranged from 23.2 to 30.7 °C in summer and from 9.2 to 23.6 °C in winter (Fig. 2a and b). The surface salinity was generally lower in summer (ranging from 17.2 to 34.0) than in winter (ranging from 27.1 to 34.6). Both temperature and salinity showed increasing gradients from inshore to offshore (Fig. 2, Table 1). In summer, the inshore regions of the ECS were strongly affected by freshwater discharged from the Yangtze River, resulting in a strong plume of low-salinity water spreading to near 125° E (Fig. 2c). However, the influence of the river input was much weaker in winter (Fig. 2d). A dramatic increase of surface temperature can be observed in the Kuroshio region in winter, which was about 10.5 and 4.5 °C higher than that of coastal and mixing regions, respectively.

The surface $\text{NO}_3^- + \text{NO}_2^-$ (N) and PO_4^{3-} (P) concentrations were much higher in winter (median value of $5.27\,\mu\text{mol}\,\text{L}^{-1}$ for N and $0.37\,\mu\text{mol}\,\text{L}^{-1}$ for P) than in summer (median value of below detection limit for both N and P) due to strong vertical mixing in winter (Fig. 2e–h). Affected by river discharge, the nutrient concentrations in the plume region were very high (with average surface N concentration of 9.69 and $23.34\,\mu\text{mol}\,\text{L}^{-1}$ in summer and winter, respectively), and showed a clear decreasing trend from the inshore plume region to offshore Kuroshio region (Fig. 2e and f; Table 1). The N / P ratio was generally lower in summer than in winter, and showed different regional patterns in both seasons (Fig. 2i and j). The area of P limitation indicated by N / P ratio > 16 was mostly confined in the coastal regions in the ECS, especially in the Yangtze River plume region, while the offshore region is mainly N-limited. Generally, the area of P limitation was larger in summer than that in winter.

The surface Chl a concentration was highly variable in summer (ranging from 0.07 to $35.3\,\mu\text{g}\,\text{L}^{-1}$ with median value of $0.20\,\mu\text{g}\,\text{L}^{-1}$), decreasing dramatically across the shelf (Fig. 2k). However, the Chl a concentration in winter was generally evenly distributed (ranging from 0.29 to $0.97\,\mu\text{g}\,\text{L}^{-1}$ with median value of $0.44\,\mu\text{g}\,\text{L}^{-1}$) (Fig. 2l).

We selected the PN transect, a well-studied transect from the Yangtze River estuary, across the continental shelf, to the offshore region, to illustrate the vertical pattern of hydrographical parameters (Fig. 3). In summer, strong stratification was observed, with higher temperature, lower salinity and lower nutrient concentrations in the surface layer, whereas in winter the hydrographic parameters were homogenous through the whole water column due to strong-wind-induced mixing. This pattern led to different depths of the DCM layer in the two seasons: a clear DCM could be found in the subsurface layer (at ~ 20 m in the plume region and ~ 50 m in transitional and Kuroshio regions) in

summer, whereas the vertical difference of Chl in winter was not clearly observed.

3.2 Distribution patterns of picophytoplankton abundance

Pro – On average, the surface average abundance of Pro was 5.6×10^4 cells mL^{-1} (ranging from 0 to 3.2×10^5 cells mL^{-1}) in summer, which was about 4 times higher than the value of 1.6×10^3 cells mL^{-1} (ranging from 0 to 2.3×10^5 cells mL^{-1}) in winter. Their distribution was largely confined in oligotrophic waters in the transitional and Kuroshio regions, but absent in the plume zones (Fig. 4a and b). The surface abundance of Pro in the transitional and Kuroshio zones was comparable in summer, but much higher in the Kuroshio region than in the transitional region in winter (independent t test, $p < 0.01$) (Table 1). Spearman rank correlation test (all surface data points) revealed that Pro abundance was significantly negatively correlated with nutrient concentrations and positively correlated with salinity in both seasons (Table 2). A negative correlation between Pro abundance and Chl a concentration was observed in summer for the whole area, while a positive correlation was obtained in winter (Table 2). Vertically, taking the PN transect as an example, the abundance of Pro exhibited a subsurface maxima around 20 m in the transitional region, and 40 m in the Kuroshio region in summer; they were distributed homogenously from 0 to 60 m in the offshore region during winter (Fig. 5a and b).

Syn – The surface average abundance of Syn was also much higher in summer (ranging from 7.0×10^3 to 3.8×10^5 cells mL^{-1} with average value of 7.6×10^4 cells mL^{-1}) than in winter (ranging from 561 to 9.1×10^4 cells mL^{-1} with average value of 1.0×10^4 cells mL^{-1}), which was similar to the trend found for Pro. Higher surface abundance was observed in the plume region than the transitional (independent t test, $p < 0.01$) and Kuroshio (independent t test, $p < 0.05$) regions in summer (Fig. 4c). Inversely, in winter, the highest abundance was observed in the Kuroshio region (Table 1), exceeding that in the plume (independent t test, $p < 0.01$) and transitional (independent t test, $p < 0.01$) regions by about 32- and 5.6-fold, respectively (Fig. 4d). No significant correlations were observed between Syn abundance and environmental factors in summer (Table 2), while Syn abundance showed significant positive correlation with temperature and salinity and negative correlation with nutrient concentration in winter.

Peuk – The abundance of Peuk was comparable in the two seasons with average value of about 4×10^3 cells mL^{-1}. Though they were less abundant compared with Pro and Syn, they contributed significantly to picophytoplankton biomass (Table 1). Peuk exhibited very different distribution patterns in the two seasons: in summer, the average abundance decreased by 2.8 and 4.4 times from the plume region to the transitional and Kuroshio regions, whereas in winter it increased dramatically by 4.6 and 9.2 times from the plume region to the transitional and Kuroshio region, respectively (Table 1; Fig. 4e and f). In contrast to Pro, in summer, Peuk tended to distribute in regions with lower salinity and higher nutrient and Chl a concentration, indicated by correlation analysis (Table 2). In winter, the distribution of Peuk was similar to that of Pro and Syn, which was positively correlated with temperature, salinity and Chl a concentration, but negatively correlated with nutrient concentrations. The highest abundances of Syn and Peuk occurred at around 10 to 20 m depths in the inshore region and 40 m in the offshore the region in summer, while in surface in winter (Fig. 5c–f).

3.3 Growth and grazing of picophytoplankton populations and their associations with environmental variables

Seawater-dilution-experiment-based estimates for growth rate and grazing mortality of picophytoplankton populations were depicted spatially in Fig. 6, with the details of each experiment in the supplemental data of Table S1. Out of 49 experiments (24 in summer and 25 in winter), positive mortality rates ($m > 0.05$ d^{-1}) were obtained in 18 experiments for Pro (Pro only existed at 22 stations of all experiments), 40 for Syn, and 42 for Peuk; and most of the negative or near-zero mortality rates were observed in winter (Table S1).

Pro – The average growth rate for Pro was 0.36 d^{-1} in summer and 0.46 d^{-1} in winter (Table 4), and showed little nutrient limitation indicated by $\mu_0/\mu_n \approx 1$ or > 1 except in the Kuroshio region in summer ($\mu_0/\mu_n = 0.87$) (Table 3). Vertically, higher average growth rates were observed at DCM in summer, but at the surface in winter (Fig. 6a-A and D; Table 4). Average grazing mortality for Pro was 0.46 d^{-1} in summer, which accounted for 84 % of Pro production. In winter, average grazing mortality deceased to about half (0.25 d^{-1}) of the summer rates, accounting for just 45 % (ranged from 12 to 133 %) daily production on average (Table 4). Grazing mortality showed a general decreasing trend from the transitional region to the Kuroshio region, except high values in the surface layer in summer and the DCM layer in winter at Kuroshio station DH13. Negative correlation was observed between grazing mortality and salinity ($r = -0.576$, $p < 0.001$; Table 5) when pooling all data points together. Microzooplankton consumed a higher proportion of Pro production in the transitional region during summer and in the Kuroshio region during winter (Table 3).

Syn – The average growth rate for Syn was 0.81 d^{-1} in summer and 0.58 d^{-1} in winter. The growth rate was positively correlated with ambient N concentrations ($r = 0.343$, $p < 0.05$; Table 5) and negatively correlated with salinity ($r = -0.312$, $p < 0.05$; Table 5) when pooling all data points together. Nutrient limitation occurred in summer, especially in the Kuroshio region with a μ_0/μ_n ratio of 0.64 and in the surface layer of all regions with a μ_0/μ_n ratio of
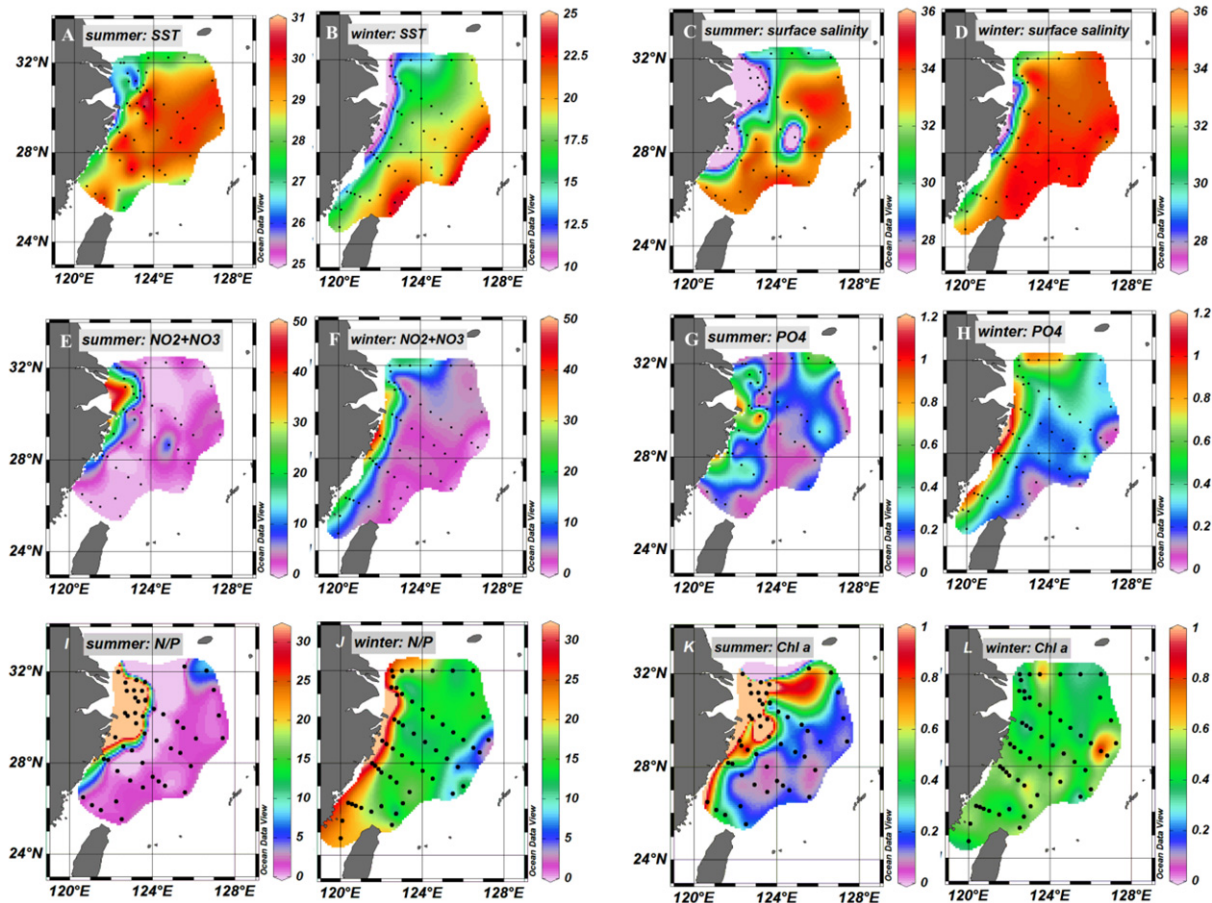
Fig. 2. Spatial distributions of sea surface **(A, B)** temperature, **(C, D)** salinity (psu), **(E, F)** $NO_2^- + NO_3^-$ concentration ($\mu g\,L^{-1}$), **(G, H)** PO_4^{3-} concentration ($\mu g\,L^{-1}$), **(I, J)** N/P molar ratio, and **(K, L)** Chl a concentration ($\mu g\,L^{-1}$) in summer (18–27 August 2009) and winter (14 December 2009–4 January 2010).

0.83. Grazing mortality of Syn averaged $0.63\,d^{-1}$ in summer, which was almost 3 times higher than the value of $0.22\,d^{-1}$ in winter, equaling 79 % (ranged from 21 to 235 %) and 47 % (ranged from 24 to 82 %) of daily Syn production in summer and winter, respectively. It was positively correlated with temperature ($r = 0.447$, $p < 0.01$; Table 5) and abundance of ciliates and dinoflagellates ($r = 0.451$, $p < 0.01$; Table 5), and negatively correlated with salinity ($r = -0.481$, $p < 0.01$; Table 5) when pooling all data points together. Both growth and grazing rates declined from the plume to Kuroshio region in two seasons (Fig. 6a and b, Table 3), with a high growth rate of $2.58\,d^{-1}$ observed at plume station YZ13 in summer.

Peuk – The average growth rate for Peuk was similar to that of Syn: $0.90\,d^{-1}$ in summer and $0.56\,d^{-1}$ in winter. The growth rate was negatively correlated with salinity in both seasons (Table 5) and positively correlated with ambient N concentration ($r = 0.433$, $p < 0.01$; Table 5) when pooling all data points together. Microzooplankton grazing rate was $0.68\,d^{-1}$ in summer, which was 3 times higher than $0.23\,d^{-1}$

in winter, consuming 74 % (ranged from 24 to 119 %) and 57 % (ranged from 11 to 144 %) of Peuk production in summer and winter, respectively. Higher growth rate and grazing mortality were observed in the surface layer and in the plume region (Fig. 6a and b, Table 3). The grazing mortality of Peuk was positively correlated with temperature ($r = 0.415$, $p < 0.01$; Table 5) and abundance of ciliates and dinoflagellates ($r = 0.408$, $p < 0.01$; Table 5) and negatively correlated with salinity ($r = -0.507$, $p < 0.02$; Table 5) when pooling all data points together. The percentage of Peuk production consumption was similar in three regions in both seasons (Table 3), which was different from that of Pro and Syn.

Growth and grazing estimates of Syn and Peuk were significantly and positively correlated (Fig. 7b and c). The slope of linear regression curve for Syn was close to one (0.87), and larger than that of Peuk (0.56).

Negative correlations were obtained between grazing mortality and abundance for Syn and Peuk, and the correlation was significant in summer for Peuk (Fig. 8b and c). There was no overall relationship found between the grazing

Table 1. Summary of environmental factors and biomass of picophytoplankton populations in the three water systems in the ECS. The values reported here are averages of surface data, with the range of the values in parentheses.

	Summer			Winter		
	Coastal	Mix	Kuroshio	Coastal	Mix	Kuroshio
Temperature	28.32	29.54	28.92	10.93	17.01	21.45
(°C)	(23.19–30.73)	(28.49–30.28)	(27.45–29.87)	(9.24–12.69)	(13.23–19.32)	(18.65–23.55)
Salinity	28.17	33.38	33.80	29.87	33.41	34.51
	(17.22–32.04)	(32.40–33.68)	(33.53–34.03)	(27.05–31.37)	(30.5–34.41)	(34.19–34.62)
[Chl a]	2.73	0.22	0.13	0.44	0.45	0.52
(μg L^{-1})	(0.30–35.3)	(0.07–0.93)	(0.08–0.20)	(0.34–0.56)	(0.32–0.68)	(0.29–0.97)
$NO_2^- + NO_3^-$	9.69	0.11	0.10	23.34	7.92	2.05
(μmol L^{-1})	(0.10–40.94)	(0.10–0.22)	(0.10–0.11)	(13.43–40.06)	(2.61–22.68)	(0.29–4.49)
PO_4^{3-}	0.26	0.09	0.17	0.79	0.46	0.15
(μmol L^{-1})	(0.08–0.71)	(0.08–0.17)	(0.08–0.66)	(0.54–1.24)	(0.23–0.93)	(0.08–0.32)
SiO_3^{2-}	9.69	2.99	1.09	24.17	10.50	2.97
(μmol L^{-1})	(0.20–36.08)	(1.18–6.95)	(0.67–1.98)	(15.49–37.83)	(4.54–26.30)	(0.83–5.67)
Pro abund	4.2	115.8	119.7	0	0.4	57.0
(10^3 cells mL^{-1})	(0–42.1)	(0–318.0)	(18.8–231.5)		(0–4.8)	(0.8–234.2)
Syn abund	97.6	58.6	25.0	0.8	4.9	27.2
(10^3 cells mL^{-1})	(7.0–380.2)	(17.8–152.5)	(8.3–62.0)	(0.6–1.1)	(0.7–14.0)	(6.8–90.6)
Peuk abund	6.5	2.1	1.5	1.0	4.7	9.5
(10^3 cells mL^{-1})	(0.8-64.3)	(0.8–7.8)	(1.4–1.8)	(0.7–1.8)	(1.1–19.5)	(3.6–19.1)

Detection limit of 0.1 μmol L^{-1} and 0.08 μmol L^{-1} were used for [$NO_2^- + NO_3^-$] and [PO_4^{3-}] calculation, respectively, when the concentration was undetectable.

Table 2. Spearman's rank correlation coefficient between environmental factors and abundances of picophytoplankton populations.

	Temp	Sal	N	P	Chl	Pro_abund	Syn_abund	Peuk_abund
Summer								
Pro_abund	0.277	0.782**	−0.696**	−0.354*	−0.801**	1	−0.145	−0.247
Syn_abund	0.036	−0.236	−0.008	0.004	0.147	−0.145	1	0.552**
Peuk_abund	−0.195	−0.419**	0.415**	0.315*	0.396**	−0.247	0.552**	1
Winter								
Pro_abund	0.846**	0.869**	−0.770**	−0.791**	0.337*	1	0.837**	0.666**
Syn_abund	0.905**	0.857**	−0.818**	−0.843**	0.456**	0.837**	1	0.874**
Peuk_abund	0.778**	0.668**	−0.626**	−0.660**	0.515**	0.666**	0.874**	1

Surface data were used for the correlation test; summer: $N = 44$; winter: $N = 47$. * Correlation is significant at 0.05 level; ** correlation is significant at 0.01 level.

mortality and cell abundance for Pro, although a significant negative correlation existed for the winter data. Interestingly, growth rates for all three populations were negatively correlated with their abundances, though the relationship was not always significant (Fig. 8d–f).

3.4 Seasonal and spatial variations of picophytoplankton biomass and their contribution to microzooplankton carbon consumption

The standing stock of picophytoplankton carbon biomass (C_0) in summer and winter showed opposite distribution patterns. In three regions from inshore to offshore, C_0 decreased with average value of 10.96, 8.77 and 3.64 ng mL^{-1} in summer, and increased with average value of 1.58, 5.83 and 6.00 ng mL^{-1} in winter, respectively (Fig. 9a). In summer, the proportion of Syn biomass decreased from the plume region to the Kuroshio region, while that of Pro demonstrated an increasing trend. In winter, Peuk dominated in all three regions, with its carbon biomass occupying 66–90 % of total biomass.

Similar to C_0, the amount and composition of carbon biomass that was consumed by microzooplankton (G) varied in both seasonal and spatial scales. Microzooplankton consumed 8.80, 2.92, and 1.30 ng C mL^{-1} in coastal, transitional and Kuroshio regions in summer, and 1.54, 2.03, and 1.21 ng C mL^{-1} in winter, respectively (Fig. 9b). Among the total picophytoplankton carbon consumed by microzooplankton, $\sim$ 50 % was contributed by Peuk and the remaining

Table 3. Comparison of m, μ, m/μ_0, and μ_0/μ_n of three populations in plume, transitional and Kuroshio regions in summer and winter.

	Summer			Winter		
	Plume	Transitional	Kuroshio	Plume	Transitional	Kuroshio
Pro_m		0.34 (0.15)	0.92 (0.85)		0.19 (0.13)	0.28 (0.13)
Pro_μ_0		0.35 (0.18)	0.38 (0.01)		0.50 (0.37)	0.44 (0.17)
Pro_m/μ_0		1.09 (0.63)	0.46 (0.51)		0.39 (0.04)	0.47 (0.41)
Pro_μ_0/μ_n		1.25 (0.10)	0.87		0.97 (0.12)	1.11 (0.37)
Syn_m	1.11 (0.76)	0.41 (0.23)	0.38 (0.17)	0.44	0.18 (0.14)	0.26 (0.20)
Syn_μ_0	1.08 (0.72)	0.74 (0.40)	0.55 (0.40)	1.04	0.67 (0.40)	0.39 (0.43)
Syn_m/μ_0	1.08 (0.58)	0.57 (0.20)	0.88 (0.82)	0.43	0.34 (0.28)	0.26 (0.12)
Syn_μ_0/μ_n	1.25 (0.65)	0.85 (0.32)	0.64 (0.15)	1.21	1.03 (0.12)	1.10 (0.44)
Peuk_m	1.18 (0.72)	0.35 (0.17)	0.76 (0.56)	0.72	0.18 (0.07)	0.25 (0.16)
Peuk_μ_0	1.53 (0.79)	0.45 (0.32)	0.92 (0.37)	1.21	0.61 (0.51)	0.37 (0.53)
Peuk_m/μ_0	0.76 (0.10)	0.74 (0.33)	0.66 (0.59)	0.60	0.54 (0.43)	0.62 (0.29)
Peuk_μ_0/μ_n	1.20 (0.32)	0.81 (0.27)	0.98 (0.14)	0.96	1.03 (0.15)	0.79 (0.26)
Abundance of ciliates and dinoflagellates (cells L^{-1})	3995 (4295)	1514 (1525)	1285 (667)		1069 (1767)	649 (662)

Values of $\mu_0 < 0.05$ and $m < 0.05$ were removed when calculating the average m and μ_0.
Values of $\mu_0 < 0.1$ and $m < 0.1$ were removed when calculating m/μ_0.
The numbers in parenthesis are standard errors of the mean.

Table 4. Comparison of m, μ, m/μ, and μ_0/μ_n of three populations at the surface and in DCM layers in summer and winter.

	Summer			Winter		
	Surface	DCM	Avg.	Surface	DCM	Avg.
Pro_m	0.52 (0.50)	0.34 (0.17)	0.46 (0.40)	0.29 (0.03)	0.19 (0.19)	0.25 (0.13)
Pro_μ_0	0.31 (0.11)	0.39 (0.17)	0.36 (0.14)	0.61 (0.20)	0.32 (0.15)	0.46 (0.22)
Pro_m/μ_0	0.82	0.85 (0.71)	0.84 (0.62)	0.36 (0.18)	0.51 (0.54)	0.45 (0.38)
Pro_μ_0/μ_n		1.15 (0.21)	1.15 (0.21)	1.04 (0.02)	1.07 (0.28)	1.08 (0.20)
Syn_m	0.72 (0.70)	0.49 (0.28)	0.63 (0.56)	0.21 (0.16)	0.23 (0.23)	0.22 (0.19)
Syn_μ_0	0.84 (0.66)	0.64 (0.29)	0.81 (0.54)	0.71 (0.42)	0.40 (0.15)	0.58 (0.37)
Syn_m/μ_0	0.91 (0.62)	0.82 (0.31)	0.79 (0.51)	0.47 (0.24)	0.45 (0.23)	0.47 (0.22)
Syn_μ_0/μ_n	0.83 (0.32)	1.13 (0.61)	0.94 (0.48)	1.09 (0.36)	1.05 (0.13)	1.07 (0.28)
Peuk_m	0.85 (0.68)	0.41 (0.31)	0.68 (0.59)	0.27 (0.17)	0.19 (0.17)	0.23 (0.17)
Peuk_μ_0	1.07 (0.83)	0.57 (0.42)	0.90 (0.73)	0.74 (0.52)	0.30 (0.18)	0.56 (0.46)
Peuk_m/μ_0	0.80 (0.26)	0.71 (0.30)	0.74 (0.27)	0.48 (0.28)	0.66 (0.46)	0.57 (0.37)
Peuk_μ_0/μ_n	0.90 (0.20)	1.11 (0.41)	0.99 (0.32)	0.95 (0.14)	1.00 (0.25)	0.96 (0.18)
Abundance of ciliates and dinoflagellates (cells L^{-1})	2785 (3840)	1639 (1242)	2303 (2878)	1118 (1841)	659 (613)	878 (1361)

Values of $\mu_0 < 0.05$ and $m < 0.05$ were removed when calculating average m and μ_0.
Values of $\mu_0 < 0.1$ and $m < 0.1$ were removed when calculating m/μ_0.
The numbers in parenthesis are standard errors of the mean.

$\sim 50\%$ came from Pro and Syn in summer, while in winter the consumption of Peuk became more important, reaching $\sim 80\%$. Spatially, in summer, Pro was a more important food for microzooplankton in the Kuroshio region than in the transitional region, accounting for 25 and 15 % in total picophytoplanton carbon loss, respectively. The grazing loss of Syn exhibited the opposite trend, accounting for 47, 38, and 19 % in total picophytoplankton carbon loss in the plume,

Table 5. Spearman's rank correlation coefficient between environmental factors and m and μ of picoplankton populations. The numbers in parenthesis are N values of correlation analysis.

	Temperature	Salinity	Chl	N	P	Abundance of ciliates and dinoflagellates
Pro_m	0.321	−0.576**	−0.260	0.653*	0.086	0.179
	(19)	(19)	(19)	(14)	(14)	(21)
Syn_m	0.447**	−0.481**	0.100	0.136	0.055	0.451**
	(46)	(46)	(45)	(38)	(34)	(45)
Peuk_m	0.415**	−0.507**	0.032	0.037	0.081	0.408**
	(47)	(47)	(46)	(39)	(35)	(46)
Pro_μ_0	−0.411	0.077	0.601*	0.235	−0.014	0.324
	(14)	(14)	(14)	(14)	(12)	(16)
Syn_μ_0	0.043	−0.312*	0.049	0.343*	0.200	0.218
	(45)	(45)	(44)	(38)	(34)	(44)
Peuk_μ_0	0.051	−0.520**	0.125	0.433**	0.246	0.288
	(41)	(41)	(40)	(35)	(31)	(40)

N: $NO_3^- + NO_2^-$; P: PO_4^{3-}
* Correlation is significant at 0.05 level; ** correlation is significant at 0.01 level.
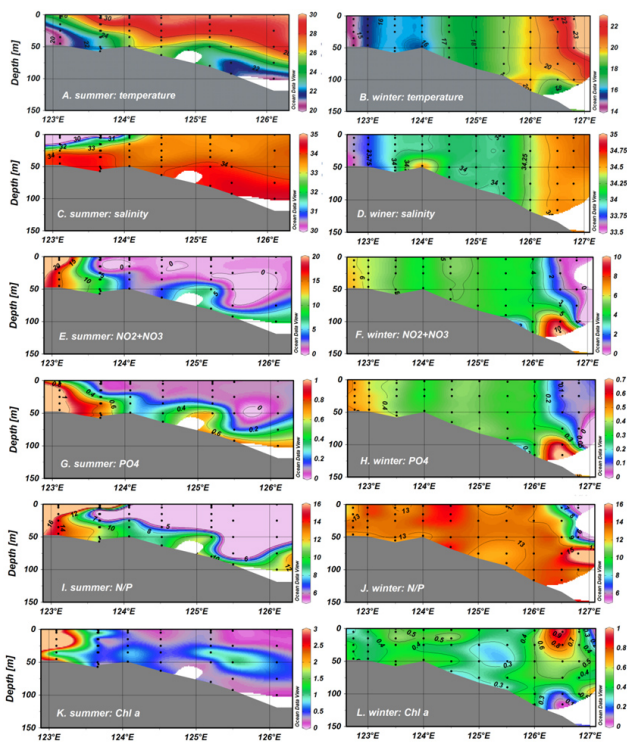
Fig. 3. Vertical distributions of **(A, B)** temperature, **(C, D)** salinity (psu), **(E, F)** $NO_3^- + NO_2^-$ concentration ($\mu g \, L^{-1}$), **(G, H)** PO_4^{3-} concentration ($\mu g \, L^{-1}$), **(I, J)** N / P, and **(K, L)** Chl a concentration ($\mu g \, L^{-1}$) in summer and winter along PN transect.

transitional and Kuroshio regions, respectively. In winter, the composition of biomass grazing loss was similar in the three regions, with Peuk dominating.

4 Discussion

4.1 Effect of environmental factors on picophytoplankton distribution and population transitions

A critical issue in marine plankton ecology is whether the distributions of water masses determine the distributions of plankton. Our study area covers a broad range of biogeochemical provinces and bears strong seasonality. Generally, three water systems could be clearly observed: the Yangtze River plume region with relatively low temperature and low salinity; the offshore Kuroshio region with relatively warm and saline water; and the transitional region covering the shelf between the two regions. The diverse hydrographical conditions significantly influenced the distribution of picophytoplankton, causing dramatic changes in the distribution patterns both spatially and seasonally.

Being abundant in summer and in the nutrient-depleted Kuroshio region and absent in the eutrophic plume region, Pro showed their preference of stratified, oligotrophic and warm habitat, which is in accordance with findings in other tropical and subtropical regions (Campbell et al., 1997; Liu et al., 1998; Flombaum et al., 2013). It has been suggested that Pro lack genes responsible for nutrient-uptake regulation and lost their capability to utilize NO_3 (Rocap et al., 2003; Martiny, 2006; Martiny et al., 2009). Moreover, it is reported that elevated nutrient concentration and trace metal from mixing or atmospheric deposition in the coastal region could be toxic to them (Worden and Binder, 2003; Paytan et al., 2009). At the same time, high grazing pressure resulting from high protist abundance in the coastal area led to efficient consumption

of Pro. All of the above factors may have contributed to the disappearance of Pro in the coastal area.

The abundance of Syn was also much higher in summer than in winter in the ECS, which is in agreement with previous studies conducted in the ECS and other coastal regions (Agawin et al., 1998; Jiao et al., 2005; Chen et al., 2009). On the other hand, high Syn abundance has been observed in winter in some tropical and subtropical oceans (Olson et al., 1990; Campbell et al., 1997; Liu et al., 2007). Such different patterns may be attributed to the combined effects of temperature and nutrient level. Compared with Pro, Syn were reported to be more nutrient dependent and adapted to environment with intermediate nutrient level (Liu et al. 1998; Chen et al., 2011). Although they were able to grow in response to nanomolar addition of nitrate (Glover et al., 2007), the optimal N concentration for Syn growth was 0.25 μM in the Mediterranean Sea and 0.1–3 μM in the Arabian Sea, and high N concentration of about > 8 μM could inhibit Syn growth (Liu et al., 1998; Agawin et al., 2000a). In addition to nutrient level, Syn growth has also been shown to increase with enhancement of temperature (Agawin et al., 1998; Li, 1998; Jiao et al., 2005). Therefore, in some highly stratified tropical and subtropical oligotrophic oceans, the enhanced nutrient availability by winter mixing can trigger a "bloom" of Syn (Olson et al., 1990; Liu et al., 2007). In the ECS, however, due to dramatic seasonal variation of temperature, the abundance of Syn in winter was much lower than that in summer. Unlike Pro and Peuk, which have their distribution significantly correlated with nutrient concentrations, we did not observe any correlation between Syn abundance and environmental factors in summer, suggesting adaptation of Syn in intermediate trophic environments. However, the population transition of Pro and Syn was not only driven by nutrients; it also depended on the balance between growth and grazing mortality. A higher average growth rate of Syn can be observed in the transitional region than in the Kuroshio region and that of Pro in the transitional region. The higher growth potential of Syn is an important advantage for them to dominate when conditions are favorable. At the same time, however, the increased phytoplankton biomass and growth rate in the inshore area also drives the increase of grazing pressure, as indicated by the close couplings between growth rate and grazing mortality of picoplankton populations. Therefore, the mortality effect on slower-growing taxa like Pro can exceed a sustainable growth-rate level, and Syn can replace Pro as the dominant population with increasing nutrient concentrations.

Different from Pro and Syn, which showed significant seasonal variations, the average abundance of Peuk was almost the same in the two seasons. Therefore, it was most likely that succession of Peuk species composition in response to hydrographic conditions in different seasons resulted in a relatively stable bulk abundance due to the high diversity nature of Peuk (Diez et al., 2001; Worden, 2006). Peuk were more abundant in coastal areas than oceanic areas in summer, with
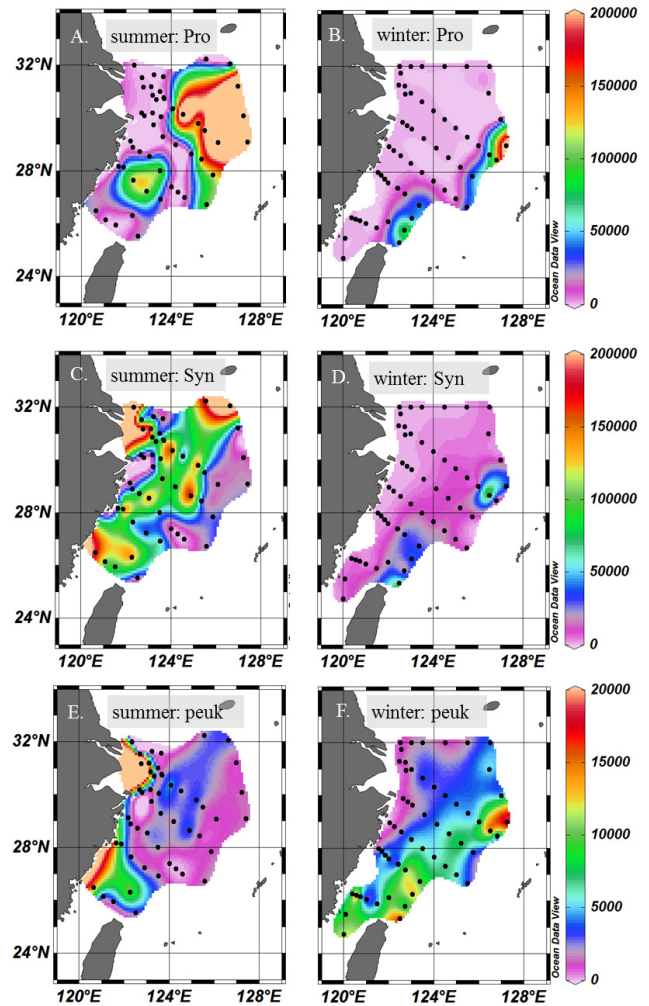
Fig. 4. Spatial distributions of surface abundances (cells mL^{-1}) of **(A, B)** *Prochlorococcus* (Pro), **(C, D)** *Synechococcus* (Syn), and **(E, F)** picoeukaryotes (Peuk) during summer and winter.
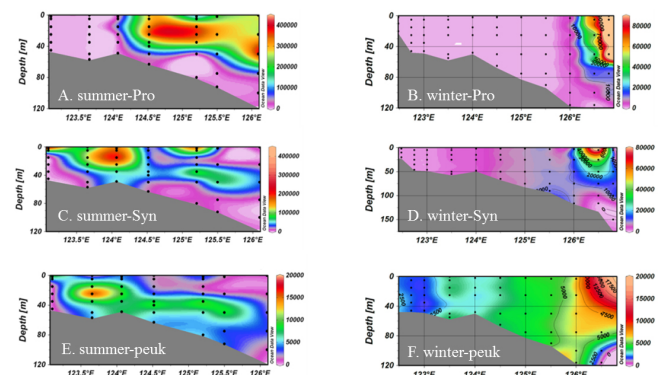

Fig. 5. Vertical profile of abundances (cells mL^{-1}) of **(A, B)** *Prochlorococcus* (Pro), **(C, D)** *Synechococcus* (Syn), and **(E, F)** picoeukaryotes (Peuk) during summer and winter along the PN transect.
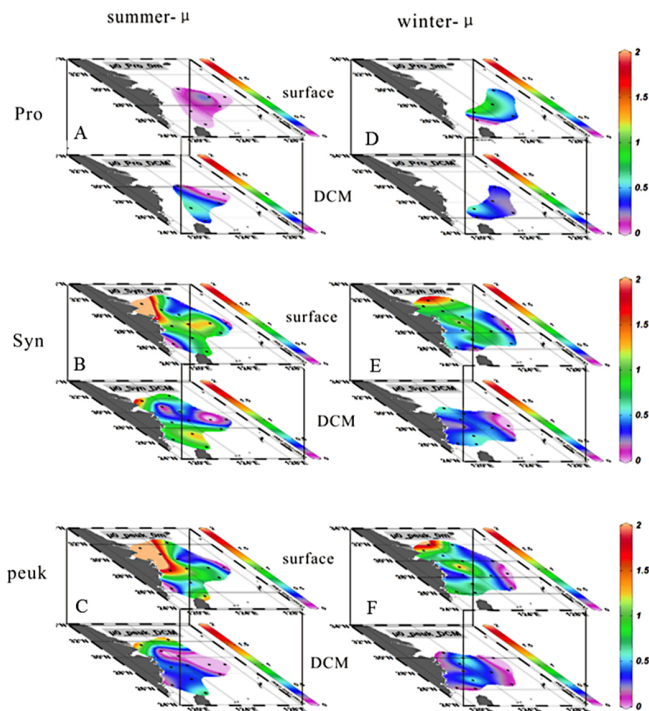
Fig. 6a. Spatial pattern of the growth rate (μ) of the three picophytoplankton populations in **(A–C)** summer and **(D–F)** winter. The two layers are surface and deep chlorophyll maximum (DCM) layer.
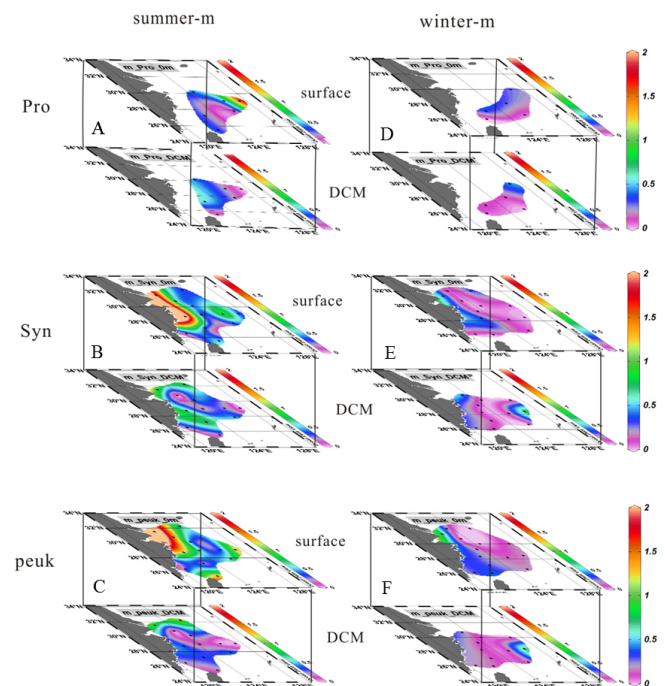
Fig. 6b. Spatial pattern of the grazing mortality (m) of the three picophytoplankton populations in **(A–C)** summer and **(D–F)** winter. The two layers are surface and deep chlorophyll maximum (DCM) layer.

an opposite trend in winter. This pattern can be captured most obviously in areas near the Yangtze River estuary, where Peuk abundance was highest in summer, but lowest in winter. The low Peuk abundance in the plume region in winter may be due to either low-temperature- or high-turbidity-induced light limitation.

4.2 Effect of environmental factor on picophytoplankton growth rate

The average growth rates over the study area for Pro, Syn and Peuk were 0.36, 0.81 and 0.90 d^{-1} in summer and 0.46, 0.58 and 0.56 d^{-1} in winter, respectively. The values are within the range of recent studies conducted in marginal or coastal seas (Hirose et al., 2007; Chen et al., 2009), while they are generally higher than the values for the open ocean (Landry et al., 1995, 2003; Liu et al., 2002b; Paterson et al., 2007) and the study on the ECS in the last decade (Chang et al., 2003).

Seasonally, consistent with the abundance of picophytoplankton, the average growth rates of Syn and Peuk were higher in summer than in winter due to the factor of temperature, which has often been identified as the major factor causing the seasonal variations in growth rates (Agawin et al., 1998; Agawin et al., 2000b). Spatially, however, high abundance of picophytoplankton populations did not coincide with high growth rate, indicated by the negative associations between growth rate and abundance of picophytoplank-

ton populations (Fig. 8). As the nutrient concentration in the seawater decreased along the freshwater plume from inshore to offshore, the growth rate estimates of Syn and Peuk also exhibited trends of decrease indicated by the negative correlation between growth rate and salinity (Table 5). A high average growth rate of $> 1\,\mathrm{d}^{-1}$ for Syn and Peuk was observed in the plume region in summer, with a maximum value of $> 2.5\,\mathrm{d}^{-1}$ at station YZ13 located in the Yangtze River estuary. This observation is consistent with previous findings of high growth rates in the river plume regions (Chang et al, 2003; Chen et al., 2009).

That nutrient availability in the ambient environment exerts influence on population-specific growth rates of picophytoplankton can also be reflected by μ_0/μ_n. The ratio was higher in the plume regions than in the other two regions, which is consistent with the patterns of nutrient concentrations. For example, in summer, μ_0/μ_n for Syn and Peuk in the nutrient-rich plume region were mostly > 1, but dropped to around 0.8 in the transitional and Kuroshio regions where nutrient concentration was depleted to levels that might suppress picophytoplankton growth. In winter, the ratio in offshore regions was around 1, and it was higher than that in summer, indicating no apparent nutrient limitation due to more nutrient supply by mixing. Nonetheless, under the same hydrographical condition, the growth of the three picophytoplankton populations demonstrated different nutrient requirement. For example, in the transitional region in summer,
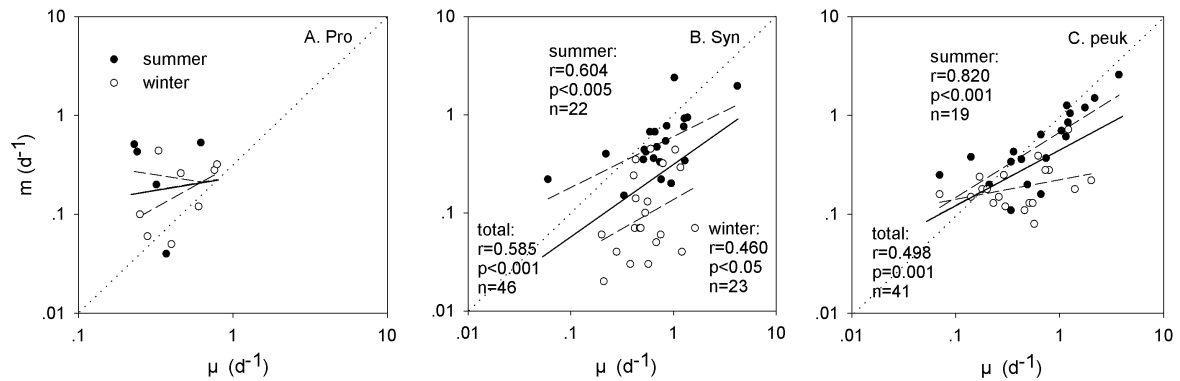
Fig. 7. Relationship between grazing mortality (m) and growth rate (μ) for **(A)** *Prochlorococcus* (Pro), **(B)** *Synechococcus* (Syn) and **(C)** picoeukaryotes (Peuk). The dotted line is a 1 : 1 line for the two parameters. The short dash line and solid line are the regression curves for each season separately and two seasons together, respectively. r, p and n are correlation coefficients, statistical significance levels and number of data points.
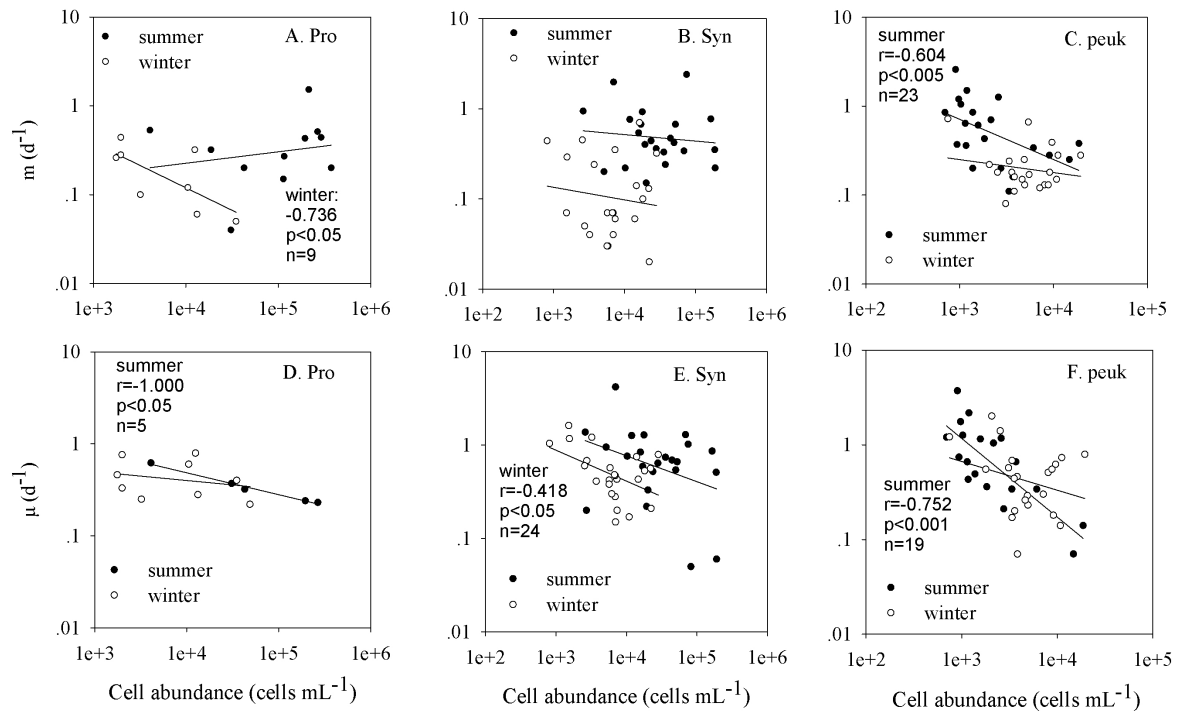

Fig. 8. Relationship between cell abundance and **(A–C)** grazing mortality (m) and **(D–F)** growth rate (μ). Solid lines are the regression curves of each season. r, p and n are correlation coefficients, statistical significance levels and number of data points.

μ_0/μ_n were 1.24, 0.85 and 0.81 for Pro, Syn and Peuk, respectively; in the Kuroshio region in winter, on the other hand, μ_0/μ_n were 0.79 for Peuk and about 1.10 for both Pro and Syn. These ratios suggest that the growth of Peuk was more likely being limited by nutrient concentration, while the growth of Pro might be negatively affected by nutrient amendment. The different nutrient requirement by Pro, Syn and Peuk could also be evidenced by the differentiated correlation coefficient between growth rate and nutrient concentrations of different picophytoplankton populations (Table 5).

4.3 Effect of environmental factor on spatial and seasonal pattern of grazing mortality

Similar to the growth rate, the grazing mortality of picophytoplankton populations also showed a decreasing trend from the inshore to offshore region. The rate was negatively correlated with seawater salinity that co-varied with nutrient concentration. Nutrient status has been reported to exert a strong influence on grazing activities of grazers (Worden and Binder, 2003). The increased nutrient concentrations in
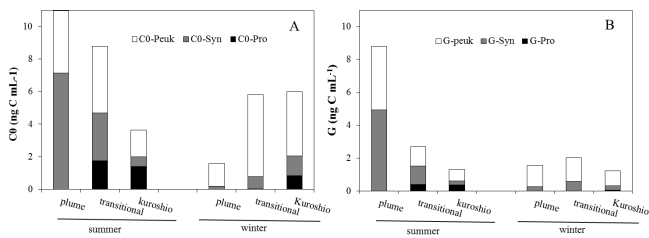
Fig. 9. Seasonal and regional composition of **(A)** picophytoplankton carbon biomass (C_0) and **(B)** their contribution to microzooplankton carbon consumption (G).

the plume area could improve the food quality of picophytoplankton as prey, indicated by larger cell size and high cellular Chl *a* fluorescence in the plume area than in the Kuroshio region (data not shown), and thus enhance the grazing activity of microzooplankton (Worden and Binder, 2003). It is generally believed that microzooplankton biomass is the major factor affecting grazing rate. The significant positive correlations between microzooplankton abundance (ciliate and dinoflagellates) and grazing mortality of Syn and Peuk in this study support this point of view. The highest grazing mortality ($1.97\,\mathrm{d^{-1}}$ for Syn and $2.58\,\mathrm{d^{-1}}$ for Peuk) was captured at station YZ13, where abundance of ciliates and dinoflagellates ($14\,000\,\mathrm{cells\,L^{-1}}$) was 1–2 orders of magnitude higher than that at other stations. However, Pro did not exhibit such a relationship with abundance of ciliates and dinoflagellates, suggesting that their major grazer is nanoflagellates rather than ciliates (Christaki et al., 1999; Guillou et al., 2001; Callieri et al., 2002).

Temperature affected the seasonal variation of protist grazing rate of Syn and Peuk, suggested by significant and positive association between the two parameters when pooling all data points together. Increased microzooplankton grazing during warm season and reduced grazing during cold season have been reported in many studies (e.g., Calbet et al., 2008; Lawerence and Menden-Deuer, 2012). The effect of temperature on grazing mortality could be due either to numerical response of protist grazers or to functional response of increased protist metabolic activities resulting from higher temperature (Peters, 1994). On average, about 84, 79 and 74 % and 45, 47 and 57 % of biomass production of Pro, Syn and Peuk in summer and winter were consumed by microzooplankton, respectively. The lower proportion in winter could be the result of a larger degree of decrease in grazing than in growth, which suggested a weaker top-down control in winter. A number of studies have pointed out that the temperature dependency of metabolic rates is different for autotrophs and heterotrophs (Allen et al. 2005; Lopez-Urrutia et al. 2006; Rose and Caron 2007; Chen et al., 2012). According to previous studies, the activation energy of heterotrophic metabolism ($0.65\,\mathrm{eV}$) is about twice that of autotrophic phytoplankton ($0.32\,\mathrm{eV}$).

4.4 Top-down controls on picophytoplankton abundance by microzooplankton grazing in the ECS

Highly correlated picophytoplankton growth rate and grazing mortality suggests a close coupling between picophytoplankton production and consumption, underlying the capacity of protists to rapidly respond to variations in their prey resources and highlighting the importance of microzooplankton grazing as a top-down control on picophytoplankton (Landry et al., 2000; Chen et al., 2009). The strong grazing control of picophytoplankton may be because of the numerical dominance of small heterotrophic flagellates ($< 20\,\mathrm{\mu m}$) in protist communities, whose abundance were one to several orders of magnitude higher than ciliates (Sherr et al., 1997; Chiang et al., 2003; Tsai et al., 2010; Choi et al., 2012). It is believed that nanoflagellates rather than ciliates are the major grazers of Pro and Syn (Christaki et al., 1999; Guillou et al., 2001; Callieri et al., 2002).

The mechanism that controls the abundance of the three picophytoplankton populations appears different. For Peuk, the seasonal change of abundance was very small, which was very different from Pro and Syn. Besides the high species diversity which may result in different Peuk taxa growing well in different seasons, high grazing pressure in summer that can keep the abundance of Peuk in check may be also one of the reasons, supported by higher m/μ_0 in summer and the negative correlation between grazing mortality and Peuk abundance. However, although the seasonal pattern of m, μ_0 and m/μ_0 of Syn was similar to that of Peuk, its grazing mortality did not demonstrate significant negative relationship with its abundance. On the other hand, there was no apparent correlation between Pro abundance and mortality rate. Such a different significance level of relationships was possibly due to the feeding preference on the three picophytoplankton populations by different grazers (Christaki et al., 1999; Guillou et al., 2001; Callieri et al., 2002). The physiological characters of the three populations, such as cell size, cell surface properties, and nutritional content, could also influence the feeding behavior of microzooplankton (Christaki et al., 1998; Monger et al., 1999). Considering the clear seasonal change in the abundance of Pro and Syn and their narrower spatial distribution compared with Peuk, we believe that the environmental factors might be more important in determining their population distributions.

There are very few studies focused on the vertical pattern of grazing mortality of picophytoplankton populations. We found a higher average growth rate of the three picophytoplankton populations in the surface layer than the DCM layer in both seasons, indicating light extinction greatly diminishes picophytoplankton growth rate in DCM layers. Our observation was consistent with previous studies that showed decreased growth rates with decreasing light levels (Landry et al., 1995, 2011b; Verity et al., 1996). However, the degree of decrease for grazing mortality at DCM compared

with surface was not as significant as that of growth rate, particularly in winter. Therefore, microzooplankton grazed a higher proportion of picophytoplankton production in the DCM layer in winter, which may be one of reasons for the higher abundance of picophytoplankton in the surface layer. As the strong-wind-induced mixing during winter resulted in a homogenous physical and chemical condition in the surface mixing layer, the protist community may be similar in the surface and DCM layers. Although light has been reported to stimulate the grazing activity of some protists (Strom, 2001), this stimulatory effect should not be as strong as the light effect on phytoplankton growth rate. In summer, the percentage of protist-consumed picophytoplankton production was similar in the surface and DCM layer because growth rate and grazing mortality decreased by a similar degree in the DCM layer compared with the surface layer. Different protist composition and lower temperature resulting from stratification may be the reasons for reduced grazing mortality in the DCM layer.

5 Summary

In summary, a comprehensive investigation on the abundance, growth rate and grazing mortality of three picophytoplankton populations in surface and DCM layers was conducted in contrasting seasons crossing different regions in the dynamic ECS. On average, microzooplankton grazing consumed ~ 60–$70\,\%$ of the daily production of the three picophytoplankton populations, with ~ 74–$84\,\%$ in summer and lower proportions of ~ 45–$57\,\%$ in winter due to a steeper decrease in grazing than in growth rates in winter. The three picophytoplankton populations occupied different ecological niches and showed different distribution patterns (especially in summer), which is, however, not coincident with their maximum growth rate. The distribution and population transition of picophytoplankton is therefore a result of the balance between growth and grazing mortality. Our study systematically described the growth and grazing rates of different picophytoplankton populations in the ECS, which provides useful data sets for better understanding the population dynamics of picoplankton and trophic transfer in microbial food webs in highly dynamic shelf ecosystems and the aquatic environment in general. As the ECS possesses a great deal of variability in both spatial and temporal scales on physical, chemical and biological parameters, understanding the interactive mechanism of bottom-up and top-down control in regulating picophytoplankton biomass and composition, and consequently the dynamics of biogeochemical cycling of carbon in the subtropical marginal seas, remains a challenge.

Supplementary material related to this article is available online at http://www.biogeosciences.net/11/ 1847/2014/bg-11-1847-2014-supplement.pdf.

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