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Seasonal Variability of Phytoplankton Community Structure in Relation to Different Nitrogen-Phosphorus Ratios in the Southern Coastal Waters of Zhejiang, China

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Abstract With the rapid development of economy and increase of population in the drainage areas, the nutrient loading has increased dramatically in the Changjiang estuary and adjacent coastal waters. To properly assess the impact of nutrient enrichment on phytoplankton community, seasonal microcosm experiments were conducted during August 2010 – July 2011 in the coastal waters of Zhejiang Province. The results of the present study indicated that the chl *a* concentration, cell abundance, diversity indices, species composition and community succession of the phytoplankton varied significantly with different N/P ratios and seasons. Higher growth was observed in the 64:1 (spring), 32:1 (summer), 16:1 (autumn) and 128:1, 256:1 (winter) treatments, respectively. The values of Shannon-Wiener index (H') and Pielou evenness index (J) were lower in the 8:1 and 16:1 treatments in autumn test, while H' value was higher in the 128:1 and 8:1 treatments in winter test. A definite community succession order from diatoms to dinoflagellates was observed in the autumn and winter tests, while the diatoms dominated the community throughout the culture in the spring and summer tests.

Key words phytoplankton community succession; nutrient enrichment; Shannon-Wiener index; Pielou evenness index; nitrogen-phosphorus ratio

1 Introduction

Eutrophication due to anthropogenic nutrient loading in coastal marine waters, which is characterized by noxious phytoplankton blooms, oxygen depletion and benthic mortality, is a growing problem worldwide (Justić *et al*., 1995; Schöllhorn and Granéli, 1996; Vuorio *et al*., 2005; Zhou *et al*., 2008). Estuaries may be specially enriched by nutrients from river water and organic pollution (locally within the estuary or remotely through runoff) and by the entrainment of coastal waters in a subsurface countercurrent transporting nutrients into the estuary (Gao and Song, 2005; Sarthou *et al*., 2005). In addition to increasing concentrations of nutrients, a shift in the proportions between the essential nutrients, which induce unbalanced nutrient ratios as compared with the Redfield ratio (Redfield, 1958), has been proved to influence the growth, physiological state and community structure of phytoplankton (Carlsson and Granéli, 1999; Egge and Aksnes, 1992; Gobler *et al*., 2006; Lagus *et al*., 2004; Lie *et al*., 2011; Rhee, 1978; Turner and Rabalais, 1991). As a result, changes in phytoplankton growth and community structure due to eutrophication have become a global phenomenon (Smith *et al*., 1999).

With the rapid development of economy and increase of population in the drainage areas, the nutrient loading has increased dramatically in Changjiang estuary and adjacent coastal waters. As a result, increased nitrogen-phosphorus (N/P) ratio and decreased silicon- nitrogen (Si/N) ratio took place in this region: the N/P ratio in the Changjiang Estuary is reported to have increased from 17.6 in 1959 to 35.0 in 2002, while Si/N ratio decreased from 3.9 in 1959 to 0.94 in 2005 (Zhang *et al*., 2007). Worse yet, this tendency is expected to continue (Zhou *et al*., 2008). The disequilibrium of nutrients is suspected as an important reason for the change of the phytoplankton community composition (Hillebrand, 2011). Previous researches indicated that the phytoplankton biomass in the Changjiang estuary and Zhejiang coastal waters is primarily contributed by diatoms, with a minor contribu-

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tion by dinoflagellates (Wang *et al*., 2004). Nonetheless, the average percentage of diatoms abundance in this region decreased from 99.5% during 1985–1986 to 75.5% during 2004–2005 (Jiang *et al*., 2010). Meanwhile, the species number and abundance of dinoflagellates increase constantly and dinoflagellates become one of the most common bloom-forming species. For example, blooms caused by *Prorocentrum donghaiense* and *Prorocentrum triestinum* occurred frequently during the spring off the coast of Zhejiang in recent investigations (Li *et al*., 2011; Zhou *et al*., 2008).

The relationship between nutrient supply and the dynamics of phytoplankton community is an issue of extreme complexity and an important topic in oceanographic research. However, it is strange that studies on the effect of N/P ratio on phytoplankton community structure are still limited so far (De Tezanos Pinto *et al*., 2010; Huang *et al*., 2012). In this experimental study, the natural phyto- plankton community in the coastal waters of Dongtou Island was maintained in different N/P ratios as observed during 2010–2011. The cell abundance, biomass, diversity indices and community structure of the phytoplankton cultured in different treatments were monitored in order to assess the effect of these nutrient manipulations on the growth and succession of phytoplankton communities.

2 Materials and Methods

2.1 Study Site and Phytoplankton Culture

Dongtou Island is located off the southern coast of Zhejiang Province, about 50 km outside the Oujiang estuary (Fig.1). It has a subtropical climate and semidiurnal tide; the average surface water temperatures range from 7 to 30℃. The hydrologic condition is mainly controlled by the Taiwan Warm Current and Minzhe Coastal Current (Zhu *et al*., 2013). The experiments were performed in spring (May, 2011), summer (August, 2010), autumn (November, 2010) and winter (January, 2011). The surface water (Table 1) containing natural phytoplankton community (the initial species composition of the phytoplankton community is shown in Table 2) was pumped from a station 1 000 m offshore into a tank. To decrease the grazing effect caused by meso- and/or macro- zooplankton as much as possible, the water was filtered through a 505 μm mesh. The water had been mixed thoroughly with an air-bubbling system for 2 h before it was transferred to test polyethylene cylinders (diameter, 60 cm; height, 80 cm; capacity, 200 L). The cylinders were then randomly spaced and immersed in an indoor pool supplied with running water to maintain the temperature inside the cylinders similar to that of the surrounding sea. Each cylinder contained 150 L of test seawater and oxygen was gently supplied. Natural light (illuminance of 31.27–63.83 mmol photons $m^{-2} s^{-1}$ during the daytime in different seasons) was provided throughout the culturing process.

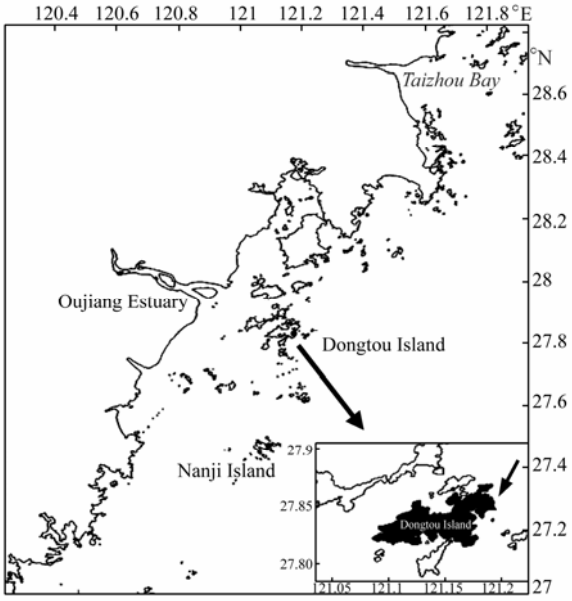

Fig.1 Map of the test station on Dongtou Island in the East China Sea. ▲ indicates the location of the test station.

2.2 Experimental Design

The phytoplankton communities were exposed to different N/P ratios (1:1, 4:1, 8:1, 16:1, 32:1, 64:1, 128:1 and 256:1; Table 3); we conducted three replicates at each N/P treatment. The analytical-reagents of sodium nitrate ($NaNO_3$; purity over 99%; CAS No: 7631-99-4) and so-

Table 1 Qualities of experimental seawater in different seasons

Parameter	Spring	Summer	Autumn	Winter
Temperature (℃)	17.6±1.3	29.3±0.6	16.0±1.1	9.7±1.8
Salinity	28.1±0.2	29.8±0.1	27.0±0.3	26.8±0.2
pH	8.05±0.23	7.98±0.22	8.12±0.17	7.93±0.27
Light intensity (mmol photons $m^{-2} s^{-1}$)	43.85±17.76	63.83±15.36	34.97±17.21	31.27±15.17
NO_2-N (μmol L^{-1})	0.86±0.04	1.29±0.07	0.21±0.01	0.43±0.02
NO_3^--N (μmol L^{-1})	57.43±4.29	16.86±2.14	74.50±1.43	77.14±2.86
NH_4^+-N (μmol L^{-1})	0.64±0.21	3.93±0.43	0.86±0.29	0.50±0.14
PO_4^{3-}-P (μmol L^{-1})	1.16±0.03	0.74±0.13	1.49±0.09	1.36±0.10
SiO_3^{2-}-Si (μmol L^{-1})	38.46±1.35	19.44±1.42	49.63±0.68	38.49±0.78
N/P ratio	51:1	30:1	51:1	57:1
Chl *a* (μg L^{-1})	1.18±0.05	6.38±0.35	0.71±0.06	1.35±0.08
Cell abundance (10^6 cells L^{-1})	0.118±0.014	0.685±0.043	0.026±0.003	0.05±0.006

Table 2 Initial species composition of the phytoplankton community in different seasons

Season	Species name	Cell abundance ($\times 10^4$ cells L^{-1})	Dominance (%)
Spring	*Skeletonema costatum*	5.23	44.36
	Thalassiosira curviseriata	3.51	29.71
	Thalassiosira rotula	0.63	5.32
	Plagioselmis prolonga	0.56	4.72
	Nitzschia longissima	0.53	4.49
	Prorocentrum sigmoides	0.46	3.89
Summer	*S. costatum*	41.7	60.83
	Pseudo-nitzschia spp.	9.90	14.45
	Scrippsiella trochoidea	7.43	10.84
	Eutreptiella gymnastica	1.38	2.02
Autumn	*S. costatum*	0.77	30.2
	Thalassiosira pacifica	0.43	16.7
	Thalassiothrix frauenfeldii	0.35	13.5
	Thalassiosira curviseriata	0.48	18.8
	Protoperidinium bipes	0.53	20.8
Winter	*S. costatum*	2.93	55.33
	T. curviseriata	0.81	15.19
	Pyramimonas sp.	0.74	13.94
	S. trochoidea	0.25	4.65

Table 3 Nominal concentrations of dissolved inorganic N (DIN μmol L^{-1}) and dissolved inorganic P (DIP μmol L^{-1}) in different N/P treatments in different seasons

N/P treatment	Spring		Summer		Autumn		Winter	
	DIN	DIP	DIN	DIP	DIN	DIP	DIN	DIP
1:1	58.93	58.93	60.08	60.08	75.57	75.57	78.07	78.07
4:1	58.93	14.73	60.08	15.02	75.57	18.89	78.07	19.52
8:1	58.93	7.37	60.08	7.51	75.57	9.45	78.07	9.76
16:1	58.93	3.68	60.08	3.76	75.57	4.72	78.07	4.88
32:1	58.93	1.84	60.08	1.88	75.57	2.36	78.07	2.44
64:1	74.24	1.16	76.80	1.20	95.36	1.49	87.04	1.36
128:1	148.48	1.16	153.60	1.20	190.72	1.49	174.08	1.36
256:1	296.96	1.16	307.20	1.20	381.44	1.49	348.16	1.36

dium dihydrogen phosphate (NaH_2PO_4; purity over 99%; CAS No: 7558-80-7) (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) were used as the test chemicals. Stock solutions (100 mmol L^{-1} $NaNO_3$-N and 1 000 mmol L^{-1} NaH_2PO_4-P) were prepared by dissolving $NaNO_3$ and NaH_2PO_4 in deionized water. Based on the concentrations measured for different nutrients in the collected water (every other day), appropriate aliquots of the stock solutions were added to the filtered seawater to maintain the designated nutrient level in each experimental cylinder. The test lasted for 30 d in each season.

2.3 Sampling and Laboratory Analyses

Prior to each sampling, the sides and bottom of the cylinders were scraped and the test water was gently, but thoroughly, agitated with a polyethylene stick. A 200 mL sample was collected at 8:00 am from each cylinder every other day to analyze the nutrient concentrations. On the other hand, a 100 mL sample was collected every six days to analyze the chlorophyll *a* (chl *a*), and another 500 mL sample was collected and stored in sample bottles awaiting counting and identifying the phytoplankton present.

2.3.1 Inorganic nutrient conditions

The concentration of dissolved inorganic nutrients was determined by filtering 200 mL sample through 0.45 μm cellulose membrane filters (Whatman, USA). Afterwards, the concentrations of NO_3^--N, NO_2^--N, NH_4^+-N, SiO_3^{2-}-Si and PO_4^{3-}-P were immediately analyzed using a spectrophotometer (UNICO WFZ UV-2802PC/PCS; Shanghai, China) following the method described in the Specification for Marine Monitoring-Inorganic Nutrients Analysis in Seawater GB 17378. 4-2007 (SAPRC, 2008). Total dis- solved inorganic nitrogen (DIN) was calculated as a sum of ammonia, nitrite and nitrate. According to the measured nutrient concentrations in water samples, $NaNO_3$-N, NaH_2PO_4-P and Na_2SiO_3-Si were added to maintain the set level of nutrients until the end of tests.

2.3.2 Chlorophyll *a*

For the determination of chl *a* concentrations, a 100 mL aliquot of the samples from each cylinder was gently filtered (< 0.05 kPa) through glass fiber filters (GF/F) (Whatman, USA; precombusted at 450℃ for 3 h), and 1 mL of $MgCO_3$ suspension with 10 g L^{-1} was added to prevent degradation of chl *a*. The cells retained on the filters were extracted with 10 mL of 90% acetone in the dark (4℃) for 24 h. Then, the chl *a* concentration was determined fluorometrically with a fluorometer (Turner Designs Trilogy, USA) with the procedures described in detail by Huang *et al.* (2012). Fluorescence units were

transformed to $\mu g\,L^{-1}$ of chl *a* after spectrophotometric analysis of extracted chl *a*.

2.3.3 Species identification and cell abundance

A 500 mL sample was removed from each cylinder and fixed with Lugol's solution at a final concentration of 1%. Then, the sample was allowed to settle for at least 24 h, condensed to an appropriate volume, and preserved in polyethylene sample vessels to await microscopic analysis. After shaking to mix thoroughly, 0.1 mL of the concentrated samples was transferred to a phytoplankton counting chamber, and the cell abundance ($ind\,L^{-1}$) and species belonging to the major taxonomic groups were determined under a microscope (Nikon-E200, Japan). The population of zooplankton grazers in the cultures was not determined, since this experiment was aimed primarily at determining the phytoplankton responses to nutrient ratios. It was assumed that the impact of grazers on all cylinders was similar.

2.3.4 Analyses of community diversity indices

Based on the cell abundance, various diversity indices were computed for each of the experimental cylinders. The Shannon-Wiener index (*H'*) and Pielou evenness index (*J*) were calculated according to the formulas below:

$$H' = -\sum_{i=1}^{S} P_i \log_2 P_i,$$

$$J = \frac{H'}{\log_2 S},$$

where *S* is the species number in the community and *P* is the ratio between the cell abundance of the species (*i*) and the total cell abundance of the community. The succession of dominant phytoplankton species in the different treatments was assessed based on the percentage of each species' contribution to the overall phytoplankton abundance. A species with a dominance > 2% was considered to be the dominant species in its respective community.

2.4 Data Analysis

The data are reported as the mean ± S.E.M (standard error of the mean), and they were checked for assumptions of normality using the Kolmogorov-Smirnov one-sample test and for homogeneity of variance using Levene's test. Wherever both assumptions were met, data were analyzed by ANOVA followed by Tukey's multiple comparison tests. Data were analyzed by the nonparametric Kruskal-Wallis test followed by Mann-Whitney *U*-test wherever either of the assumptions was not met. Difference was considered significant at $P < 0.05$. All statistical analyses were run by SPSS 15.0 for Windows (Statistical Program for Social Sciences, Chicago, IL, USA).

3 Results

3.1 Phytoplankton Biomass and Cell Abundance

The chl *a* concentration reached a peak of $11.60\mu g\,L^{-1}$ in the 1:1 treatment on day 6 in spring test; however, no significant difference was observed among different N/P ratios (ANOVA, $P > 0.05$; Fig.2a). In contrast, the chl *a*

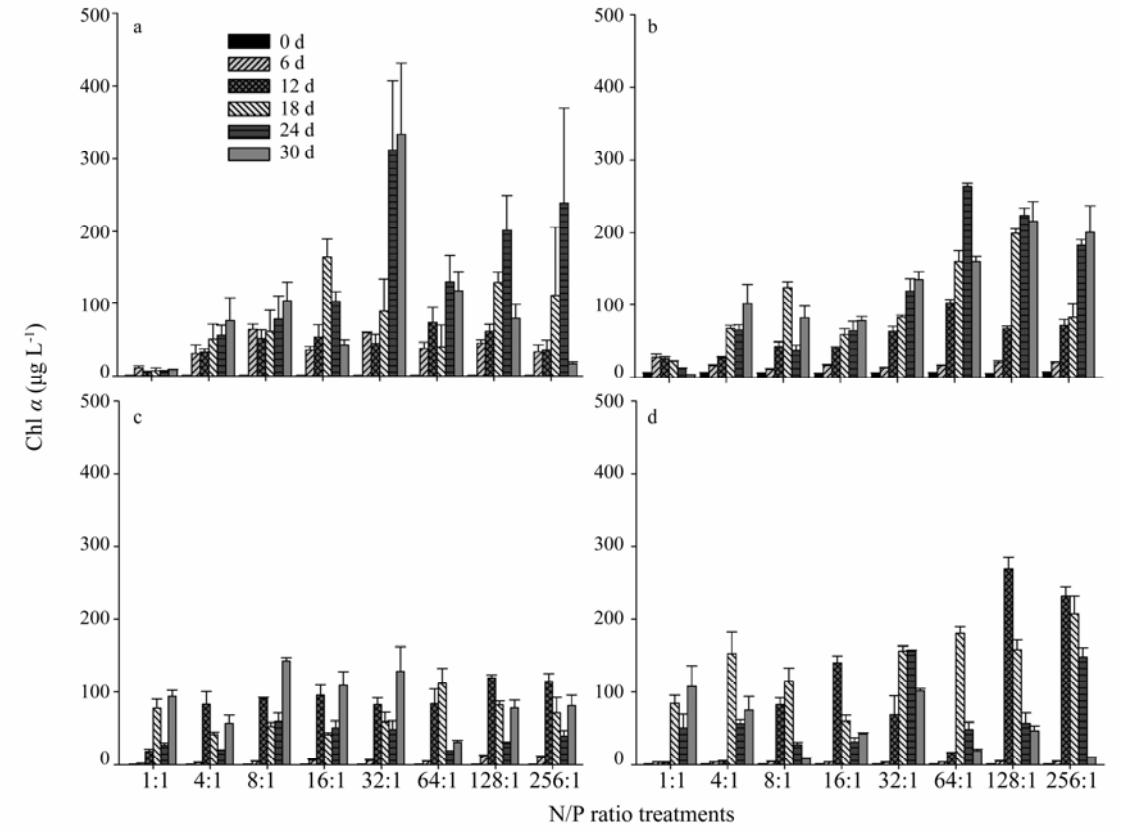

Fig.2 Changes in chl *a* concentration ($\mu g\,L^{-1}$, mean ± S.E.M, $n = 3$) of phytoplankton community cultured in different N/P ratios for 30 d in (a) spring, (b) summer, (c) autumn and (d) winter.

concentration in the 1:1 treatment (5.96μg L^{-1}) was significantly lower than those in all the other treatments (33.30–73.47μg L^{-1}) on day 12 (ANOVA Tukey's test, $P < 0.05$). In summer test, the chl *a* concentration in the 1:1 treatment (28.28μg L^{-1}) was significantly higher than that in the 8:1 treatment (11.33μg L^{-1}) on day 6; the value in the 128:1 treatment (199.32μg L^{-1}) was significantly higher than those in the 1:1, 4:1, 8:1, 16:1, 32:1 and 256:1 treatments (22.48–124.01μg L^{-1}) on day 18; the value in the 1:1 treatment (3.47μg L^{-1}) was significantly lower than any other treatments (77.83–216.16μg L^{-1}) at the end of summer test (ANOVA Tukey's test, $P < 0.05$ for each comparison; Fig. 2b). The initial chl *a* concentration was extremely low (0.71μg L^{-1}) in autumn test, and no significant difference was observed for different N/P ratio treatments (ANOVA, $P > 0.05$; Fig.2c). Nonetheless, the chl *a* concentration in the 128:1 treatment (12.29μg L^{-1}) was significantly higher than the value in the 1:1 treatment (1.84μg L^{-1}) on day 6; the value in the 1:1 treatment (18.21μg L^{-1}) was significantly lower than those in the other treatments (82.37–119.68μg L^{-1}) on day 12 (ANOVA Tukey's test, $P < 0.05$ for each comparison). In winter test, the chl *a* concentrations in the 8:1, 16:1, 32:1, 128:1 and 256:1 treatments (82.53–269.01μg L^{-1}) were significantly higher than those in the other treatments (3.39–16.58μg L^{-1}) on day 12; the concentrations in the 1:1 and 4:1 treatments (107.65 and 74.60 μg L^{-1}, respectively) were significantly higher than those in the 64:1 and 256:1 treatments (19.26 and 9.34μg L^{-1}, respectively) on day 30 (Kruskal-Wallis test, $P < 0.05$ for each comparison; Fig.2d).

The cell abundance of the phytoplankton community was also significantly affected by N/P ratios in spring test (ANOVA, $P < 0.05$; Fig.3a). The cell abundance in the 8:1 treatment (4.24×10^6 cells L^{-1}) was significantly higher than in the 1:1, 16:1, 32:1, 64:1, 128:1 and 256:1 treatments(($0.45–2.29$) $\times10^6$ cells L^{-1}) on day 6; the values in the 32:1, 64:1 and 128:1 treatments (($1.53–1.68$)$\times10^6$ cells L^{-1}) were significantly higher than in the other treatments (($0.11–0.93$) $\times10^6$ cells L^{-1}) on day 30 (Tukey's test, $P < 0.05$ for each comparison). The initial cell abundance was as high as 0.69 $\times10^6$ cells L^{-1} in summer test, while there was no significant difference in the treatments on day 6 (ANOVA, $P > 0.05$; Fig.3b). The value in the 32:1 treatment (6.57 $\times10^6$ cells L^{-1}) was significantly higher than in the 4:1 treatment (1.08 $\times10^6$ cells L^{-1}) on day 12 (Tukey's test, $P < 0.05$). On the other hand, the cell abundances in the 64:1, 128:1 and 256:1 treatments (1.24, 2.52 and 3.03 $\times10^6$ cells L^{-1}, respectively) were significantly higher than in the other treatments (($0.41—1.11$) $\times10^6$ cells L^{-1}) on day 6; the values in the 8:1, 16:1 and 32:1 treatments (7.95, 8.35 and 8.08 $\times10^6$ cells L^{-1}, respectively) were significantly higher than in the other treatments (($1.58–5.90$)$\times10^6$ cells L^{-1}) at the end of autumn test (ANOVA Tukey's test, $P < 0.05$ for each comparison; Fig.3c). The cell abundance in the 4:1 treatment (0.11 $\times10^6$ cells L^{-1}) was significantly lower than in the other treatments (($0.53–1.34$)$\times10^6$ cells L^{-1}) on day 6; the value in the 128:1 treatment (7.84 $\times10^6$ cells L^{-1}) was significantly higher than in the 1:1 and 8:1 treatments (2.36 and 2.69 $\times10^6$ cells L^{-1}, respectively) on day 18; the values in the 128:1 and 256:1 treatments (2.92 and 1.80 $\times10^6$ cells L^{-1}, respectively) were significantly higher than in the 1:1, 4:1 and 8:1 treatments (($0.25–0.50$) $\times10^6$ cells L^{-1}) on day 30 in winter test (ANOVA Tukey's test, $P < 0.05$ for each comparison; Fig.3d).

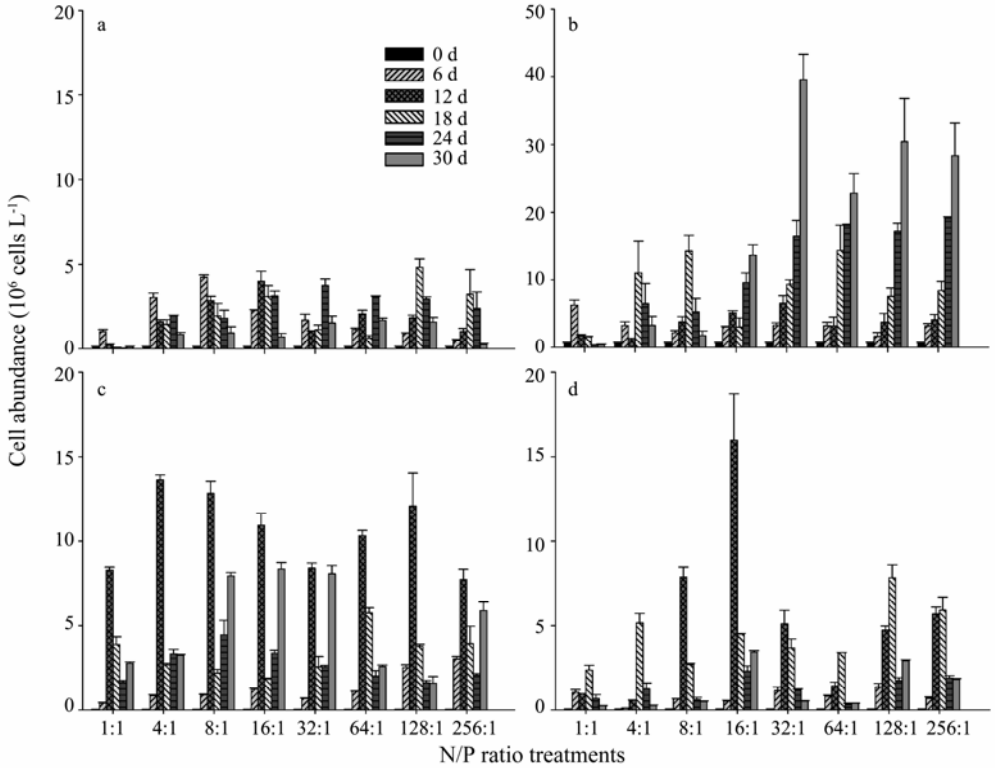

Fig.3 Changes in cell abundance (10^6 cells L^{-1}, means ± S.E.M, $n = 3$) of phytoplankton community cultured in different N/P ratios for 30 d in (a) spring, (b) summer, (c) autumn and (d) winter.

3.2 Diversity Indices

In the present study, significant differences in the Shannon-Wiener index (H') and Pielou evenness index (J) were observed in different N/P treatments (ANOVA, $P < 0.05$; Fig.4, Fig.5). The index H' in the 128:1 and 256:1 treatments (1.68 and 1.74, respectively) were significantly higher than in the other treatments (0.62 – 1.30) on day 6 in spring test (ANOVA Tukey's test, $P < 0.05$; Fig.4a). Similarly, the index J in the 256:1 treatment (0.75) was

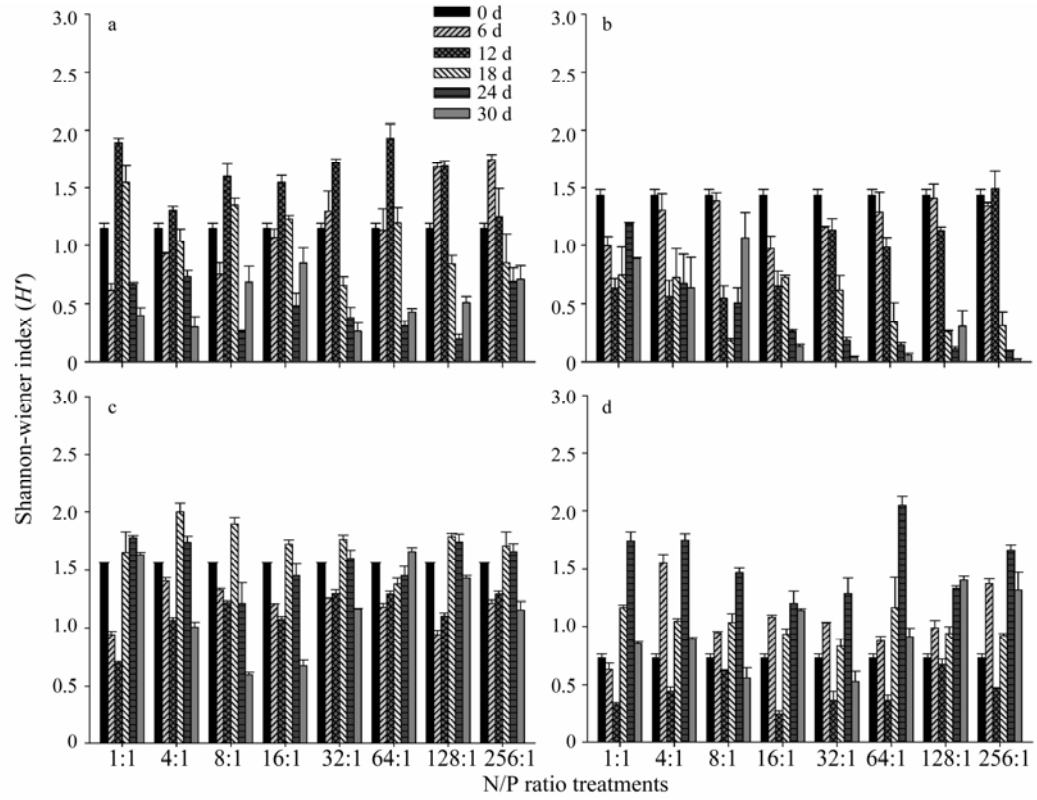

Fig.4 Changes in Shannon-Wiener index (H') (means ± S.E.M, n = 3) of phytoplankton community cultured in different N/P ratios for 30 d in (a) spring, (b) summer, (c) autumn and (d) winter.

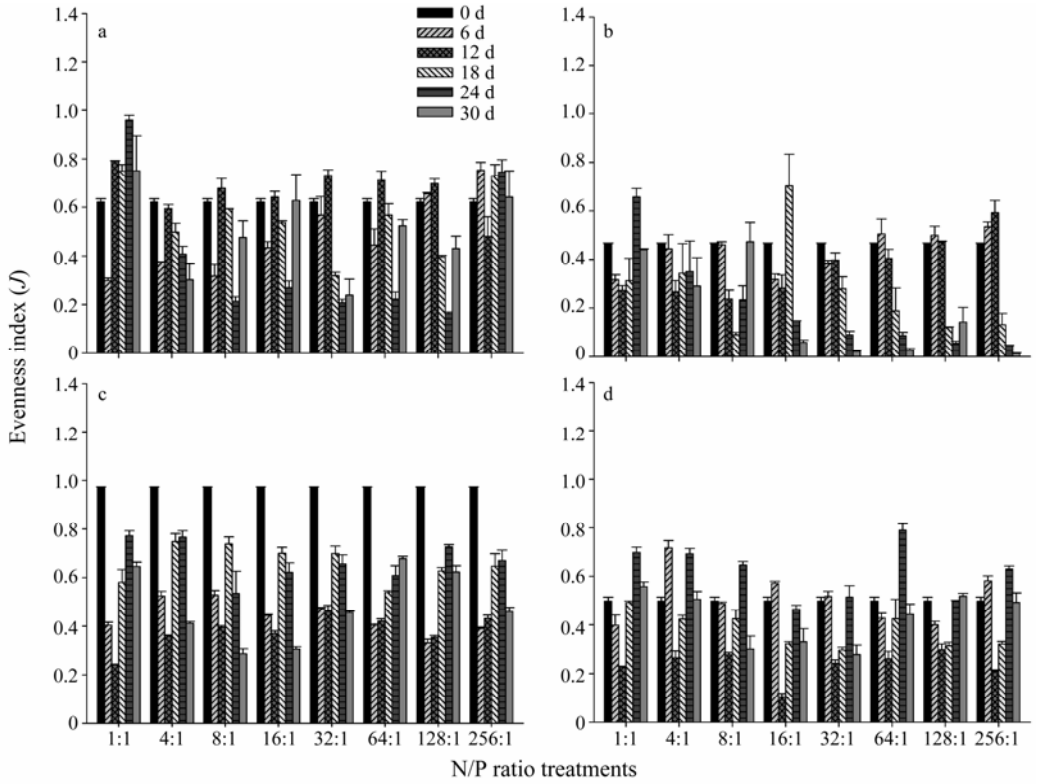

Fig.5 Changes in evenness index (J) (means ± S.E.M, n = 3) of phytoplankton community cultured in different N/P ratios for 30 d in (a) spring, (b) summer, (c) autumn and (d) winter.

significantly higher than in the 1:1, 4:1 and 8:1 treatments (0.30 – 0.37) on day 6; the index *J* in the 32:1 treatment (0.32) was significantly lower than in the other treatments (0.40 – 0.75) on day 18 (ANOVA Tukey's test, $P < 0.05$ for each comparison; Fig.5a). In summer test, the index *H′* in the 256:1 treatment (1.51) was significantly higher than in the 1:1, 4:1, 8:1 and 16:1 treatments (0.55 – 0.65) on day 12 (ANOVA Tukey's test, $P < 0.05$; Fig.4b). The index *J* in the 128:1 and 256:1 treatments (0.47 and 0.59, respectively) were significantly higher than in the 1:1, 4:1, 8:1 and 16:1 treatments (0.24 – 0.28) on day 12 (ANOVA Tukey's test, $P < 0.05$; Fig.5b). In autumn test, the index *H′* in the 1:1 and 128:1 treatments (0.93 and 0.94, respectively) on day 6, in the 1:1 treatment (0.69) on day 12, and in the 8:1 and 16:1 treatments (0.59 and 0.67, respectively) on day 30, were significantly lower than in any other treatments in the same period (ANOVA Tukey's test, $P < 0.05$ for each comparison; Fig.4c). The *J* in the 1:1, 64:1, 128:1 and 256:1 treatments (0.33 – 0.40) were significantly lower than in the 4:1 and 8:1 treatments (0.52 and 0.53, respectively) on day 6; the value in the 1:1 treatment (0.24) on day 12, and values in the 8:1 and 16:1 treatments (0.29 and 0.30, respectively) on day 30, were significantly lower than any other treatments in the same period (ANOVA Tukey's test, $P < 0.05$ for each comparison; Fig.5c). In winter test, the *H′* in the 1:1 treatment (0.60) was significantly lower than in any other treatments (0.88 – 1.55) on day 6; values in the 1:1 and 64:1 treatments (1.17 for both) were significantly higher than in the other treatments (0.83 – 1.05) on day 18; the value in the 64:1 treatment (2.04) was significantly higher than in the 16:1, 32:1 and 128:1 treatments (1.20 – 1.33) on day 24 (ANOVA Tukey's test, $P < 0.05$ for each comparison; Fig.4d). Moreover, *J* in the 4:1 treatment (0.72) was significantly higher than in the 1:1, 8:1, 32:1, 64:1 and 128:1 treatments (0.40 – 0.52) on day 6; the value in the 64:1 treatment (0.79) was significantly higher than in the 16:1, 32:1 and 128:1 treatments (0.46, 0.51 and 0.50, respectively) on day 24 (ANOVA Tukey's test, $P < 0.05$ for each comparison; Fig.5d).

3.3 Species Composition

In spring test, *Skeletonema costatum* (proportion of 48.5% – 83.7%) dominated the phytoplankton communities in all test treatments on day 6. The proportion of *S. costatum* decreased while that of *Nitzschia longissima* increased with increasing N/P ratio (Fig.6a). Then the proportion of *S. costatum* (4.6% – 6.8%) decreased dramatically while that of *Thalassiosira rotula* (4.3 % – 38.0%) increased and became the dominant species in all treatments on day 12. *Protoperidinium bipes* was the main dinoflagellate species, constituting 3.4 % – 17.4% of the total cell abundance in the communities (Fig.6b). On day 18, *Guinardia delicatula* and *T. rotula* dominated the community together in all the test treatments except in the 1:1 treatment (Fig.6c). Afterwards, the proportion of *Cerataulina pelagica* increased to 77.1% – 96.7% in all treatments on day 24 (Fig.6d). At the end of test, the proportion of *G. delicatula* in the 8:1, 16:1, 64:1 and 128:1 treatments increased to 78.3% – 86.8%; in contrast, *C. pelagica* (51.7% – 78.9%) still dominated the community in the other treatments (Fig.6e).

In summer test, the succession of phytoplankton com-

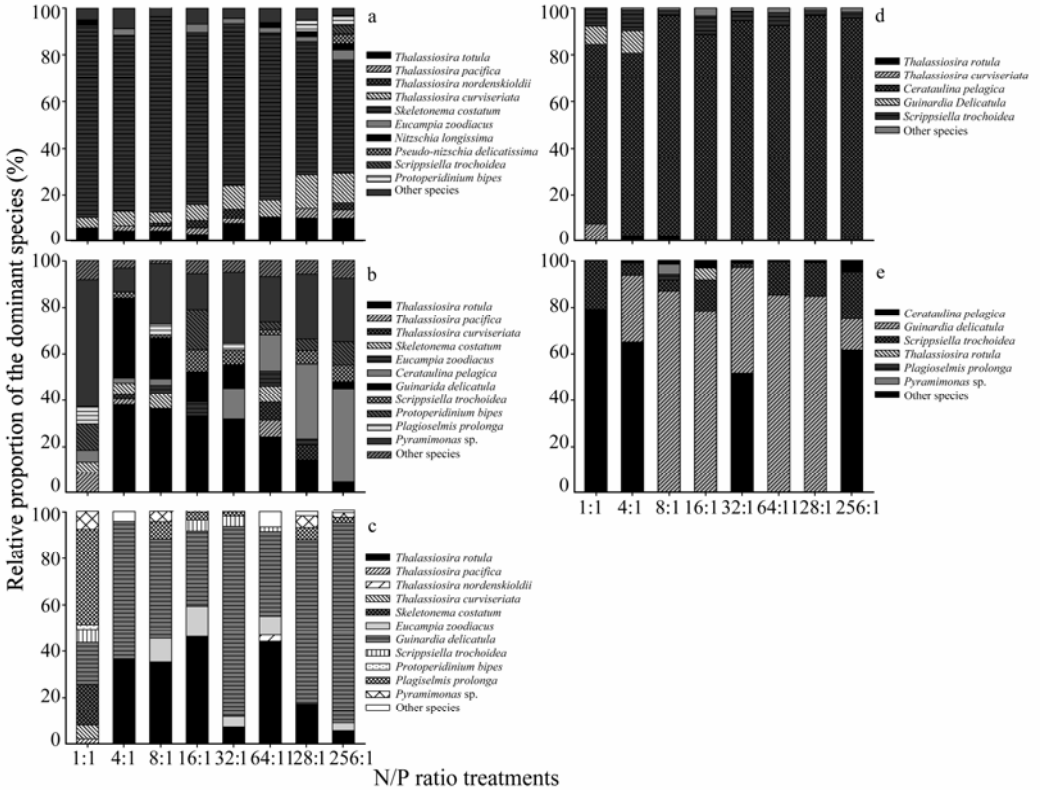

Fig.6 The proportion of dominant species (%) cultured in different N/P ratios for (a) 6 d, (b) 12 d, (c) 18 d, (d) 24 d and (e) 30 d in spring test.

munity was quick due to high water temperature. On day 6, *S. costatum* (53.4%–76.7%) and *Pseudo-nitzschia* spp. (11.8%–26.4%) were the dominant species in all N/P treatments; *Thalassiosira curviseriata* also constituted a high proportion (12.5%–16.5%) in the 1:1, 4:1, 8:1, 16:1, 32:1 and 64:1 treatments. In contrast, the proportion of dinoflagellate was extremely low (Fig.7a). On day 12, *C. pelagica* (54.4%–88.0%) replaced *S. costatum* (0–4.0%) and became the dominant species in all treatments, while *Pseudo-nitzschia* spp. still accounted for a high proportion (20.6%–29.1%) in the 32:1, 64:1, 128:1 and 256:1 treatments (Fig.7b). Thereafter, the proportion of *C. pelagica* increased constantly and reached 68.6 – 99.8% in all treatments by the end of test (Fig.7c-e).

In autumn test, *S. costatum* (63.0%–78.7%) and *T. curviseriata* (2.4%–14.6%) were the dominant species in all treatments; *Thalassiosira pacifica* also constituted a high proportion (2.0%–5.1%) in the 1:1, 4:1, 8:1, 16:1 and 32:1 treatments on day 6. As for the dinoflagellates, *Proro- centrum triestinum*, which constituted 2.2% of the total cell abundance, was the dominant species in the 1:1 treatment (Fig.8a). Afterwards, the decrease of the *S. costatum* (8.4%–62.5%) proportion was accompanied by an increase in the dominance of *T. curviseriata* (13.8%–72.9%) in all treatments on day 12. *Thalassiothrix frauenfeldii* also became the dominant species in the 4:1, 8:1, 16:1, 32:1, 64:1, 128:1 and 256:1 treatments (2.0%–6.3%) (Fig.8b). On day 18, *T. curviseriata* (7.1%–45.7%), *S. costatum* (3.3% – 26.9%), *T. frauenfeldii* (12.7% – 24.3%), *P. bipes* (3.7%–27.3%), *Scrippsiella trochoidea* (2.0% – 18.9%) and *P. triestinum* (2.7 %– 16.3%) were dominant species in all treatments, and the proportion of dinoflagellates exceeded that of diatoms in the 4:1, 16:1 and 32:1 treatments (Fig.8c). Thereafter, a further increase in the proportion of dinoflagellates took place in all treatments on day 24, and *P. triestinum* (18.9%–35.9%), *P. bipes* (3.0% –27.3%), *S. trochoidea* (5.8%–18.9%) and *T. frauenfeldii* (10.3%–28.0%) existed as co-dominants (Fig.8d). At the end of test, *G. delicatula* (44.4%–84.5%) dominated the community in the 4:1, 16:1 and 32:1 treatments, while *P. triestinum* (36.2%–70.5%) dominated the community in the other treatments (Fig.8e).

In winter test, a community succession order from diatoms to dinoflagellates also appeared in all treatments. On day 6, *S. costatum* (50.7–76.2%) and *Plagioselmis prolonga* (4.7–45.0%) were the dominant species in all treatments, and *T. curviseriata* (9.4–21.5%) also constituted a high proportion in treatments except in the 4:1 treatment (Fig.9a). *S. costatum* (83.9–94.1% and 68.3 –78.8%) was the predominant species during the subsequent phase of test (day 12 and day 18), while *T. curviseriata* decreased constantly in all treatments (Figs.9b, c). Thereafter, the dominant species and their dominance varied with different N/P ratios during the late phase of test. On day 24, *P. bipes* (22.7%), *S. costatum* (21.9%) and *T. rotula* (21.8%) dominated the community in the 4:1 treatment, the proportion of *P. bipes* was above 50% in the 8:1, 16:1 and 32:1 treatments, and *G. delicatula* (28.9%) and *S. costatum* (41.1%) dominated their respective communities in the 64:1 and 256:1 treatments

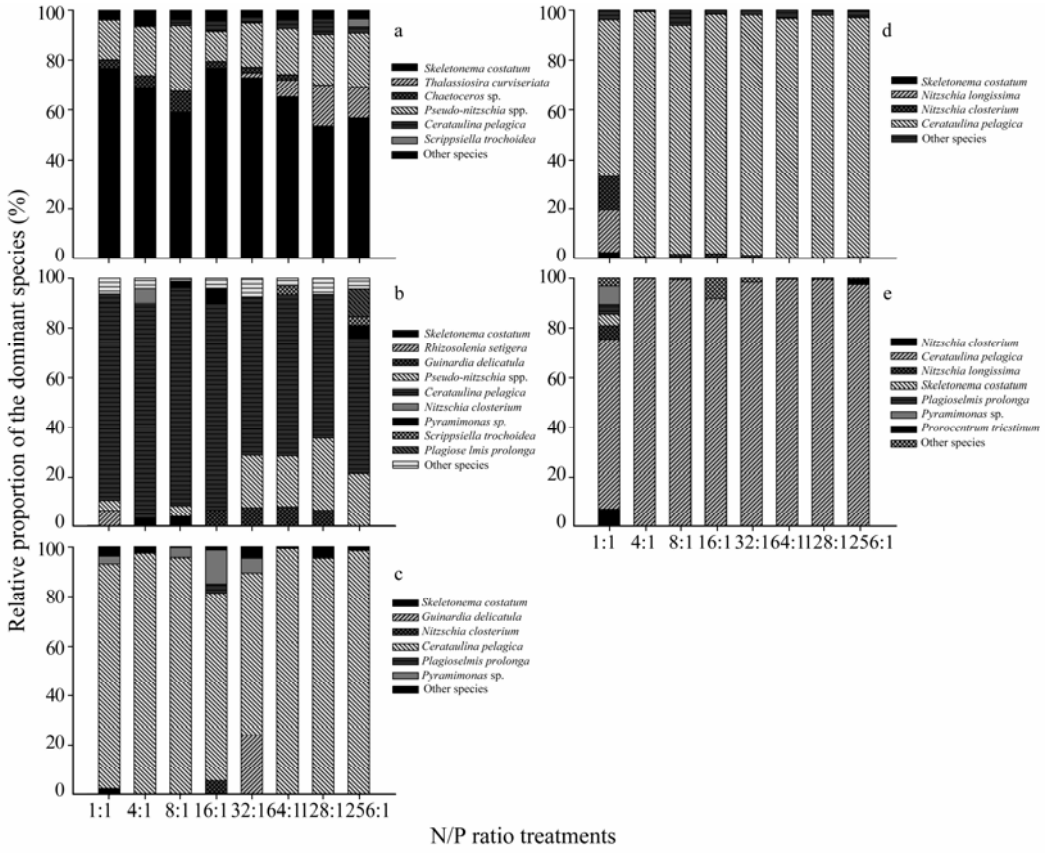

Fig.7 The proportion of dominant species (%) cultured in different N/P ratios for (a) 6 d, (b) 12 d, (c) 18 d, (d) 24 d and (e) 30 d in summer test.

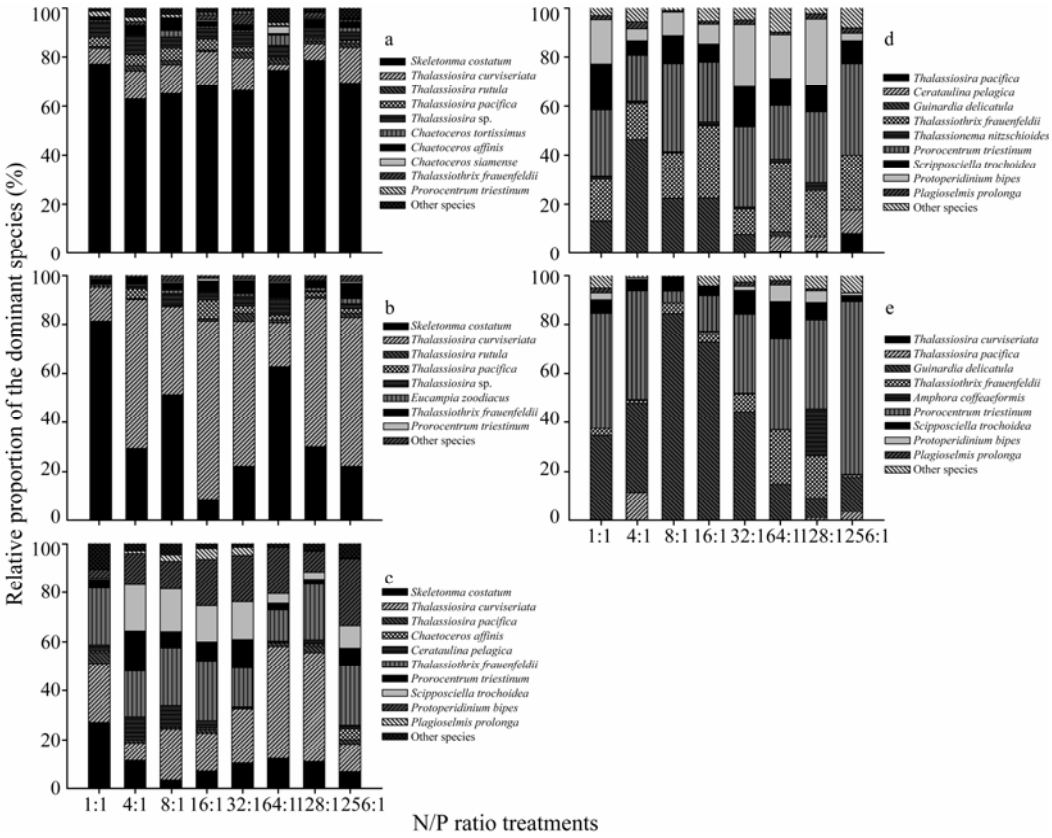

Fig.8 The proportion of dominant species (%) cultured in different N/P ratios for (a) 6 d, (b) 12 d, (c) 18 d, (d) 24 d and (e) 30 d in autumn test.

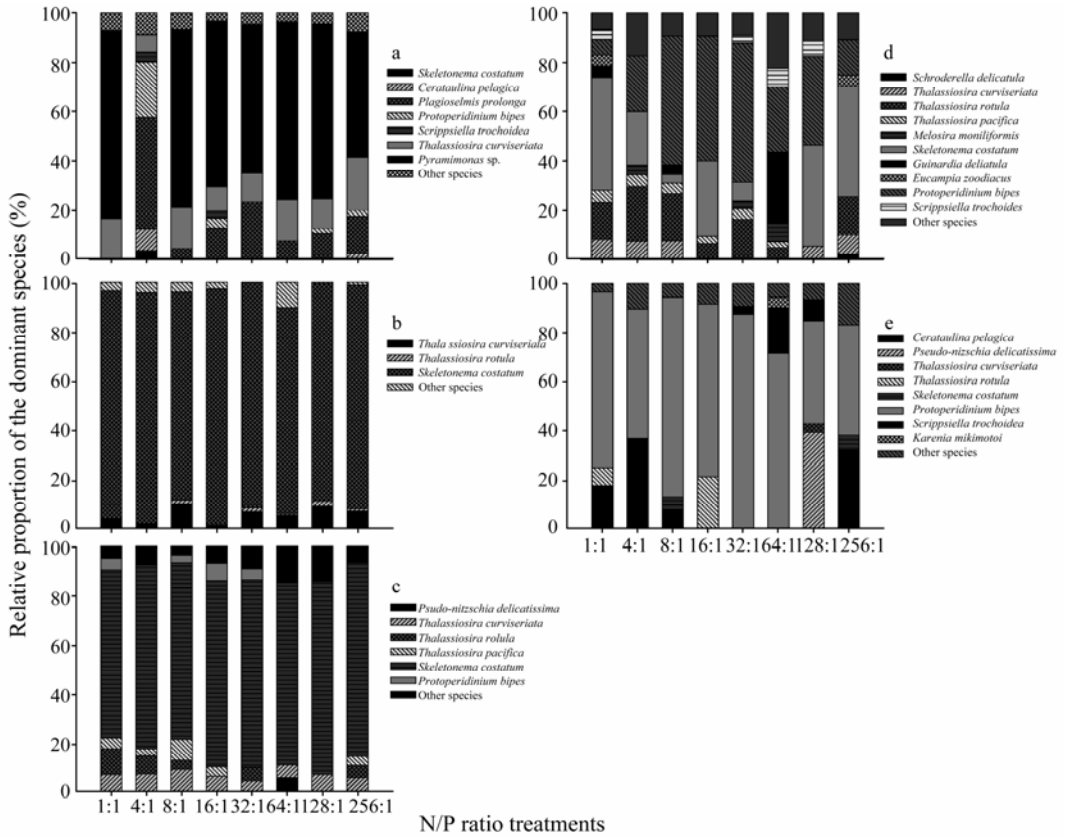

Fig.9 The proportion of dominant species (%) cultured in different N/P ratios for (a) 6 d, (b) 12 d, (c) 18 d, (d) 24 d and (e) 30 d in winter test.

(Fig.9d). On day 30, *P. delicatissima* (39%) and *C. Pelagica* (32%) dominated the communities in the 128:1 and 256:1 treatments, respectively, while *P. bipes* (52.9% – 87.6%) dominated the communities in the other treat-

ments (Fig.9e).

4 Discussion

The Changjiang Estuary and Zhejiang coastal waters are characterized, in general, by phosphorus limitation and by a high proportion of *S. costatum* abundance during all seasons (Wang *et al*., 2004). Our data was also in keeping with this conclusion; *S. costatum* was detected to contribute to 34.5%–60.8% of the initial community in different season tests. Moreover, our result suggested that the growth of phytoplankton community is significantly affected by N/P ratios, and the responses varies with different seasons. The cell abundance in the low N/P ratio treatments (*e.g*. 1:1) was higher than in the high N/P ratio treatments (*e.g*. 128:1) at the early phase (day 6) of spring and summer tests. This is in accordance with the experiment conducted by Harrison *et al*. (1990) off the Xiamen coast, and *Chaetoceros calcitrans* was found to have a higher growth rate in the low N/P ratio treatment (16:1 *vs*. 656:1). This is probably due to the fact that diatoms have higher phosphorus demands with respect to dinoflagellates and thus are more readily affected by phosphorus supply (Egge, 1998; Riegman *et al*., 1996). As the Chang jiang Estuary and adjacent coastal waters are phosphate-limited (Lai *et al*., 2011; Wong *et al*., 1998; Zhou *et al*., 2008), phosphate enrichment may especially benefit the growth of diatoms at the initial phase of spring and summer tests. On the contrary, both the cell abundance and chl *a* concentration indicated that the growth of phytoplankton in the high N/P ratio treatments (*e.g*. 128:1 and 256:1) was higher than in the low N/P ratio treatments (*e.g*. 1:1 and 4:1) at the same period of autumn and winter tests. This may relate to the high initial P concentrations of the test water (1.49, 1.36 in autumn and winter *vs*. 1.16, 0.74 in spring and summer).

In the present study, the diversity indices H' and J of phytoplankton community respond sensitively to different N/P ratios. Compared with other indices, H' depends less on the species richness (S), but is more sensitive to the uniformity of species distribution in the community (Sun *et al*., 2004). This could explain the lower H' and J values in the 8:1 and 16:1 treatments than in any other treatments on day 30 of autumn test. The relative proportions of *G. delicatula* (predominant species) were as high as 84.5% and 72.9% in 8:1 and 16:1 treatments at that moment. In contrast, there were several species (*P. triestinum*, *G. delicatula* and *T. frauenfeldii*) served as co-dominant species in the other treatments. Therefore, shifts in community structure from several co-dominants to only one or two dominants due to competitive advantage brought about by the changes in nutrients supply may result in the decline of community uniformity and eventually lead to a lower H' value (Li *et al*., 2010). Qu *et al*. (2000) reported that the treatments with close to Redfield ratio have higher diversity index value relative to unbalanced N/P ratio. However, the H' and J values in the 8:1 and 16:1 treatments were lower than in the other treatments in our autumn test. The mechanism for this difference is unclear, but the difference in nutrients addition manner (single pulse *vs*. continuous pulse) and the experimental duration (5 d *vs*. 30 d) between the tests may result in different competitive patterns and thus the dominant species, which may be an important reason.

The nutrient requirements of individual species vary depending on species-specific nutrient uptake kinetics, assimilation and storage capacities (Tilman *et al*., 1982). Therefore, the N/P ratio may not only affect the phytoplankton growth, but also regulate dominant species due to their different nutrient uptake rates and preferences (Li *et al*., 2012). The species identified in our different treatments were generally similar in all season tests. However, the dominance of some species varied with different N/P ratios. Furthermore, the succession of dominant species also differed among the N/P treatments. Thus, the N/P ratios also have a significant effect on phytoplankton succession. At the early phase of winter test (day 6), high N/P ratio (128:1 and 256:1) were more favorable for the growth of diatoms, while high (256:1) or low (4:1) N/P ratios stimulated the growth of dinoflagellates at the late phase of test (day 30). A series of previous field and mesocosm experiments also suggested that N/P ratio has an important role in phytoplankton community succession. For example, Billen *et al*. (1988) reported high N/P ratio diatoms gain competitive advantage in German Bight. In Tolo Harbour, the dinoflagellate replaced diatom as the dominant species was found accompanied by a significant decrease of N/P ratio (Yung *et al*., 1997). The replacement of diatom by dinoflagellate also took place in the Bohai Sea accompanied by increased N/P and decreased Si/N ratio (Wei *et al*., 2004). Therefore, the changes of nutrient structure will lead to variation in species composition, community structure and even affect zooplankton food structure, and thus change the diversity and stability of marine ecosystem (Piehler *et al*., 2004).

The difference of phytoplankton nutrient utilization and growth features is the key factor that affects their ecological strategy and community structure (Anderson *et al*., 1991). Our four-season test data suggested that diatoms compete better with dinoflagellates in conditions of nutrient enrichment, at least in the early phase of tests. Compared with other genera, most diatoms (*e.g. S. costatum*) have lower Ks (half-saturation constant for nutrient uptake), but higher growth rates; these groups are classified as *r*-strategists (Billen *et al*., 1988; Li *et al*., 2010). The higher growth rate potential of diatoms likely allows them to increase their relative abundance quickly and become predominant easily under nutrient adequacy conditions in early phase of succession. In contrast, most dinoflagellates (*e.g. P. donghaiense* and *P. triestinum*) which have much larger nutrient pools but lower growth rate are typical *k*-strategists (Anderson *et al*., 1991; Li *et al*., 2011). These groups could reserve excess nutrients inside the cell through 'luxury consumption', and utilize the nutrients in the intracellular pools to acquire competitive advantage during times of nutrient limitation in late phase of succession (Li *et al*., 2008). However, this succession order will be disrupted if there are continuous

nutrient supply and better water exchange, and diatoms could maintain high population levels and hinder the dinoflagellates to thrive (Lin *et al*., 1994). Therefore, this may be the reason why dinoflagellates failed to dominant the community in our spring and summer tests. However, the major nutrients (N, P and Si) were also added constantly and no nutrients limitation took place in our autumn and winter tests, and dinoflagellates still replaced diatoms and became the dominant species in late phase of succession. This result may relate to the heterotrophy feature of dinoflagellates. Most phototrophic dinoflagellates have been considered to be mixotrophic, which could utilize nutrients from particulate food or directly feed on co-occurring diatoms in nutrient-limited conditions (Jeong *et al*., 2004; Lagus *et al*., 2004; Sun and Guo, 2011). For example, Yoo *et al*. (2009) observed that *P. donghaiense* and *P. triestinum* could feed on *S. costatum*. Thus, the feeding relationship and nutrient- utilizing characters of dinoflagellates could also determine the competition of community and thus the sequences of succession to some extent.

Due to the complexity of the marine ecosystem and high nutrient concentrations in coastal waters, the results of experiments (indoor or enclosure) conducted in different seas are not exactly the same, or even contrary. This may be due to the fact that bioassays are performed over a number of days under controlled conditions, and the growth and succession of phytoplankton are affected by a variety of environmental factors such as light, temperature, salinity, pH and grazing, other than nutrients supply (Beardall *et al*., 2001; Holland *et al*., 2004; Montagnes and Franklin, 2001; Wang *et al*., 2004). Therefore, the combined effects of these factors need to be considered and the experimental conditions should be strictly controlled to ensure the reliability of the results when such experiments are performed.

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