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Picophytoplankton abundance and community structure in the Philippine Sea, western Pacific*

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Abstract Flow cytometric determinations of the abundance distribution and community structure of picophytoplankton (i.e., *Prochlorococcus* spp., orange fluorescence *Synechococcus* spp. and picoeukaryotes) were used for samples taken from the Philippine Sea in the western tropical Pacific Ocean from September to October of 2004. A fluorescence probe was employed to detect Chlorophyll *a* (Chl *a*). Abundances of *Prochlorococcus* spp., orange fluorescence *Synechococcus* spp. and picoeukaryotes ranged from 0.1 to 58×10^3 cells ml^{-1}, 0.38 to 17×10^2 cells ml^{-1} and 0.42 to 26×10^2 cells ml^{-1}, respectively. *Synechococcus* spp. and picoeukaryotes co-occurred in relatively shallow water with the maximum abundance observed at 50 to 70 m depth, while *Prochlorococcus* spp. only occurred in the 70 to 200 m layer. *Prochlorococcus* spp. was the dominant picophytoplankton population in terms of abundance and biomass. The cell size and carbon biomass content were estimated for the three picophytoplankton groups. In addition, among the three groups of picophytoplankton, the relative contribution of red fluorescence to the total red fluorescence varied with depth. The fluorescence and light scatter properties of individual cells indicated that in the upper 100 m layer, picoeukaryotes were a major contributor to total red fluorescence, while at the depth below 100 m, *Prochlorococcus* spp. and *Synechococcus* spp. made an important contribution to the total red fluorescence.

Keyword: picophytoplankton; community structure; flow cytometry; carbon biomass; Philippine Sea

1 INTRODUCTION

The Philippine Sea is a marginal sea to the east of the Philippines. It is part of the western Pacific, encompassing an area from Palau to Japan. The ecosystem of the western tropical Pacific is mostly oligotrophic and is often likened to that of the Pacific warm pool. Mesotrophic waters of the Pacific cold tongue in the East, which are cold and saline and have high-nutrient, low-chlorophyll (less than 0.1 mg m^{-3}) (HNLC) conditions, are surrounded by the oligotrophic waters of the warm pool to the west. The warm pool is characterized by a sea-surface temperature (SST) higher than 29°C, low surface salinity and oligotrophic conditions (Mackey et al., 1995; Radenac et al., 1996; Messié et al., 2006).

Picophytoplankton (<2 μm) is composed of three groups including the cyanobacteria *Prochlorococcus* spp., *Synechococcus* spp. and small eukaryotic algae. These tiny microorganisms are major primary producers, contributing more than 50% of the biomass and production in warm oligotrophic tropical and sub-tropical open oceans (Agawin et al., 2000). *Prochlorococcus* spp. has been found to be more abundant in oligotrophic than in eutrophic water, and *Synechococcus* spp. is ubiquitous in the upper layers of temperate and warm oceans. These two phytoplankton groups, together with picoeukaryotes, display fast growth rates matched by high mortality losses, making them fundamental components of the biomass and primary production

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of marine ecosystems, and major contributors to nutrient regeneration and cycling in the ocean. The community structure and abundance of picophytoplankton is therefore of great importance for the characterization of marine ecosystems (Pan et al., 2005).

The distribution of picophytoplankton in the Philippine Sea at the edge of the South China Sea has been the subject of past research (Agawin et al., 2003; Yang et al., 2004). However, little data has been published regarding the abundance of picophytoplankton in the Pacific side of the Philippine Sea. This study investigated the distribution and community structure of picophytoplankton in the Philippine Sea in the western Pacific. Cell size and carbon content of picophytoplankton were estimated using flow cytometry and the contributions of three different picophytoplankton components to primary biomass were calculated. In addition, the relationship between the variation of light scattering and fluorescence properties of individual cells in the depth profile was discussed.

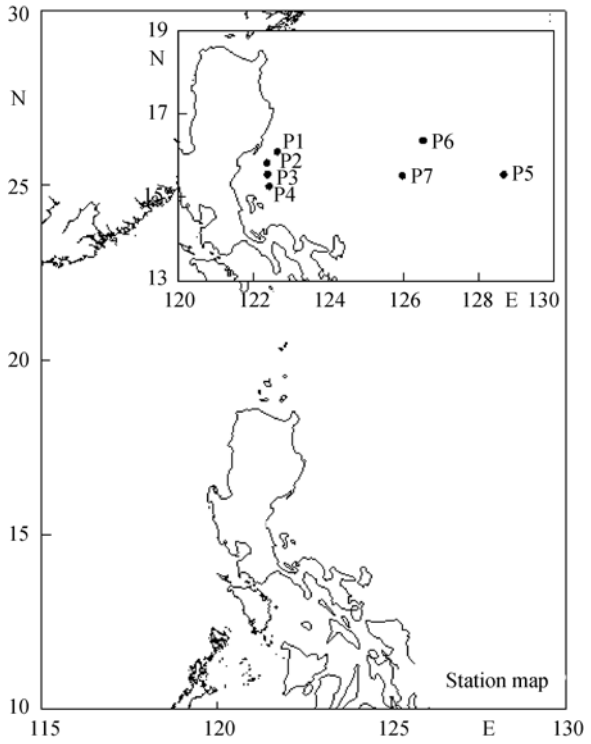

Fig.1 Sampling stations

2 METHODS

2.1 Study area and sampling

This study was carried out in the Philippine Sea (15°15′–16°20′N; 122°18′–128°38′ E) in the western Pacific (Fig.1) between September 8 and October 3, 2004. Seawater from five to six discrete depths was collected using a Rosette equipped with a CTD (Conductivity, Temperature and Depth, SeaBird-General Oceanic) and 5-liter Niskin bottles. Samples for flow cytometry analysis were collected and treated immediately with paraformaldehyde (final conc., 1%) and preserved at -20°C for later analysis.

2.2 Hydrographic and chlorophyll *a* measurements

Temperature and salinity were recorded throughout the water column using a Seabird CTD. Chl *a* signature fluorescence was measured using a fluorometer attached to the Seabird CTD to generate a continuous profile of in vivo fluorescence. Chl *a* concentrations were calculated from the Chl *a* fluorescence data with the equation (Chl *a* concentration = 0.751× Chl *a* Fluorescence intensity +0.22) which was calibrated with samples using an in vitro fluorometer (Turner Design, Model 10-005R) following acetone extraction (Parsons, 1984).

2.3 Flow cytometry analysis

The fixed seawater samples were analyzed using a FACSVantage SE flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with water-cooled argon laser (488 nm). Several picophytoplankton populations were differentiated using their FL2 (orange fluorescence) vs. FL3 signatures (red fluorescence). Yellow-green fluorescent beads (2.0 μm) (Polyscience Inc., catalogue #18338-5) were employed as an internal standard to normalize the size and cell fluorescence to allow a comparison of the different phytoplanktonic groups (Blanchot et al., 1996). Flow cytometry data were collected in list mode and analyzed using CELLQUEST Software (Becton Dickinson, CA, USA).

2.4 Estimation of mean cell size and carbon content

The Mie theory predicted a diameter d dependence of forward scattering (FS) in d^{β} with β between 4 and 6 (Morel, 1991). For laboratory cultures of reasonably spherically shaped cells, there was always a strong correlation between FLS and cell size (Olson et al., 1989; Stramski et al., 1995). Durand (1995) obtained a β value of 5.4 from 4 strains of *Synechococcus* spp. at two different times of day, while Chisholm (1992) indicated a value of 5.1 in *Synechococcus* spp. On the other hand, Binder (1996) interpreted FS variation from dawn to dusk and reported a value of 5.4 in *Prochlorococcus* spp. A similar value was determined for both

Prochlorococcus spp. and picoeukaryotes (Blanchot et al., 1997). Moreover, Blanchot (2001) reported a practical way to estimate the mean cell size, using beads as an internal reference, and found similar results. In the present study, we followed this method. Namely: $d_{cell} = d_{bead}\,(FS)^{1/5}$.

The resulting cell sizes were then converted to carbon using the conversion factors of 470 fg C cell^{-1} for prokaryotes and 433(cell volume)$^{0.866}$ pg C cell^{-1} for eukaryotes (Verity et al., 1992).

2.5 Data analysis and graphics

Graphics were generated using Surfer v8.0 and MS-EXCEL. Correlation analysis of the mean cellular forward angle light scatter (FALS) data versus the red fluorescence data was carried out using correlation and regression modules of MS-EXCEL.

3 RESULTS

3.1 Hydrological conditions and chlorophyll *a* concentrations

In this survey, the hydrological conditions and Chlorophyll *a* concentrations were similar among stations P1, P2, P3 and P4, which were near shore and also similar between stations P6 and P7 which were offshore. Hydrological conditions and Chlorophyll *a* concentrations at station P5, which was far offshore, were different from all other stations. Stations P3, P7 and P5 were thus selected as examples (Fig.2).

The temperature decreased from 29.7 to 17.2°C from the upper layer to the bottom (Fig.2). Salinity ranged from 33.2 to 35.1 with a maximum value observed between 75 m and 120 m depth (Fig.2). Chlorophyll *a* concentrations varied between 0.02 and 0.62 mg m^{-3}, with a comparably low Chl *a* concentration observed at the surface (less than 0.1 mg m^{-3}) and high values observed from 70 to 85 m depth for the stations near shore and between 100 m to 120 m depth for the stations far offshore (Fig.2).

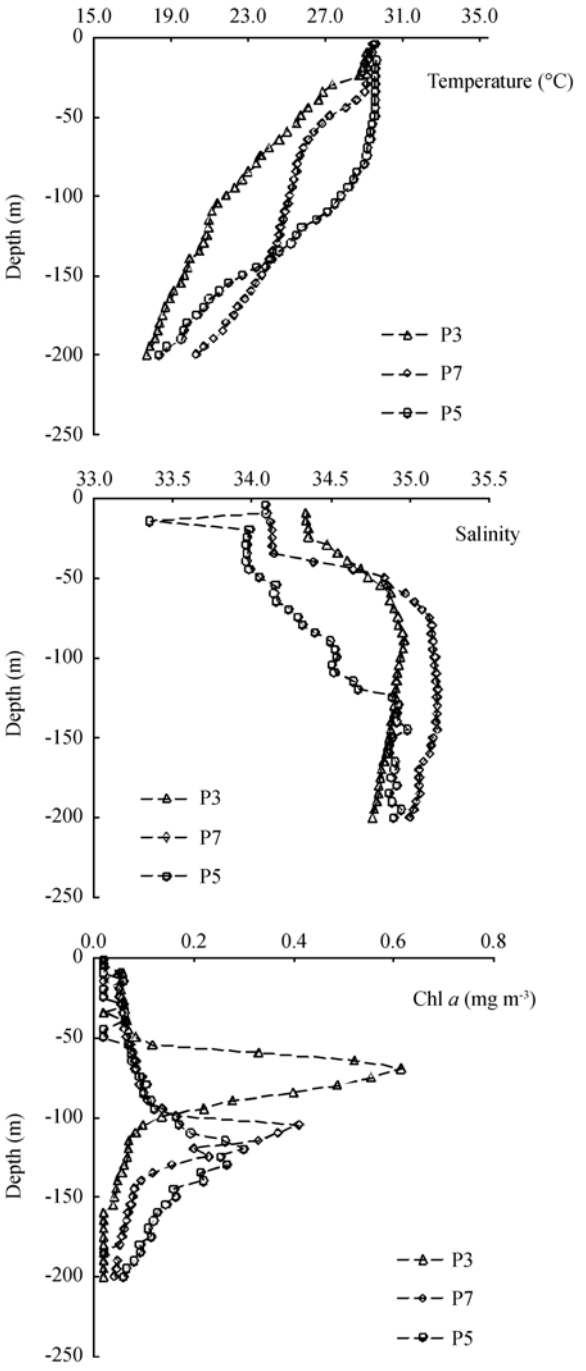

Fig.2 The distribution of temperature (°C), salinity and Chlorophyll *a* (mg m^{-3}) at three typical stations in the western Pacific region of the Philippine Sea during the survey

3.2 Vertical variation in abundances of picophytoplankton groups

Flow cytometric analysis revealed that the picophytoplankton community in the Philippine Sea was comprised of orange fluorescence *Synechococcus* spp., picoeukaryotes and *Prochlorococcus* spp.. All three groups of picophytoplankton showed strong vertical patterns in abundance (Fig.3).

The concentration of *Synechococcus* spp. ((0.38–17)×10^2 cells ml^{-1}) and picoeukaryotes ((0.42–26)×10^2 cells ml^{-1}) were comparable. The distribution of the two groups of picophytoplankton mainly occurred in upper 150 m. However, the depths of maximum cell abundance varied between the two groups. For the picoeukaryotes, the region of maximum cell abundance started at 50 m and increased with depth to about 75 m at inshore stations whilst it extended to a depth of 100 m at offshore stations (Fig.3). The vertical distribution

pattern of *Synechococcus* spp. was similar to that of picoeukaryote, except for the obvious differences in the depth of maximum cell abundance. For *Synechococcus* spp. the maxima extended started at a greater depth of 75 m depth increased with depth to 100 m in coast area and extended to greater depths (125 m) at offshore stations (Fig.3). The *Prochlorococcus* spp. population mostly occurred and predominated from 70 m to 200 m depth in the water column. The concentration of *Prochlorococcus* spp. was about one to two orders of magnitude higher than that of *Synechococcus* spp. ((0.38–17)×10^2 cells ml^{-1}) and picoeukaryotes. The population of *Prochlorococcus* spp. occurred at highest concentrations from 75 m depth (near the coast) to 100 m or even deeper (125 m) at offshore stations (Fig.3). The cell abundance maximum layer of *Prochlorococcus* spp. seemed to be located at the same depth as that of *Synechococcus* spp. (Fig.3).

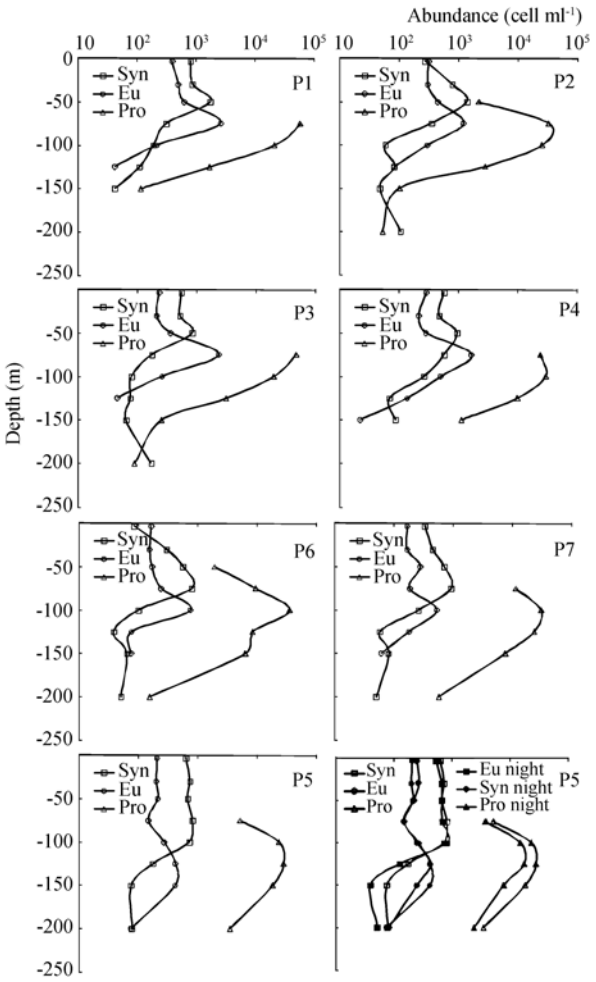

Fig.3 The vertical distribution of picoeukaryotes, *Synechococcus* spp. and *Prochlorococcus* spp. in the western Pacific region of the Philippine Sea during the survey (Unit: cells ml^{-1})

3.3 Cell size and carbon biomass estimates

Cell size and carbon biomass were estimated using the empirical relationship between the forward angle light scatter (FALS) and cell size as previously described. *Prochlorococcus* spp., *Synechococcus* spp. and picoeukaryote cell diameters averaged 0.69 μm (in the range from 0.60 to 0.76 μm), 1.14 μm (in the range from 1.00 to 1.29 μm) and 1.33 μm (in the range from 1.26 to 1.45 μm), respectively. The mean carbon concentration, based on the diameter calculated in this study, reached 81 *f*g C $cell^{-1}$ for *Prochlorococcus* spp., 369 *f*g C $cell^{-1}$ for *Synechococcus* spp., and 554 *f*g C $cell^{-1}$ for picoeukaryotes (Table 1). The calculated percentage biomass contributions to total picophytoplankton were 65%, 15% and 20% for *Prochlorococcus* spp., *Synechococcus* spp. and picoeukaryotes, respectively.

3.4 Vertical distribution of mean cellular forward angle light scatters (FALS) and red fluorescence (FL3)

The mean cellular FALS vertical profiles displayed different trends with depth among the three groups (Fig.4). The value of FALS per cell for the picoeukaryote group decreased slightly with the depth, indicating a slight change in cell size. For *Prochlorococcus* spp. and *Synechococcus* spp., the FALS per cell decreased from the surface to subsurface, and then increased with the depth. Moreover, a sharp increase in average FALS for *Prochlorococcus* spp. was observed from 100 m to 150 m depth (Fig.4A).

The mean cellular red fluorescence of the three-phytoplankton groups in related to depth was shown in Fig.4B. For picoeukaryotes the mean cellular fluorescence increased slightly with depth. This increasing trend was much more obvious for *Synechococcus* spp., with a steady increase from a depth of 50 m. For *Prochlorococcus* spp., the mean cellular fluorescence increased dramatically with depth from 100 m to 150 m and subsequently decreased slightly from 150 m to 200 m depth.

4 DISCUSSION

4.1 Picophytoplankton abundance, biomass and cell size

The current research shows that within the picophytoplankton, the abundances and biomass of picoeukaryote and *Synechococcus* spp. were relatively low, suggesting that picoeukaryote and

Table 1 *Prochlorococcus* spp. (Pro), *Synechococcus* spp. (Syn) and Picoeukaryotes (Peu) in the western Pacific region of Philippine Sea

Depth (m)	FS (AU)			D (μm)			C_c (fg C cell^{-1})		
	Pro	Syn	Peu	Pro	Syn	Peu	Pro	Syn	Peu
0	—	0.076	0.142	—	1.16	1.32	—	387	504
30	—	0.065	0.137	—	1.13	1.31	—	351	495
50	—	0.061	0.149	—	1.11	1.33	—	338	517
75	0.004 3	0.037	0.168	0.66	1.00	1.36	70	249	551
100	0.004 2	0.041	0.160	0.65	1.03	1.35	68	269	538
125	0.004 9	0.085	—	0.67	1.19	—	75	411	—
150	0.006 3	0.095	—	0.71	1.21	—	87	441	—
200	0.006 6	—	—	0.71	—	—	89	—	—

Vertical profiles of Forward Scatter (AU: arbitrary units), estimated cell diameter (D), and carbon content per cell (C_c) in the station P7 determined using the conversion factor of Verity et al. (1992). ("—" means no data)

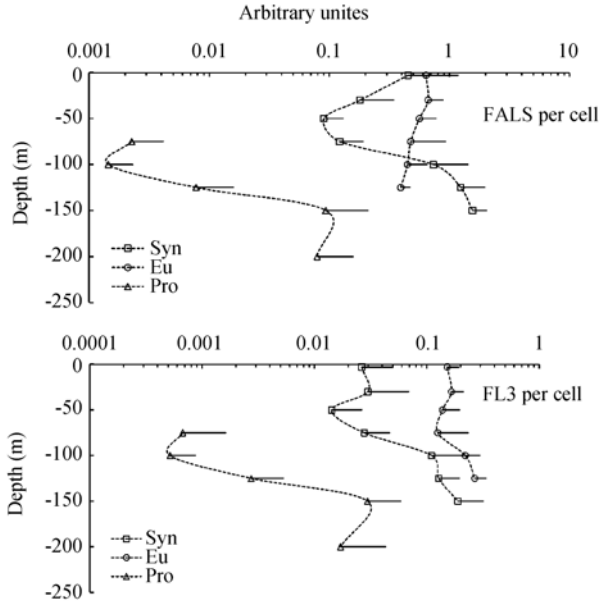

Fig.4 Vertical distribution of mean cellular forward angle light scatter (FALS) and red fluorescence (FL3) among stations

Synechococcus spp. are likely to be minor contributors to this tropical phytoplankton community. Although *Prochlorococcus* spp. abundances were likely to be underestimated due to the low fluorescence of the cells near the surface (Chisholm et al., 1988; Olson et al., 1990), *Prochlorococcus* spp. was still the dominant group in this ecosystem. This group contributed 65% of the total picophytoplankton standing stock. Such a significant contribution to the biomass emphasizes the importance of *Prochlorococcus* spp. in oligotrophic environments.

Moreover, the high abundances of *Prochlorococcus* spp. and low abundances of *Synechococcus* spp. and picoeukaryotes in this sea area demonstrate the greater adaptation of *Prochlorococcus* spp. for nutrient assimilation. This is consistent with the findings of previous studies (Kremer et al., 1994; Landry et al., 1996).

In the stratified oligotrophic water, picoeukaryotes and *Synechococcus* spp. dominated the phytoplankton community in the upper layers, while *Prochlorococcus* spp. dominated in deeper layers. This switch between taxa of picophytoplankton appeared to be related to the ambient nutrient concentrations. In the warm pool of the western Pacific, a deep thermocline and nutricline, combined with a barrier layer produced by shallow haloclines, prevent the upwelling of dissolved inorganic carbon (DIC) and nutrient-rich water into the euphotic zone, even if surface winds from the east are favorable (Borgnea et al., 2002).

One of the main characteristics of tropical ecosystems is low temporal variability (Walsh, 1976). In this study, the change of picophytoplankton abundance, distribution and community structure between noon and evening were investigated at station P5. Cell concentrations and vertical distributions of autotrophic eukaryotes, *Synechococcus* spp. and *Prochlorococcus* spp. in the upper 200 m water column exhibited the same distribution in noon and evening samples, apart from a slight decrease in the concentration of *Prochlorococcus* spp. in the evening (Fig.3). It was clear that there was little variation in both picophytoplankton abundances and community structure. Such a steady state requires equilibrium between proliferation of picophytoplankton and grazing or prey production and predator ingestion.

To precisely evaluate the contribution of biomass to primary biomass among the three taxa the cell

diameter and biomass was estimated using a simplified empirical relationship between FALS and cell size (Blanchot et al., 2001). The cell diameter of picoeukaryotes calculated in this study was a little smaller than that of Blanchot et al. (2001), due to the warm pool and high-nutrient, low-chlorophyll conditions (HNLC) of the equatorial Pacific, where size was estimated to range from 1.93±0.02 μm to 2.07±0.09 μm (Blanchot et al., 2001). However, sizes from this study agreed with those of Worden (Worden et al., 2004), who reported that the picoeukaryote cell diameter was about 0.7–1.2 μm at a Pacific Ocean coastal site in the Southern California Bight. *Synechococcus* spp. cell diameter averaged 1.14 μm (in the range of 1.00–1.29 μm), which was slightly larger than found previously (Blanchot et al., 2001) for warm pool and HNLC waters. However, this result was similar to one recorded for the China Yellow Sea, where direct estimation of sizes of *Synechococcus* spp. isolates (about 1.2 μm in diameter) were made using scanning electron microscopy (Wang et al., 2006). *Prochlorococcus* spp. cell diameter has been reported to range between 0.6–0.8 μm in many reports (Chisholm et al., 1988; Blanchot et al., 2001). Results from this study (average 0.69 μm; in the range from 0.60–0.76 μm) were roughly of the same order as for previous studies. Overall, the results obtained for diameter estimation of picophytoplankton indicate that the simplified empirical relationship between FALS and cell size, described by Blanchot (Blanchot et al., 2001), was applicable for measurement of picophytoplankton diameters.

4.2 Flow cytometric total red fluorescence

Flow cytometric total red fluorescence has been shown to be reasonably correlated with measured bulk chlorophyll *a* in the field (Li et al., 1993; Blanchot et al., 2001). Therefore, it can be useful for estimating the relative contribution of different groups to total chlorophyll *a* standing stock.

Although the proportion of red fluorescence contributed by the different groups of picophytoplankton varied with depth, the overall total red fluorescence depth profiles matched well with depth profiles of Chl *a* concentrations. Chl *a* concentration was positively correlated with the total in vivo fluorescence (r=0.65 n=55; P<0.01) in this study, which was similar to that reported previously (Blanchot et al., 2001). The percentage contribution to total red fluorescence of each of the three groups changed with the presence of picoeukaryotes. In the upper 100 m layer, picoeukaryotes were a major contributor to total red fluorescence (79% in average), while at depths below 100 m, *Prochlorococcus* spp. and *Synechococcus* spp. were the major contributors to total red fluorescence. The contribution was 49% and 51%, respectively, for *Prochlorococcus* spp. and *Synechococcus* spp. populations.

4.3 The relation between FALS and red fluorescence per cell

FALS per cell was positively correlated with red fluorescence per cell in all three picophytoplankton groups. Among the three groups (Fig.5), the value of FALS per cell in *Prochlorococcus* spp. and *Synechococcus* spp. exhibited a highly positive relationship with red fluorescence per cell, while for picoeukaryotes it was slightly lower (Fig.5).

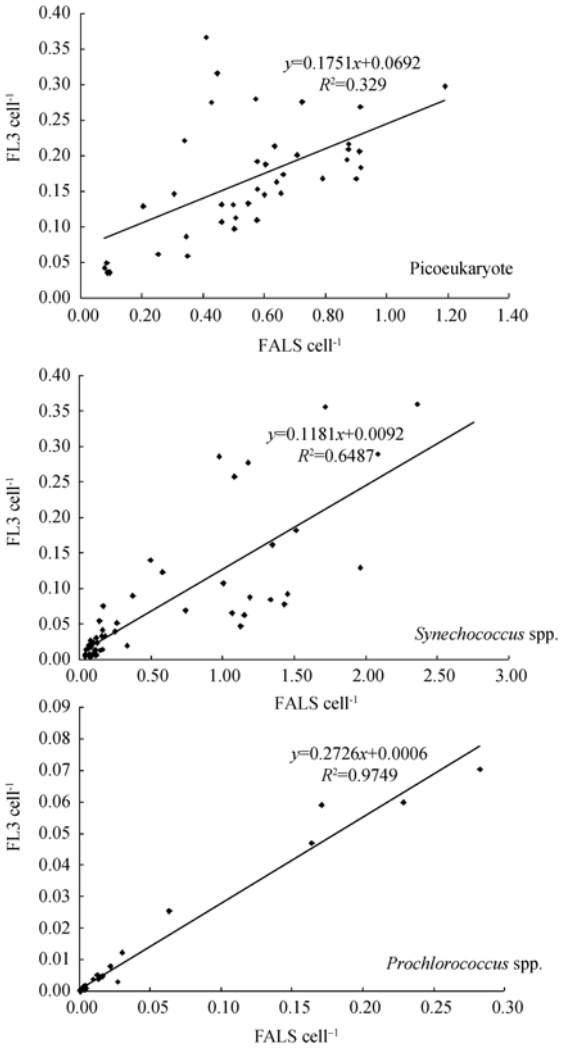

Fig.5 Relationship between mean cellular forward angle light scatter (FALS) and red fluorescence (FL3) per cell

Because the change of FALS with depth profile represented changes in cell size, the positive correlation between FALS per cell and fluorescence per cell indicated that, in addition to light adaptation to the availability or intensity of light (Olson et al., 1990; Rocap et al., 2003), there was also an increase in cell size with depth through which the picophytoplankton increased their chlorophyll *a* content. Goericke et al. (1998) proposed that the increase in chlorophyll *a* with depth could be almost entirely attributed to photoacclimation, since phytoplankton carbon did not vary with depth. DuRand et al. (2001) found that carbon per cell (based on FALS) does increase with depth, but that chlorophyll fluorescence per cell increases more. Our results indicate that the increase in cell size with depth was also an important reason for the increase in fluorescence in picophytoplankton with depth.

5 CONCLUSION

The Philippine Sea was a marginal sea in the Western Pacific, while the distribution of picophytoplankon in it had many characterizations, which were observed in typical Western Pacific. The cell size of *Prochlorococcus* spp. *Synechococcus* spp. and picoeukaryotes were 0.69 μm, 1.14 μm and 1.33 μm, respectively. The flow cytometric analysis of Chl *a* signature fluorescence shown that in the upper layer the Picoeukaryotes were major contributor of Chl *a* (up to 79%), while under the depth of 100 m, *Prochlorococcus* spp. and *Synechococcus* spp. became the major contributors. The increase of cell size with depth coincided with the increase of Chl *a* concentration in each cells.

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