

# Ecological studies on *Prochlorococcus* in China seas

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**Abstract** *Prochlorococcus*, a tiny oxygenic photosynthetic picoplankton with unique pigment composition, has been found to be ubiquitous and abundant in the world oceans, and has been recognized to be closely related to living resources and environmental issues. It has attracted the interest of marine biologists since its discovery, and field data on it over global oceans have accumulated rapidly in the past 10 years. In China, we have studied *Prochlorococcus* for 8 years, achieving a basic ecological understanding. The presence of *Prochlorococcus* in China seas, marginal seas of the west Pacific, was confirmed, and its distribution patterns were also brought to light. *Prochlorococcus* is very abundant in the South China Sea and the offshore regions of the East China Sea. It is seasonally present in the southeast part of the Yellow Sea and absent in the Bohai Sea. Temporal and spatial variations of the abundance of *Prochlorococcus* and their affecting factors, physiological and ecological characteristics of *Prochlorococcus* and their relationships to the other groups of picoplankton, and the importance of *Prochlorococcus* in total biomass and possible roles in living resources and environmental problems are discussed. In the future, isolation of different *Prochlorococcus* strains from the China seas and their physiological characteristics, genetic diversity, phylogenies and gene exploitation, etc. are important issues to be addressed.

**Keywords:** *Prochlorococcus*, divinyl-chlorophyll, picoplankton, ecology, China seas.

*Prochlorococcus* is the smallest known photosynthetic organism in the world. Besides its tiny cell size (0.6—0.7  $\mu\text{m}$ ), it has unique pigment composition<sup>[1-3]</sup> and challenges the previous knowledge about the phylogeny of algae pigment system and thus the classic algae taxonomy. It is the only wild species that has divinyl-chlorophyll as the major photosynthetic pigment. The absorption maximum of divinyl-chlorophyll shifts 8—10 nm to the red end of the spectrum compared with that of normal chlorophyll, which helps it to use dim light efficiently at the bottom of the euphotic zone. Furthermore, it possesses  $\alpha$ -carotene, a characteristic not assumed to belong to prokaryotes previously. Some strains of *Prochlorococcus* even have phycoerythrin<sup>[3]</sup>. This unusual pigment system makes *Prochlorococcus* different from the other two prochlorophytes, *Prochloron* and *Prochlorothrix*, which contain normal chlorophyll and  $\beta$ -carotene, but without phycoerythrin. On the other hand, molecular biological studies found that the *rbcL*

gene encoding the large subunit of Rubisco in some strains of *Prochlorococcus* resembles that of a proteobacteria more than that of most cyanobacteria<sup>[4]</sup>. Other studies showed that some strains of *Prochlorococcus* lack the gene encoding nitrate reductase and thus could not use { EMBED Equation.DSMT4 }-N<sup>[5]</sup>. These unique points are challenging in the phylogeny and systematics of *Prochlorococcus*.

*Prochlorococcus* is distributed ubiquitously from the surface to the bottom of the euphotic zone in the oceans between 40°N and 40°S. It numerically dominates the autotrophs in these regions (generally 10<sup>5</sup> cells/mL in oceanic waters) and is the most abundant photosynthetic autotroph in marine ecosystems. Moreover, it is an important contributor to the biomass and primary production. In the Sargasso Sea, 30% of chlorophyll and 25% of primary production were provided by *Prochlorococcus* annually<sup>[6]</sup>. In the north Pacific, *Prochlorococcus* contributes 35% of depth integrated carbon biomass for 200 m water column<sup>[7]</sup>. In the central equatorial Pacific, *Prochlorococcus* contributes 27%—41% of photosynthetic carbon<sup>[8]</sup>. In the Mediterranean Sea, 31% of bulk chlorophyll was contributed by divinyl-chlorophyll, the diagnostic pigment of *Prochlorococcus*<sup>[9]</sup>. Besides the very abundant distribution in the above regions, *Prochlorococcus* can actually reach 60°N in the subarctic sea<sup>[10]</sup>, and the marginal seas even bays such as the Mediterranean Sea, the Red Sea and shelf waters in the East China Sea<sup>[9, 11-13]</sup>. Moreover, as indicated by cell cycle analysis, growth rates of *Prochlorococcus* are very high in natural waters. Generally the field populations of *Prochlorococcus* divide one time a day (1 div/d)<sup>[14-17]</sup>. Some studies also revealed the ultradian growth of 1.4 div/d in the Arabian Sea<sup>[18]</sup>. The ubiquitous distribution, high abundance and biomass and high growth rate make *Prochlorococcus* a considerable contributor to the recycling of global carbon and other biogenic nutrients, and an important organism in marine living resources and global climate changes.

However, studies on *Prochlorococcus* are not evenly developed around the world, mainly restrained by equipment and facilities. Early studies were only done in a few famous laboratories, and mainly confined to the oceanic waters where water is clean and community structures are relatively simple. Some important issues, e.g. why *Prochlorococcus* disappears in most coast waters, remain unclear so far. A better understanding of the distribution patterns and their regulation factors, phylogeny and taxonomy, ecological importance of *Prochlorococcus* on the global scale requires more investigations and studies in marine waters around the world. With one of the world's largest continental shelf waters, the East China Sea, and one of the world's deepest marginal seas, the South China Sea, and covering tropical, subtropical and temperate climatic zones, the China seas are important marginal seas of

the Pacific, and ideal for marine scientific research. Ecological studies on *Prochlorococcus* in the China seas are not only necessary for the understanding of microbial community structures, the role of *Prochlorococcus* in living resources, biogenic elements cycling in the local area, but also helpful in providing data on forming a global frame of *Prochlorococcus* related ecological knowledge. We started to investigate *Prochlorococcus* in China seas from 1994, and made progress in ecological understandings about this organism as summarized below.

1 Presence of *Prochlorococcus* in the China seas

The earliest record of *Prochlorococcus* in the China seas can be traced back to 1984 when Takahashi and Hori<sup>[19]</sup> found, by the transmission electron microscopy, an organism sized 0.6—0.9 μm in diameter, with tightly appressed 5—7 layers of thylakoids along the cell membrane, numerically dominated in the algae community of the 70 m samples in the South China Sea. It was, as seen from the architecture, actually the typical structure of *Prochlorococcus* found and denominated by Chisholm et al. several years later<sup>[1, 2]</sup>. However, at that time it was not recognized as an ecologically important new species and was deemed to be *Synechococcus* type II after Johnson et al.<sup>[20]</sup>. It is a pity that this dominant species in the South China Sea was ignored for 10 years until Jiao's team initiated the studies of *Prochlorococcus* in the China seas. Presence of *Prochlorococcus* in the East China Sea was first recorded in 1994 by the detection of its diagnostic pigment, divinyl-chlorophyll by high performance liquid chromatography<sup>[21]</sup> (fig. 1), and then was further confirmed by flow cytometry in 1997<sup>[22]</sup> (fig. 2). Wide distribution in the South China Sea was later verified.

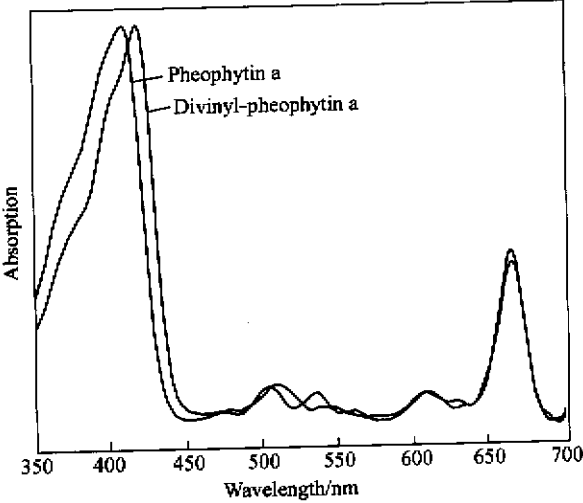


Fig. 1. The UV-VIS absorption spectra of divinyl-pheophytin a and pheophytin a, derivations of divinyl and normal chlorophyll a after acidification (Note the red-shift of divinyl-pheophytin a in the blue part).

2 Distribution of *Prochlorococcus* in the China seas

( i ) Distribution boundary. From 1994 to 2001,

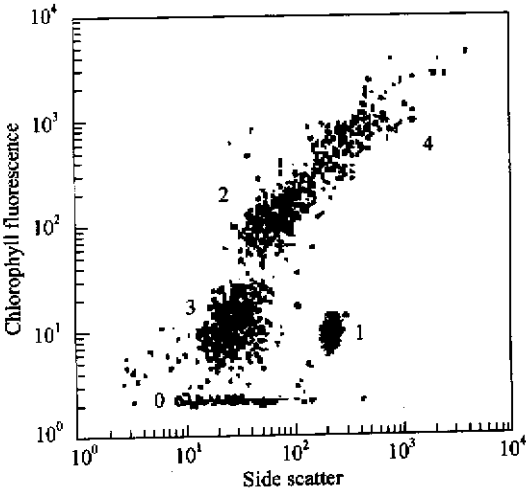


Fig. 2. Flow cytogram plots of marine picophytoplankton. 0, Noise; 1, beads; 2, *Synechococcus*; 3, *Prochlorococcus*; 4, picoeukaryotes.

investigations were carried out in the four major seas, the Bohai Sea, the Yellow Sea, the East China Sea and the South China Sea, and their adjacent coastal waters such as Jiaozhou Bay, Zhifu Bay and the Yangtze River estuary on more than 40 cruises including 9 major multi-disciplinary cruises to the Yellow Sea, the East and South China Seas. And 248 stations were investigated in these areas. Based on the data collected from the above cruises that covered most of the China seas, it is now clear that *Prochlorococcus* is present in almost the whole sea (except the very coasts) of the South China Sea, in most of the East China Sea, and in the southeast region of the Yellow Sea. There is no *Prochlorococcus* in the Bohai Sea. The north distribution boundary is the south edge of the Yellow Sea (fig. 3). The coast-ward distribution boundary in the East China Sea is around 50 m isobath in summer (fig. 3, dashed line a), but back to the Kuroshio Current and adjacent waters in winter (fig. 3, dashed line b).

( ii ) Abundance. Averaged depth integrated cell abundance of *Prochlorococcus* varied between  $10^4$ — $10^5$  cells/mL in the South China Sea, with the highest values occurred in the south part of the Luzon Strait between Taiwan and Philippines. Abundance of *Prochlorococcus* changed significantly in different regions of the East China Sea with a general pattern of increasing in offshore directions. The highest was recorded in the south part of the Kuroshio Current with an averaged depth (150 m) integrated cell abundance of  $9.8 \times 10^4$  cells/mL (fig. 3).

(iii) Vertical distribution. In the shelf waters of the East China Sea, vertical distribution of *Prochlorococcus* could reach 50—100 m depth, whereas in the Kuroshio Current and most of the South China Sea, *Prochlorococcus* was actually distributed down to 150 m. Depth profiles of *Prochlorococcus* cell abundance in the China Seas

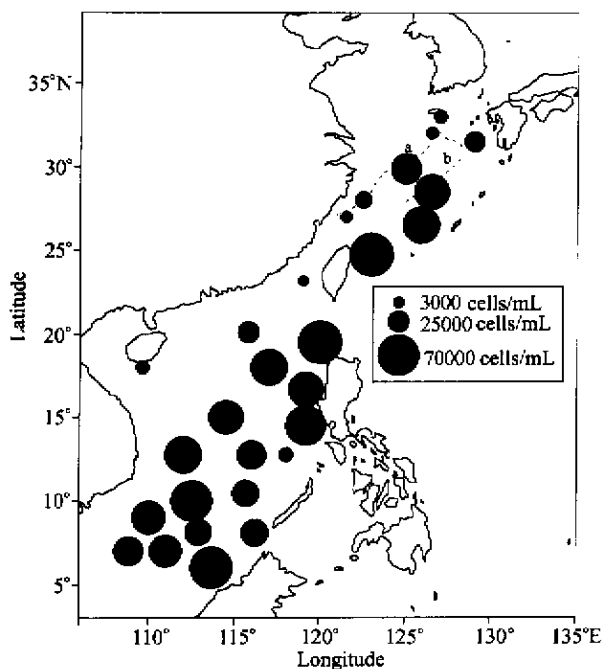


Fig. 3. Distribution of *Prochlorococcus* in the China seas (averaged depth integrated cell abundance). Dashed line a, Summer distribution boundary; dashed line b, winter distribution boundary.

can be generally summarized into three types (fig. 4): One is characterized by surface maximum and gradual decrease with depth, such as the case at station 905 (122° E, 26.5° N) in the East China Sea (fig. 4(a)). This type was often found in *Prochlorococcus* distribution boundary areas and/or where different water masses or currents mix and interact, as well as upwelling areas. Another type is characterized by the subsurface maximum (usually around 50 m), such as station 211 (120° E, 17.5° N) in the South China Sea (fig. 4(b)). This is a typical pattern of oligotrophic oceans, often presenting in the tropical and subtropical oceanic regions where thermocline usually well developed. The third type is as demonstrated by that at station

206 in the East China Sea (128.5° E, 29° N, fig. 4(c)) with no typical abundance maximum layer or maximum layer spreading over a large depth range. This pattern was often seen in the Kuroshio Current, the Taiwan Warm Current areas, and other well mixed waters.

(iv) Seasonal variation. Little seasonal difference in the distribution of *Prochlorococcus* was observed in the South China Sea which is located in the subtropical and tropical zones, while in the East China Sea located in temperate zone, seasonal variations were significant (fig. 3). Activities of the major currents (Kuroshio Current and Taiwan Warm Currents), as well as variations in water temperature are thought to be mainly responsible for the seasonal differences. In winter, distribution of *Prochlorococcus* in the the East China Sea was basically confined to the Kuroshio Current and its adjacent area. While in summer, it expanded even to the Yangtze River estuary, covering most area of the sea. On the other hand, nutrients, trace metals and biological interaction are all the possible factors affecting or regulating the spatial and temporal distribution of *Prochlorococcus* in the China seas (Jiao et al., unpublished data).

### 3 Relationship between *Prochlorococcus* and other groups of picoplankton in the China seas

Besides *Prochlorococcus*, there are other groups of marine picoplankton, such as *Synechococcus*, picoeukaryotes and heterotrophic bacteria. Using flow cytometry, we carried out the simultaneous measurements on the above four groups<sup>[23]</sup> and found complicated and interesting relationships among them.

When seen on a large scale, both spatially and temporally, the relationship between phytoplankton and heterotrophic bacteria is usually positive, because the former provides organic carbon to the latter and the latter provides inorganic nutrients to the former, i.e. they are

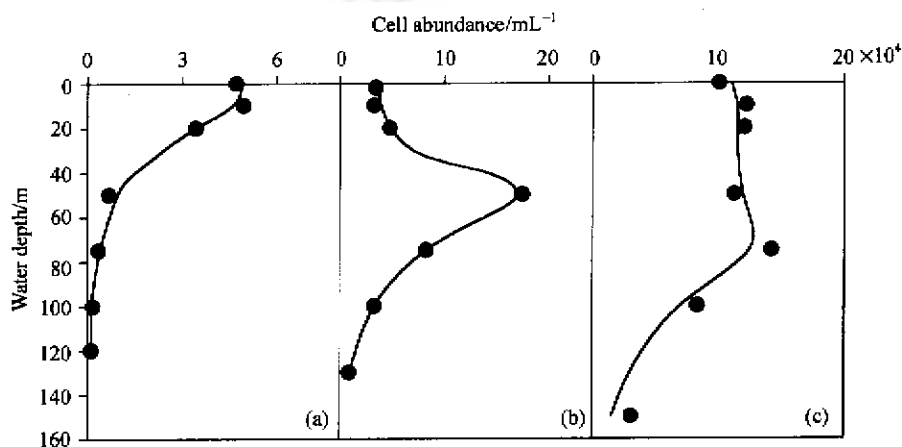


Fig. 4. Three typical vertical distribution patterns of *Prochlorococcus* in the China seas. (a) Station 905, the East China Sea (122° E, 26.5° N); (b) station 211, the South China Sea (120° E, 17.5° N); (c) station 206, the East China Sea (128.5° E, 29° N).

5° N); (b) station 211, the South China Sea (120° E, 17.5° N); (c) station 206, the East China Sea (128.5° E, 29° N). recip rocal to each other. However, the relationship between *Prochlorococcus* and heterotrophic bacteria is more complicated. Negative correlations could often be observed between the two along trophic gradients, e.g. along the Kuroshio Current from south to north, as the current became weaker, nutrient availability became higher, biomass of heterotrophic bacteria increased with decreasing biomass of *Prochlorococcus* (fig. 5). It appears that heterotrophic bacteria partially took over the role of *Prochlorococcus*, suggesting some kind of niche overlap between the two. This seems unusual, but actually, physiological studies also revealed that some strains of *Prochlorococcus* could use organic P directly as their main P source<sup>1)</sup>. Along with these field observations, there is some molecular evidence for this point as well, e.g. sequence of *rbcL* gene in some strains of *Prochlorococcus* is of high similarity to a purple bacterium<sup>[4]</sup>.

Although *Prochlorococcus* is taxonomically classified as Prochlorophyta, it is closer to *Synechococcus* than to the other two prochlorophytes, *Prochloron* (symbiont, marine environments) and *Prochlorothrix* (free living, fresh water) in terms of niche, morphology and physiological ecology. *Prochlorococcus* is closely related to *Synechococcus* in phylogeny. And they are much similar both in physiological ecological character and in geographical distribution patterns. For example, they are both oxygenic photosynthetic prokaryotes, both the major members of pico-autotrophs in the oceanic waters, and some strains of *Prochlorococcus* also have phycobilin<sup>[3]</sup>. Nevertheless, distinct differences in physical and ecological features of the two were also observed. There is obvious niche differentiation between them. *Synechococcus* usually presents in the upper euphotic zone, whereas *Prochlorococcus* could be distributed abundantly in the lower and the bottom of the euphotic zone. In the South China Sea and the Kuroshio Current region in the East China Sea, vertical distribution of *Prochlorococcus* could actually reach 150 m, a depth *Synechococcus* never reached (fig. 6). On the other hand, they have quite different nutrient requirements. Generally speaking, *Synechococcus* usually requires higher level of nutrient, and responds more quickly and significantly to the changes of nitrogenous nutrients whereas *Prochlorococcus* does not and some strains even could not assimilate  $\text{NO}_3^- \text{-N}$ <sup>[5]</sup>. A field case in the South China Sea illustrated such physiological and ecological differences clearly (fig. 6). The environmental conditions at station 32 (112.9° E, 8.1° N) and station 36 (111.7° E, 6.7° N) were similar, except that { EMBED Equation.DSMT4 }-N in the upper 50 m at station 32 was bellow detection limits whereas that at station 36 was about 0.5  $\mu\text{mol/L}$ . Consequently, significant difference in response of *Prochlorococcus* and *Synechococcus* was recorded. *Synechococcus* flourished at station 36, reaching a maxi-

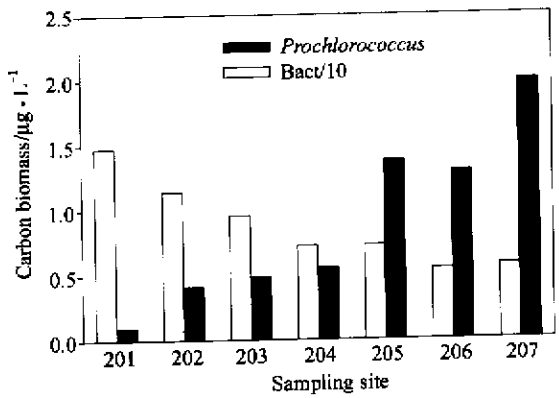


Fig. 5. Relationship between carbon biomass of *Prochlorococcus* and heterotrophic bacteria in the Kuroshio Current in the East China Sea. Stations 207—201 were distributed along the Kuroshio Current from south to north between 128° E, 28.5° N to 129° E, 31.5° N with roughly equal intervals.

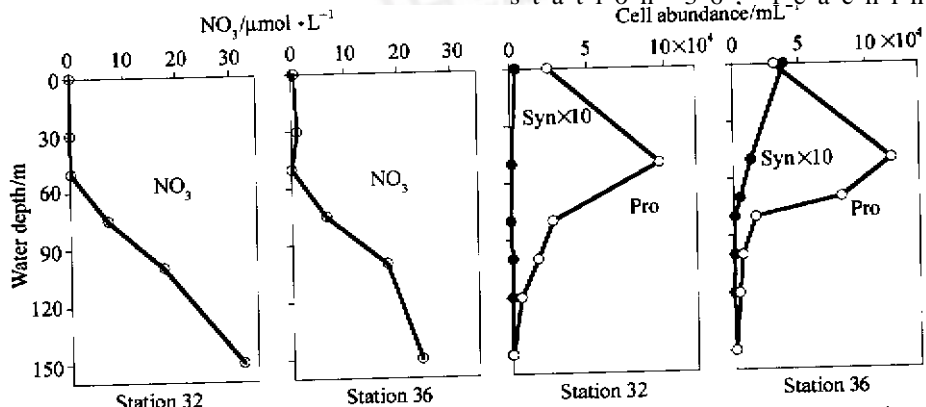


Fig. 6. Nutrient status and distribution of *Synechococcus* and *Prochlorococcus* at station 32 (112.9° E, 8.1° N) and station 36 (111.7° E, 6.7° N) in the South China Sea, indicating responses of *Synechococcus* and *Prochlorococcus* to different nitrate concentrations and the



differentiation of spaces occupied mainly by *Synechococcus* and *Prochlorococcus* respectively in the water column. mum of  $3.9 \times 10^3$  cells/mL in abundance, while the maximum abundance at station 32 was only  $4.2 \times 10^2$  cells/mL, about one tenth of that at station 36. *Prochlorococcus*, however, showed little difference between the two stations both in abundance and in vertical distribution.

Picoeukaryotes is a general designation about the pico-sized eukaryotic phytoplankton, which covers many different species. The relationship between picoeukaryotes and *Prochlorococcus* should not be simply discussed. Because species composition and the ecological character of the dominant species vary due to species succession during temporal and spatial changes, consequently their relationships with *Prochlorococcus* are not always consistent. However, there are some general recognitions, e.g. picoeukaryotes like higher nutrients can bear lower ambient temperature than *Prochlorococcus* does, and can distributed to similar depths as *Prochlorococcus* reaches. The total picoeukaryote abundance is usually 2 orders of magnitude lower than that of *Prochlorococcus*.

Experimental studies showed that there are differences in response to nutrient perturbation and adaptability to environmental changes between *Prochlorococcus* and other groups of picoplankton. Results from the experiments performed in the winter of 1997 and in the summer of 1998 in the South China Sea suggested that *Synechococcus* and picoeukaryotes had higher requirement for N and Fe, and *Prochlorococcus* was markedly stimulated by the enrichments of Co and P. Moreover, *Prochlorococcus* showed some similar responses to nutrient enrichment to that of heterotrophic bacteria. Despite its remarkable adaptability to extreme oligotrophic conditions, *Prochlorococcus* is less flexible and less tenacious to environmental changes or external perturbations than the other groups. *Prochlorococcus* is usually the most affected among picoplankton by the “bottle effects” during incubation. In field distribution, temperature is a major factor that affects the abundance and distribution of *Prochlorococcus*<sup>[24]</sup>. Although high abundance of *Prochlorococcus* is usually associated with high water temperature, when water temperature in the incubation bottle was increased from 21°C, the temperature at the bottom of the euphotic zone to 29°C of the surface water temperature (experiment at station 43 in the South China Sea, 110° E, 6° N, Nov., 1997), heterotrophic bacteria benefited most. *Prochlorococcus*, on the contrary, was inhibited<sup>1)</sup>.

4 Ecological importance of *Prochlorococcus* in the China seas

Wherever it presents, *Prochlorococcus* is the numerically dominant species among the photosynthetic populations in the China seas. In the open oceanic area, abundance of *Prochlorococcus* is generally one order of magnitude higher than that of *Synechococcus*, and 2 orders higher than that of picoeukaryotes. Moreover, it is also the major contributor to pico-autotrophic carbon biomass. By applying cellular carbon conversion factors widely used in the literature<sup>[10, 25]</sup>, the carbon biomass (µg/L) and the relative contribution of the three groups of pico-autotroph to the total in the East and South China seas were roughly evaluated (table 1). These calculations are for reference because such conversion of cell numbers to carbon biomass may introduce significant errors especially for coastal waters where picoeukaryotic species compositions are complex and diverse. However, these first hand calculations still show us, for the first time, valuable information that *Prochlorococcus* is a major contributor to the picophytoplankton biomass in most areas in the East and South China Seas. Given the importance of total pico-autotrophs in the China seas——picophyto- plankton is responsible for 23% —30% of the bulk chlorophyll a in the coastal areas and 40% —50% in the offshore regions in the East China Sea, 46% —89% in the oceanic waters in the South China Sea, the contribution of *Prochlorococcus* to the total autotrophic biomass in the East and South China Seas is obviously considerable.

In addition, the important ecological role of *Prochlorococcus* in the China seas was also demonstrated by its high growth rate. Cell cycle of field *Prochlorococcus* population is highly synchronized and coupling well with the daily light cycle, thus *in situ* growth rate of field *Prochlorococcus* population could be evaluated directly by the cell cycle approach. The advantages of this direct approach are: i) No culture procedures were involved, thus the consequent artificial bias could be avoided<sup>[26—29]</sup>; ii) the effects of grazers are avoided; iii) it provides the estimation at single specific species level, while the

Table 1 Carbon biomass and relative importance of pico-phytoplankton in the China seas

		Syn		Pro		Euk	
		C/µg · L <sup>-1</sup>	(%)	C/µg · L <sup>-1</sup>	(%)	C/µg · L <sup>-1</sup>	(%)
ECS	Coastal	0.4—4.0	3.4—31.2	0.0—1.0	0.0—8.0	0.9—12.3	7.5—96.6
	Kuroshio Current	0.5—2.1	16.5—64.7	1.2—2.0	35.8—61.9	0.6—0.7	16.7—21.1
SCS	Oceanic	0.3—0.4	8.4—11.1	1.6—2.8	44.3—79.8	0.5—1.5	13.5—43.1

1) Moore, L., personal communication.

a) ESC, the East China Sea; SCS, the South China Sea; Syn, *Synechococcus*; Pro, *Prochlorococcus*; Euk, picoeukaryotes.

results from isotope trace techniques are only at assemblage level. Previous studies showed that, the cell cycle of *Prochlorococcus* is 24 h, with the starting time of DNA synthesis different at different depth<sup>[14]</sup>, and natural growth rates of *Prochlorococcus* are around 0.7—1 div/d<sup>[14—17]</sup>. Similar estimations on the growth rate of *Prochlorococcus* were also achieved by the method for inhibiting prokaryotic cell division<sup>[30]</sup>. Through cell cycling approach, we estimated the growth rate of *Prochlorococcus* at the maximum chlorophyll layer in the South China Sea (station 43, 110E, 6N) to be 0.72 div/d<sup>1)</sup> (fig. 7), which was comparable to the result reported from other oceanic sites. Such a high growth rate and the relatively constant cell abundance suggest that growth of *Prochlorococcus* is balanced by grazer (mainly tiny protozoan such as flagellate and ciliates), viral lysis, etc. *Prochlorococcus*, thus, might play an important role in the nutrient cycling in the marine euphotic zone, and consequently, living resources and environment problems.

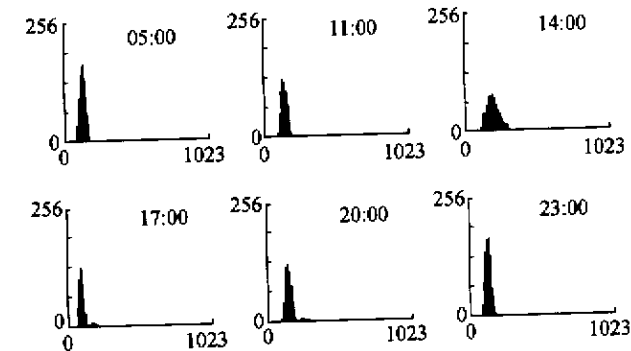


Fig. 7. Diel DNA distribution of *Prochlorococcus* at the maximum chlorophyll layer at station 43 in the South China Sea. The transverse and vertical axes are relative DNA fluorescence and cell number, respectively

5 Prospects

( i ) Natural ecology of *Prochlorococcus*. Given the importance of *Prochlorococcus* in the China seas, further field studies with application of new techniques and methods to the fundamental issues such as biomass and productivity are necessary for a better understanding of the structures and functions of the microbial food loop in the seas. Once intensive microbial data obtained, together with data from extensive field oceanographic investigations, the roles of *Prochlorococcus* in the cycling of biogenic materials, energy flow paths, marine productivity and living resources as well as environmental problems in different ecosystems in the China seas would be more accurately evaluated. And more reliable parameters would be available for the modeling at ecosystem level.

( ii ) Isolation and culture of *Prochlorococcus* strains

from the China seas. Because of the tiny cell and unique physiological nature, traditional isolation techniques seem inconvenient and ineffective for *Prochlorococcus*. Fortunately, the sorting function of flow cytometry provides an effectual technique. To isolate species/strains by this approach, the large sorting system has to be carried on board an investigation vessel for *in situ* operation. That is a difficult work, and only some overseas laboratories have succeeded in isolating and culturing *Prochlorococcus* so far. Nonetheless, we must undergo this step. Only if we succeeded in isolating and culturing *Prochlorococcus* strains of the China seas, can the following physiological ecology studies, molecular biological studies, taxonomic studies, phylogenic studies and the comparative studies of different strains be possible.

(iii) Physiological ecology. *Prochlorococcus* has many special physiological features. The structure of its novel phycoerythrin is different from the structures of all the known cyanobacterial phycoerythrins<sup>[31, 32]</sup>. The ratios of chlorophyll b to chlorophyll a differ in *Prochlorococcus* populations from different sites or water depths. It cannot use { EMBED Equation.DSMT4 }-N<sup>[5]</sup>, and so on. *In situ* experiments and field investigations also found that corresponding to the sudden changes in nutrient status, such as nutrient pulse, cellular pigment content and light scatter signals changed significantly<sup>[8,33]</sup>. The physical and chemical structures of the water columns and community structures of the shelf water, offshore water and oceanic water in the East and South China Seas are significantly different from each other. From the temperate to the subtropical and tropical; from the shelf waters, Kuroshio current in the East China Sea to the basin, the coral reef areas in the South China Sea, distribution of *Prochlorococcus* covers different climate zones and geological environments. Even at a single site, nutrients, temperature and salinity vary considerably from the surface to the bottom of the euphotic zone. In such a variety of environments, are there any adaptations in *Prochlorococcus* cell ultrastructure? And what are the responses of its ecological and physiological strategy? Answers to these questions would help illuminate the roles and mechanisms of *Prochlorococcus* in the cycling of biogenic materials and environmental problems.

(iv) Genetic diversity. Although great attention has been paid to this area already, many questions remain unanswered and puzzling so far. For example, it was found that genetic similarity among the surface *Prochlorococcus* populations from different sites is higher than that between populations isolated from the surface

1) Jiao, N. Z., Yang, Y. H., Harada, S. et al., Responses of picoplankton to nutrient perturbation in the South China Sea, with special reference to the coast-ward distribution of *Prochlorococcus*, Acta Botanica Sinica, 2002, in press.

and deep waters at the same site<sup>[34]</sup>. The ratio of divinyl-chlorophyll b/divinyl-chlorophyll a of the Sargasso Sea

strain is higher than that of the Mediterranean Sea strain, and the phycobilin gene was only found in the former but

1) Yang, Y. H., Jiao, N. Z., *In situ* growth rate of *Prochlorococcus* at the chlorophyll maximum layer in the southern South China Sea: An estimation from cell cycle analysis, Chin. J. Oceanogr. Limnol., 2002, Special Issue, in press.

are also different<sup>[33, 35]</sup>. On the other hand, some of the physiological ecological characteristics mentioned above might be just physiological acclimation to particular environments, while others might reflect intrinsic differences at strain or subspecies level. Distribution of *Prochlorococcus* in the China seas covers coastal to oceanic waters of different ecological types from 6°N to 32°N, and the highly diversified environments may gestate diverse *Prochlorococcus* strains. By examining the flow cytograms of samples from the East and South China Seas, at least two populations with different pigment content and cell size could be distinguished. Systematic diversity studies on the *Prochlorococcus* in the China seas could add valuable data on the global *Prochlorococcus* gene bank.

(V) Phylogeny and gene resources. Up to now, the position of *Prochlorococcus* on the phylogenetic tree is uncertain. Although it was classified to Prochlorophyta, the ultrastructure and pigment composition are significantly different from the other two prochlorophyte. On the other hand, it does not descend from the same ancestor as that of the most closely related *Synechococcus*. Partial sequence of ribulose-1,5-bisphosphate carboxylase/oxygenase indicates that a common ancestor of *Prochlorococcus* and  $\gamma$  purple bacteria branched off from the land plant lineage earlier than *Prochloron*, *Prochlorothrix* and cyanobacteria<sup>[4]</sup>. The capability of using organic P (Moore L., personal communication), lack of nitrate reductase encoding gene<sup>[5]</sup>, and the possible niche overlap with heterotrophic bacteria make the taxonomy study of *Prochlorococcus* more challenging. Yet the diagnostic pigment of *Prochlorococcus*—divinyl chlorophyll has provided a new idea for exploring the origin of chloroplast<sup>[32, 36]</sup>. Further studies could reveal mechanisms of biosynthesis of chlorophyll, photosynthetic physiology and molecular evolution. Besides, the strengths of *Prochlorococcus* such as the adaptability to very weak light, and the ability to use nutrients of very low concentration might correspond to some valuable genes. Transformation and expression of these genes on other economic plants could produce valuable benefits and has good potential prospects.

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## Subcellular localization and functional analyses of structural domains of COP1 in transgenic tobacco

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**Abstract** Plants have evolved an extremely exquisite light signal regulatory network to adapt to the changing ambient light conditions, in which COP1 plays a critical role of the light signal transduction. Based on the cloned pea COP1 cDNA sequence and its protein structure, four individual gene fragments encoding different structural domains of the COP1 were designed to fuse to the *GFP* gene. The plant expression vectors containing these fusion genes as well as the *COP1GFP* fusion gene were constructed and used to transform tobacco by *Agrobacterium* as confirmed by Southern analyses. Antibodies were raised against the recombinant GFP-COP1 overproduced in *Escherichia coli*. Immunoblotting results demonstrated that all of the fusion genes were constitutively expressed in transgenic tobacco plants. We systematically investigated the different subcellular localization of these fusion proteins and the resulting phenotypic characteristics of these transgenic plants under light and dark conditions. Our data show that (1) the molecular mass of the tobacco endogenous COP1 protein is 76 kD. It is constitutively expressed in all of the tested tissues and the total cellular content of COP1 protein is not noticeably affected by light conditions. (2) The nuclear localization signal of COP1 plays a critical role in regulation of its nuclear-cytoplasmic partitioning. The subcellular localization of the COP1 protein containing nuclear localization signal is regulated by light in the epidermal cells of leaves, but, it is located in nucleus constitutively in root cells. (3) The coiled-coil domain is very critical to the function of COP1 protein, while the zinc binding RING finger domain only plays a supportive role. (4) The WD-40 repeats domain is essential to the COP1 function, but this domain alone does not affect photomorphogenesis. (5) Overexpression of COP1 protein not only inhibits the photomorphogenesis of the stems and leaves of the transgenic tobacco, but also results in the generation of short and clustered roots. In contrast, overexpression of COP1 protein without WD-40 repeats domain promotes the photomorphogenesis process in the stems and leaves and lead to root elongation and lack of lateral roots. The COP1-COP1 interaction happens not only in the nucleus, but also in cytoplasm.

**Keywords:** photomorphogenesis, COP1, tobacco, GFP, subcellular localization.

Light is one of the most important environmental