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Distributions of Picophytoplankton and Phytoplankton Pigments Along a Salinity Gradient in the Changjiang River Estuary, China

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Abstract We investigated the abundance of different picophytoplankton groups and the phytoplankton pigment ratio in relation to environmental factors such as nutrients and suspended solids along a salinity gradient in the Changjiang River Estuary. The average numbers of *Synechococcus* spp. (*Syn*) and picoeukaryotes (Euk) were $(2.7 \pm 5.1) \times 10^3$ and $(1.1 \pm 1.4) \times 10^3$ cells mL^{-1}, respectively. *Prochlorococcus* spp. (*Pro*) was only found in the high-salinity brackish water with the concentration of 3.0×10^3 cells mL^{-1}. *Syn* and Euk numbers both tended to increase offshore and *Syn* showed a larger variation in cell abundance than Euk. The contribution of picophytoplankton to total phytoplankton biomass increased with increasing salinity and decreasing nutrient concentrations from the estuary to the open ocean. The response of different picophytoplankton groups to environmental variables was different. Water temperature was more important in its control over Euk than over *Syn*, while nutrients were more important in their influence over *Syn* than over Euk. Phytoplankton pigment ratios were different in the three different ecological zones along the salinity gradient (*i.e.*, freshwater zone with 0−5 range, fresh and saline water mixing zone with 5−20 range, and high-salinity brackish water zone with 20−32 range), where three different phytoplankton communities were discovered, suggesting that phytoplankton pigment ratios can be considered as a complementary indicator of phytoplankton community structure in the Changjiang River Estuary.

Key words *Synechococcus*; picoeukaryotes; phytoplankton pigment; salinity; Changjiang River Estuary

1 Introduction

Estuaries are coastal areas where fresh water from rivers and streams mixes with salt water from the ocean. Natural and anthropogenic materials are transported, deposited, and transformed in the estuary. Phytoplankton is sensitive to environmental variables and therefore considered as an important investigated object for environmental change (Stockner, 1988; Gao and Song, 2005). Over the last two decades, human activities have strongly enhanced nutrient loading in the Changjiang River Estuary, resulting in eutrophication and concomitant changes in species composition of phytoplankton, structure of food chain, and element biogeochemical cycle in the ecosystem (Gao and Song, 2005; Zhu *et al*., 2009; Jiang *et al*., 2010).

Picophytoplankton comprises prokaryotic picocyanobacteria and eukaryotic phototrophs. They are ubiquitous in both fresh water and marine ecosystems (Stockner, 1988). Nowadays, it is well known that, with the enhancement of trophic state, picophytoplankton abundance and biomass increase and its relative importance decreases (Bell and Kalff, 2001; Callieri, 2007). Numerous studies have been conducted with respect to picophytoplankton in the East China Sea (Chang *et al*., 2003; Jiao *et al*., 2005; Pan *et al*., 2005). However, few studies focus on picophytoplankton in the Changjiang River Estuary (Vaulot and Ning, 1988; Pan *et al*., 2007; Shang *et al*., 2007).

In this study we have investigated the abundance of picophytoplankton *Synechococcus* spp. (*Syn*), *Prochlorococcus* spp. (*Pro*), picoeukaryotes (Euk) and examined contents of chlorophyll *a*, *b*, *c* and carotenoid index, phaeopigment index and related environmental factors along a salinity gradient in the Changjiang River Estuary. Our aim is to elucidate the distributions of different picophytoplankton groups and phytoplankton pigment ratios in relation to environmental factors, and to discern the influencing factors on their distributions in the Changjiang River Estuary.

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2 Materials and Methods

2.1 Sampling

Sample collection was carried out on June 19–22, 2005. A total of 19 stations were investigated in the Changjiang River Estuary (Fig.1). Water samples for depth profiles were taken with 5 L Niskin bottles. Sampling depths were 0 and 7 m at stations 6 and 12; 0, 6 and 16 m at station 15; 0, 10, 20 and 30 m at station 17; and 0, 10, 20, 35 and 50 m at station 18. At other stations, water samples were collected from surface water (upper 0.5 m).

Water temperature (T), dissolved oxygen (DO), pH, and salinity were measured *in situ* using a portable multi-parameter instrument (pIONneer 65). Water samples for determination of nitrate (NO_3^-) and dissolved silicon (DSi) were filtered through 0.45 μm acid-cleaned acetate cellulose filters. The filtrates were poisoned by $HgCl_2$ and stored in the dark at 0–4℃ before analysis. NO_3^- was measured with the cadmium reduction method (Parson *et al*., 1984) and DSi silicomolybdenum blue method (Strickland and Parsons, 1968) with precision of <5%. Duplicate samples were taken to determine the amount of total suspended solids (TSS, mgL^{-1}), for which the salts trapped on the cellulose filters were removed by distilled water. TSS was calculated by the dry-weight method. Water samples for determining the abundance of different picophytoplankton groups were filtered by 53 μm nylon fabric in order to remove impurities and then were put aside in darkness for 15 min with paraformaldehyde (final concentration: 1%) and stored in liquid nitrogen till analysis in one month (Pan *et al*., 2005).

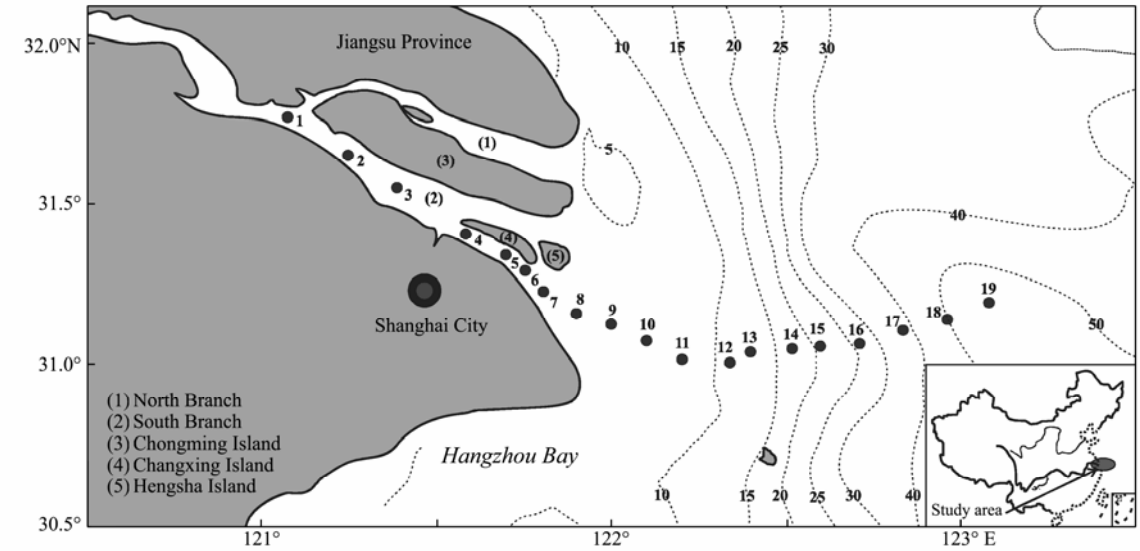

Fig.1 Map showing sampling locations and sample numbers. The dashed lines are isobaths and depth is given in meters.

2.2 Analysis of Phytoplankton Pigments

One liter of seawater was filtered by 0.45 μm acetate cellulose membrane. Phytoplankton on the membrane was soaked in 90% acetone at 4℃ in the dark for 20 h in order to adequately extract the pigments. The extract was analyzed at 410, 430, 480, 630, 647, 663, and 750 nm wavelengths, respectively, against a 90% acetone blank. The concentrations of chlorophyll *a*, *b*, *c* ($\mu g L^{-1}$, Jeffrey and Humphrey, 1975), Carotenoid index (CI; Strickland and Parsons, 1968), and Phaeopigment index (PI; Moss, 1967) were calculated according to the following equations:

$$\text{Chlorophyll } a \text{ (Chl } a) = (11.85(A_{663}-A_{750})-1.54(A_{647}-A_{750})-0.08(A_{630}-A_{750}))\cdot V_a/V_w;$$

$$\text{Chlorophyll } b \text{ (Chl } b) = (-5.34(A_{663}-A_{750})+21.03(A_{647}-A_{750})-2.66(A_{630}-A_{750}))\cdot V_a/V_w;$$

$$\text{Chlorophyll } c \text{ (Chl } c) = (-1.67(A_{663}-A_{750})-7.60(A_{647}-A_{750})+24.52(A_{630}-A_{750}))\cdot V_a/V_w;$$

$$\text{Carotenoid index (CI)} = (A_{480}-A_{750}) / (A_{663}-A_{750});$$

$$\text{Phaeopigment index (PI)} = (A_{430}-A_{750}) / (A_{410}-A_{750}).$$

where A_x is absorbance at x nm; V_a is extraction volume in milliliter; V_w is filter volume in liter.

2.3 Analysis of Picophytoplankton

Picophytoplankton samples were analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with an air-cooled argon laser (488 nm, 15 mW). Cell fluorescence emissions and light scatter signals were calibrated by adding yellowish green fluorescent beads (1.002 μm) (Polysciences Inc., catalogue # 18660). For each particle in the sample, forward light scatter, side light scatter, orange fluorescence (585 nm± 21 nm), and red fluorescence (>650 nm) were recorded and the data obtained were processed with CELLQuest™ software (Becton Dickinson, San Jose, CA, USA). According to their specific autofluorescence properties and light scatter differences, the different picophytoplankton groups could be discriminated and enumerated (Collier, 2000).

The software SPSS (version 11.5; SPSS Inc.) was used to carry out statistical analysis of the data and Pearson's correlation coefficient analysis was conducted.

3 Result

Fig.2 shows the spatial and temporal distributions of water temperature, salinity, DO, pH, TSS, DSi, and NO_3^- in the studied area. The average values were 23.9±2.3℃ (from 20.2 to 28.7℃) for water temperature, 15.5±12.5 (from 0 to 32.3) for salinity, 7.2±1.0 mg L^{-1} (from 5.4 to 9.6 mg L^{-1}) for DO, 8.0±0.4 (from 7.0 to 8.7) for pH, 77.8±111.3 mg L^{-1} (from 0.3 to 518.2 mg L^{-1}) for TSS, 48.9±33.6 μmol L^{-1} (from 13.5 to 99.9μmol L^{-1}) for DSi, and 57.3±39.2μmol L^{-1} (from 2.2 to 105.5μmol L^{-1}) for NO_3^-. pH and DO leaned to the increase of salinity. Water temperature decreased with depth and increasing salinity and therefore it showed significant correlations with

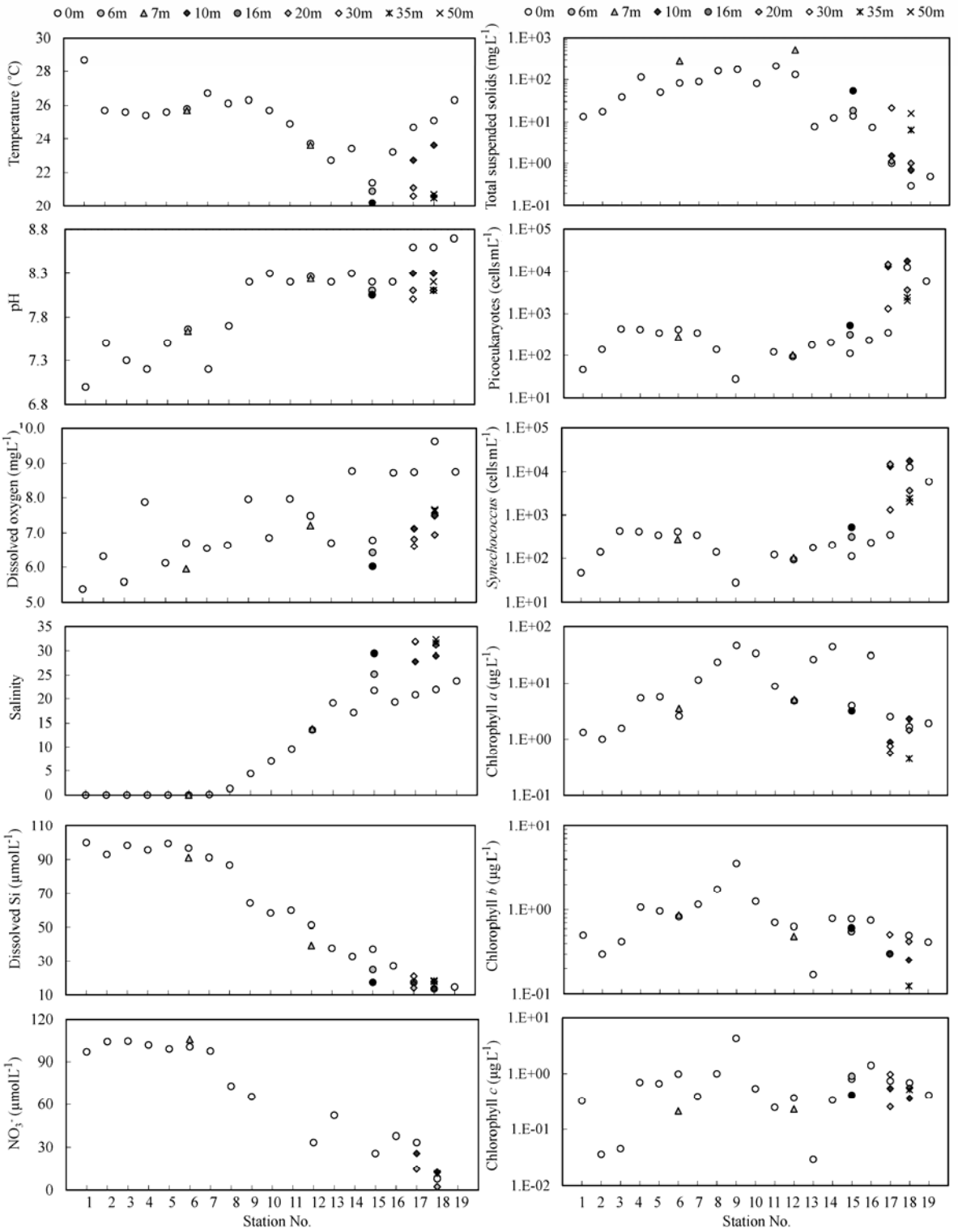

Fig.2 Distributions of temperature, pH, dissolved oxygen, salinity, dissolved Si, NO_3^-, total suspended solids, picoeukaryotes, *Synechococcus*, chlorophyll *a*, *b*, *c* in the investigated area.

depth and salinity (Table 1). At station 15, an upwelling of cold and saline water existed (Fig.2). Low DO appeared with this upwelling and this low DO did not come directly from the Changjiang River Diluted Water in the upper layer, instead it might come from the modified high saline Taiwan Warm Current Water in the deep and bottom layers (Zhao *et al*., 2001). TSS showed high values at stations 9–12 (Fig.2), which is located in the Turbidity Maximum Zone that is originated from sediment resuspension caused by salt and fresh water mixing (Pan *et al*., 1999). Since there was an inverse correlation between NO_3^- and the salinity and between DSi and the salinity, respectively (Table 1), both NO_3^- and DSi tended to decrease rapidly with the increase of the salinity (Fig.2).

Table 1 Relationships between the investigated factors in terms of Pearson's correlation coefficient analysis

	Depth	T	pH	DO	Salinity	TSS	Euk	*Syn*	Chl *a*	DSi
T	−.693**									
pH	.161	−.437*								
DO	−.038	−.049	.672**							
Salinity	.656**	−.845**	.687**	.328						
TSS	−.164	.244	−.068	−.067	−.396*					
Euk	−.301	.432*	.152	.315	−.084	−.138				
Syn	.199	−.187	.352	.258	.497**	−.311	.110			
Chl *a*	−.350	.209	.154	.270	−.242	.100	−.089	−.300		
DSi	−.483**	.730**	−.844**	−.519**	−.956**	.312	−.015	−.498**	.088	
NO_3^-	−.541*	.785**	−.849**	−.585**	−.957**	.509*	.426	−.594**	.078	.966**

Notes: **Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed); T, water temperature; DO, dissolved oxygen; TSS, total suspended solids; DSi, dissolved silicon; Euk, picoeukaryotes; *Syn*, *Synechococcus*; Chl *a*, chlorophyll *a*.

The average cell abundance was $(2.7\pm5.1)\times10^3$ cells mL^{-1} for *Syn* with the range from 0.03 to 17.8×10^3 cells mL^{-1}, $(1.1\pm1.4)\times10^3$ cells mL^{-1} for Euk with the range from 0.1 to 7.7×10^3 cells mL^{-1}, respectively. *Pro* was only found at station 19 with the concentration of 3.0×10^3 cells mL^{-1}. *Syn* and Euk numbers both tended to increase offshore and *Syn* showed a larger variation in cell abundance than Euk (Fig.2). Euk tended to decrease with depth; however, this phenomenon was not observed for *Syn* and the high cell abundance of *Syn* appeared in the 10 m layer of seawater at station 18 (Fig.2). *Syn* showed significant correlations with NO_3^- and DSi concentrations, while this was not found for Euk (Table 1).

The average concentration was 9.3 ± 13.4 μg L^{-1} for Chl *a* with the range from 0.4 to 46.1 μg L^{-1}, 0.7 ± 0.7 μg L^{-1} for Chl *b* with the range from 0.1 to 3.6 μg L^{-1}, and 0.6 ± 0.7 μg L^{-1} for Chl *c* with the range from 0.03 to 4.2 μg L^{-1}, respectively. On both sides of the upwelling area (*i.e*., station 15), one area with high Chl *a* concentration appeared. In the freshwater area (stations 1–8), Chl *a*, *b*, and *c* showed a similar variation trend (Fig.2). High Chl *a* occurred in upper low salinity area due to the dominance of Changjiang River Diluted Water with ample nutrients in this zone (Song *et al*., 2009).

4 Discussion

4.1 Distribution of the Different Picophytoplankton Groups in Relation to Environmental Factors Along the Salinity Gradient

The environmental factors in the Changjiang River Estuary are mainly influenced by the extremely high nutrient loads from the Changjiang River and the mixing between the fresh and saline water. Both water temperature and pH show significant correlations with salinity (Table 1), suggesting that they were controlled by the mixing between the fresh and saline water. In surface waters, nutrient concentrations decrease from eutrophic coastal to oligotrophic open shelf waters (Zhang *et al*., 2007; Chai *et al*., 2009; Chen *et al*., 2010) though patchy character of nutrient distribution can be produced by biological uptake and regeneration in the surface waters (Zhang *et al*., 2007). In this study, both NO_3^- and DSi showed significant negative correlations with salinity (Table 1). With the increase of surface salinity from 0 to 23.8, NO_3^- decreased from 104.3 to 7.7 μmol L^{-1} and DSi decreased from 99.9 to 14.6 μmol L^{-1}; meanwhile, chlorophyll *a* decreased from 46.1 to 1.9 μg L^{-1}, indicating a rapid change of trophic state along the salinity gradient. Phosphate was found to show similar distribution pattern to that of nitrate in the Changjiang River Estuary (Chen *et al*., 2010). Potential phosphorus limitation mainly took place where the salinity was less than 30 after 2003, while potential silicon limitation occurred in an area of salinity more than 30 (Chai *et al*., 2009).

Euk were the most competitive among the picophytoplankton in freshwater zone (0–5 salinity range, named Zone I) with high temperature and abundant nutrients (Figs.2 and 3a); they showed significant correlation with temperature and this phenomenon was not found for *Syn*, suggesting that water temperature was a more important factor controlling Euk than *Syn*. In fresh and saline water mixing zone (5–20 salinity range, named Zone II), picophytoplankton numbers decreased due to the limitations forced by high turbidity (the radiation effect). In high-salinity brackish water (20–32 salinity range, named Zone III), *Syn* numbers increased rapidly with low nutrients and clear water while Euk did not, and *Pro* was also discovered (Figs.2 and 3a), suggesting the change of composition of different picophytoplankton groups in this zone.

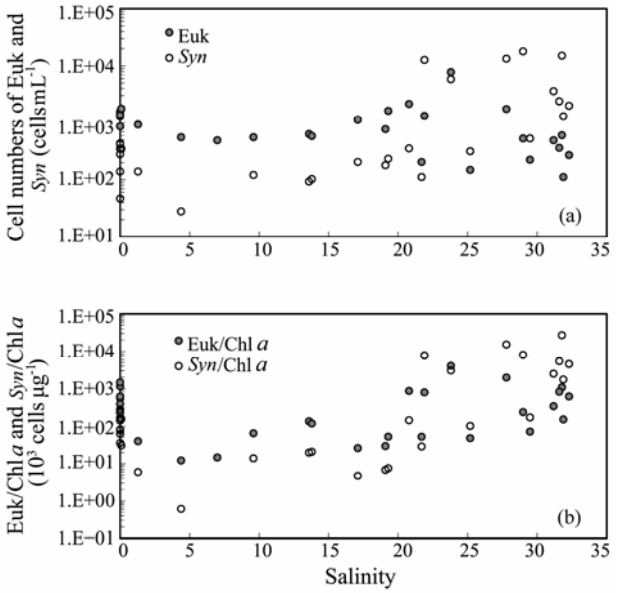

Fig.3 Picoeukaryotes (Euk) and *Synechococcus* (*Syn*) *vs* the salinity (a), and contributions of Euk and *Syn* to chlorophyll *a* (Chl *a*) *vs* the salinity (b).

Our previous study showed that *syn* had the significant negative correlation with PO_4^{3-} (Wang *et al*., 2008). As phosphate presented similar distribution pattern to that of nitrate (Chen *et al*., 2010), it can be inferred that *Syn* could had close relationship with PO_4^{3-} in the Changjiang River Estuary and nutrients could be more important factors influencing *Syn* than Euk (Table 1). *Pro* was only found at station 19 with a salinity of 23.8 due to its favorable marine environment (Partensky *et al*., 1999). These results demonstrated that the responses of different picophytoplankton groups to environment variables such as temperature, light, and nutrients are genus-specific. The picophytoplankton numbers in this study are comparable to these in previous studies conducted in the Changjiang River Estuary (Vaulot and Ning 1988; Shang *et al*., 2007; Pan *et al*., 2007). The ratios of *Syn* and Euk numbers to that of chlorophyll *a* increased with the increase of salinity (Fig.3b), suggesting the increasing importance of photosynthetic picoplankton from estuaries to the open ocean with the decreasing nutrient concentrations. This result is consistent with the studies of picophytoplankton in Southampton Water (south coast of England; Iriarte and Purdie, 1994) and in San Francisco Bay (Ning *et al*., 2000).

4.2 Distribution of Phytoplankton Pigment Composition Along the Salinity Gradient

Phytoplankton pigments absorb light over different wavelength ranges. Chlorophyll absorbs it in the 430–450 nm and 600–690 nm ranges, and carotenoid in the 400–500 nm range. Phytoplankton pigment composition can be considered as a taxonomic signature (Ston *et al*., 2002) because there are highly specific quantities and relative proportions of pigments in particular species. Adaptive divergence in pigment composition promotes phytoplankton biodiversity (Falkowski and LaRoche, 1991; Stomp *et al*., 2004).

Chlorophyta contains Chl *a* and *b*, while Bacillariophyta and Pyrrophyta have Chl *a* and *c*, diadinoxanthin and β-carotene. Phytoplankton showed lower values of Chl *a*/*b*, *a*/*c*, and *b*/*c* in Zone III and higher values of Chl *a*/*b* and *a*/*c* in Zone II (Fig.4), indicating that Chlorophyta decreased and Bacillariophyta increased with the salinity. They also showed different CI and PI in Zone II from those in Zones I and III. These results indicate that there were three different phytoplankton communities along the salinity gradient. An earlier study has distinguished these three different phytoplankton communities in accord with salinity gradient in Changjiang River Estuary according to the analyses of phytoplankton species (Table 2; Wang, 2002). Another study found that *Melosira granulata* and most of the Chlorophyta species that belong to the freshwater community predominated in Zone I, Euryhaline species such as *Skeletonema costatum* predominated in Zone II, and another euryhaline species, *Prorocentrum dentatum*, predominated in Zone III (Gao and Song, 2005). Different phytoplankton groups have different responses to the environmental variables along the salinity gradient. The optimal range of salinity for *P*. *dentatum* growth is 25–31 and that for *S*. *costatum* is 18–35.7

Table 2 Basic ecological parameters for the three different phytoplankton communities

	Phytoplankton community I		Phytoplankton community II		Phytoplankton community III	
Sampling time	June 2005	May 1998	June 2005	May 1998	June 2005	May 1998
Range	121°E−122°E	West of 122°E	122°E−122°30′E	122°E−123°E	122°30′E−123°10′E	East of 123°E
Salinity	0−5	0.11−5	5−20	5−30	20−32.3	30−34.4
Temperature (℃)	26.2	22.4	24	20.8	22.3	15.7
pH	7.5	—	8.2	—	8.3	—
DO (mg L^{-1})	6.5	—	7.5	—	7.5	—
Nitrate (μmol L^{-1})	94.7	68.0	42.8	30.2	18.9	4.6
Phosphate (μmol L^{-1})	—	1.1	—	0.69	—	0.34
DSi (μmol L^{-1})	91.6	—	46.6	—	19.4	—
Chl *a* (μg L^{-1})	10.2	0.85	20.4	0.68	3.8	1.30
Dominant species (%)	—	*S. costatum* (35.7) *L. minimus* (24.7)	—	*S. costatum* (60.5) *C. calceiformis* (15.1)	—	*P. triestinum* (71.4) *M. sulcata* (17.4)
References	This study[a]	Wang (2002)	This study[a]	Wang (2002)	This study[a]	Wang (2002)

Notes: [a]The data presented here except salinity are average values; DO, dissolved oxygen.

(Chen *et al*., 2005). *S. costatum* showes a much higher phosphatase activity and thus can assimilate phosphorus from the environment much faster than *P. donghaiense* under the same nutrient conditions (Zhao *et al*., 2009).

Our study demonstrated that phytoplankton pigment compositions were different in the three different ecological zones (Table 2; Fig.4), where three different phytoplankton communities had been earlier discovered (Wang, 2002; Gao and Song, 2005). Therefore, phytoplankton pigment ratios can be used as the indicator of phytoplankton community structure. Chl *a* and *b* can possibly be overestimated because a small part of them may originate from vascular plant detritus in turbid estuary (Lionard *et al*., 2008, Zhu *et al*., 2009); therefore, phytoplankton pigment ratios should be considered a complementary to, but not exclusive replacement for, microscopic observation for understanding the dynamics of phytoplankton.

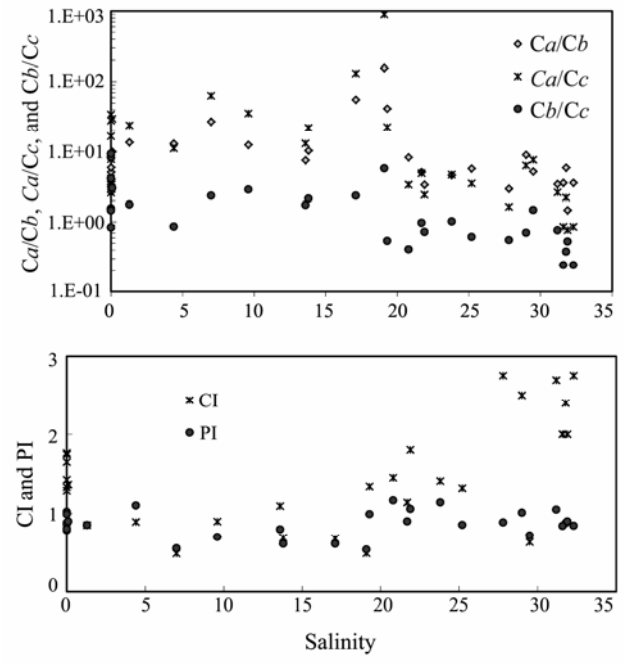

Fig.4 Phytoplankton pigment ratios *vs* the salinity. C*a*, chlorophyll *a*; C*b*, chlorophyll *b*; C*c*, chlorophyll *c*, CI, Carotenoid index; PI, Phaeopigment index.

5 Conclusion

The responses of different picophytoplankton groups to environmental variables in the Changjiang River Estuary were genus-specific. *Pro* was only found in the high-salinity brackish water. Water temperature was more important in its regulation of Euk than of *Syn*, while nutrients were more important in their influence over *Syn* than over Euk. The contribution of picophytoplankton to total phytoplankton biomass increased with increasing salinity and decreasing nutrient concentrations from estuaries to the open ocean. Phytoplankton pigment ratios were different in the three different ecological zones along the salinity gradient, indicating that they can be an indicator of phytoplankton community structure in the Changjiang River Estuary.

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