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Responses of Picoplankton to Nutrient Perturbation in the South China Sea, with Special Reference to the Coast-wards Distribution of *Prochlorococcus*

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Abstract: Responses of *Prochlorococcus* (Pro), *Synechococcus* (Syn), pico-eukaryotes (Euk) and heterotrophic bacteria (Bact) in pelagic marine ecosystems to external nutrient perturbations were examined using nitrogen- (N), phosphorus- (P), iron- (Fe), and cobalt- (Co) enriched incubations in the South China Sea in November 1997. Variations in abundance of the 4 groups of microorganism and cellular pigment content of the autotrophs during incubation were followed by flow-cytometric measurements for seven days. During the incubation, Syn and Euk showed a relatively higher demand on Fe and N, while Pro required higher levels of Co and P. The Fe was inadequate for all the organisms in the deep euphotic zone (75 m) of the study area. The experimental results also implied that biological interaction among the organisms played a role in the community structure shift during the incubation. It seemed that besides the effects of temperature, there are some other physical and chemical limitations as well as impacts from biological interactions on Pro distribution in coast waters.

Key words: *Prochlorococcus*; picoplankton; nutrients; iron; cobalt; South China Sea

Since the discovery of *Prochlorococcus* (Pro), an extremely small (mean cell size 0.6 μm), divinyl chlorophyll containing prokaryotic oxy-phototrophic autotroph[1], new perspectives are required to understand the structure of micro-communities and the relevant ecological processes in the marine ecosystems. Over the past decade, many field ecological investigations of Pro have been conducted[2-7]. Distribution of Pro has been extended from tropical and subtropical to near sub-arctic areas[8] and from oceanic waters to certain coastal waters[7,9,10]. The significance of Pro in total phytoplankton biomass, production and energy-flow pathways, especially its distribution and relationships with other picoplankton such as *Synechococcus* (Syn), pico-eukaryotes (Euk) and heterotrophic bacteria (Bact), has become a major concern of biological oceanographers. Temperature is the crucial environmental factor to the distribution of Pro[5,6], which was also verified by laboratory experiments[11]. Physical conditions such as mixing and stratification have also been proven to be important in describing depth profiles and seasonal fluctuations of Pro populations[5,12]. Chemical conditions such as nutrients are also considered to be affecting factors in the distribution of Pro[13,14], yet nutrient impacts are not as clear as the other factors. Major nutrients such as nitrogen and phosphorus have been the basic concerns in traditional concepts of limiting factors for phytoplankton in the marine environment. Recent studies have revealed that micronutrients, such as iron[15,16] and Cobalt[17,18], are crucial to phytoplankton in vast areas of the world oceans. Furthermore, in our previous studies on the field ecology of picoplankton in the East China Sea, we observed significant correlations between Pro and other picoplankton organisms such as Syn, Euk and Bact[19]. Similar relationships were also found across the Gulf Stream in the North Atlantic in summer 1996 (Jiao, unpublished data). These evidence suggested that biological interaction as a possible factor influencing the distribution of Pro cannot be ignored.

Based on the above considerations, especially the difference in nutrient composition and concentration between oceanic waters and coastal waters, this study was to explore the response of Pro as well as the other co-existing picoplankton components Syn, Euk and Bact, to enhance nutrient concentrations from oligotrophic oceanic levels to eutrophic coastal levels, and the biological interactions among these organisms during the nutrient condition shifts. We hoped to obtain some information for interpreting the distribution of Pro in the transition areas of marginal seas. We chose a typical marginal sea in the western Pacific, the South China Sea, as the *in situ* experimental field, and employed long-time (seven days) nutrient enriched incubation as the experimental strategy.

1 Materials and Methods

1.1 Experimental site

The South China Sea, 5°- 20°N and 109°- 120°E, is a marginal sea in the western Pacific covering tropical and subtropical regions. It is characterized by high temperature (surface water temperature was around 29 ℃ during the study period, November 1997) and meso-oligotrophic conditions. The nutrient enrichment experimental

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site was chosen from 32 field investigation stations, the station No. 43, located at latitude 6°N, longitude 110°E, with water depth of 1 100 m and thermocline and nitracline of 60 - 70 m. Water samples were taken for incubation from the surface layer (0 - 5 m) and 75 m, the maximum chlorophyll layer around the nitracline. Water temperatures were 28.62 ℃ and 20.85 ℃ respectively. Nutrient concentrations (μmol/L) at 5 m depth were 0.0, 0.017, 3.558 and 0.218 for NO_3-N, NO_2-N, NH_4-N and PO_4-P, respectively; those at 75 m were 14.93, 0.017, 2.738 and 0.495.

1.2 Experimental design

In order to see the influence of coastal water on Pro, all experiments were designed with respect to shifting the ecological conditions in the incubation bottles from oceanic to typical coastal status. Four elements, nitrogen (N), Phosphorus (P), iron (Fe) and cobalt (Co), were chosen for the nutrient enrichment experiments based on the following consideration. N and P are naturally more abundant in coastal than in oceanic waters and are frequently reported as limiting factors for phytoplankton; Fe is an element that phytoplankton require for synthesis of chlorophyll and nitrate reductase, and has been proven to be deficient in most oceanic waters[15,16,20], although it is much more abundant in coastal areas. Co has been reported to be important to phytoplankton metabolism[17,18]. All the nutrient enrichment levels were designed according to high concentration levels recorded in the natural coastal areas of the China Seas[18,21,22]. The concentrations added to the incubation bottles were: 50 μmol/L N (NH_4Cl), 10 μmol/L P (NaH_2PO_4), 0.117 μmol/L Fe ($FeCl_3$), and 0.005 μmol/L Co ($CoCl_2$), respectviely.

One litre glass bottles were used as incubators. The bottles were cleaned according to the procedures described by Fitzwater *et al*[23]. Water samples were taken from the Go-flo bottles with almost no exposure to air. The incubation bottles were treated individually in a small area isolated by clean plastic sheets[23]. The time lags of incubation after sampling was minimized to the best attempts.

Incubations were conducted in flowing surface water-cooled water baths on the deck of the research vessel. Blue plastic sheets were utilized as covers to provide light at ambient levels of the sampling depths. However, we could not keep the temperature of incubation bottles of the 75 m water the same as that at the sampling depth. Duplicate incubations were conducted for each treatment.

In order to observe the interactions of picoplankton under conditions shifting away from their origin, incubations were lengthened up to seven days. Bottles were shaken several times a day. 1 mL subsamples were taken from the incubation bottles every day during incubation for cell abundance and cellular pigment content examinations. Samples were placed in 1.2 mL cryogenic vials (Nalgene) and were fixed with glutaraldehyde (final concentration, 1%) in dark for 10 min, then stored in deep freezers or liquid nitrogen until analysis.

1.3 Flow-cytometric (FCM) analysis

Samples were run on an FACSCalibur flow cytometer (Becton-Dickinson) equipped with an external quantitative sample injector (Harvard Apparatus PHD 2000); the injection flow rate was set at 10 - 20 μL/min for normal enumeration. FCM data were acquired and analyzed by CellQuest 2.2 (Becton Dickinson). We used 0.474 μm fluorescent beads (Fluorescence Scientific) as the internal reference. The three autotrophs were distinguished according to their positions in plots of chlorophyll (FL3) vs. 90°-angle light scatter (SSC), and phycoerythrin (FL2) vs. SSC[5]. Cellular pigments were normalized to bead units. SYBR Green-I (Molecular Probes) was applied as the DNA stain for heterotrophic bacteria enumeration[24]. Samples for FCM enumeration of autotrophs were run separately from those for heterotrophs.

2 Results

2.1 Variations in cell abundance of the picoplankters and in cellular pigment content of the autotrophs in the surface water incubations

During the seven days incubation of control bottles, the Pro population continued to decline. Bact population was relatively stable in the first three days, and then kept increasing to the end. There was a significant inverse correlation between the two ($P < 0.01$). The Syn population followed a trend similar to that of Bact except for a significant drop on the second day. The population of Euk decreased at first and then increased to a relatively stable level at about 1/3 of the initial population (Fig. 1a). The cellular pigment content of Syn in the first half of the incubation was about twice as the initial level, then decreased from the fifth day to a level similar to the initial (Fig. 2a). Pigment levels of Pro increased a little in the first two days and then dropped down to levels similar to or lower than the initial level. Euk's cellular pigment contents dropped abruptly from the beginning of the incubation and kept a low level till the end (Fig. 2, b, c).

In the bottles supplemented with Co (Fig. 1b), Pro responded rapidly in the first 24 h and a considerable increase in cell abundance was observed. The cell abundance then decreased to the initial level where it remained for the next three days. On the fifth day another smaller drop occurred and then it maintained a level near 3×10^4 cells/mL until the end of the experiment. In contrast, the Syn population decreased in the first two days, increased rapidly after the Pro population declined, and then held a concentration of 8.6×10^3 cells/mL until the end of the experiment. The Euk population, although fluctuating, finally reached a level almost twice its initial value. Bact showed a pattern similar to that in the control bottles. In this treatment cellular pigment content of autotrophs reached high levels early or late days of the incubation. That of Pro in particular was markedly different from that in the controls (Fig. 2). This was one of the two most favorite conditions for Pro (the other being P, below) in terms of both cell abundance and cellular pigment content.

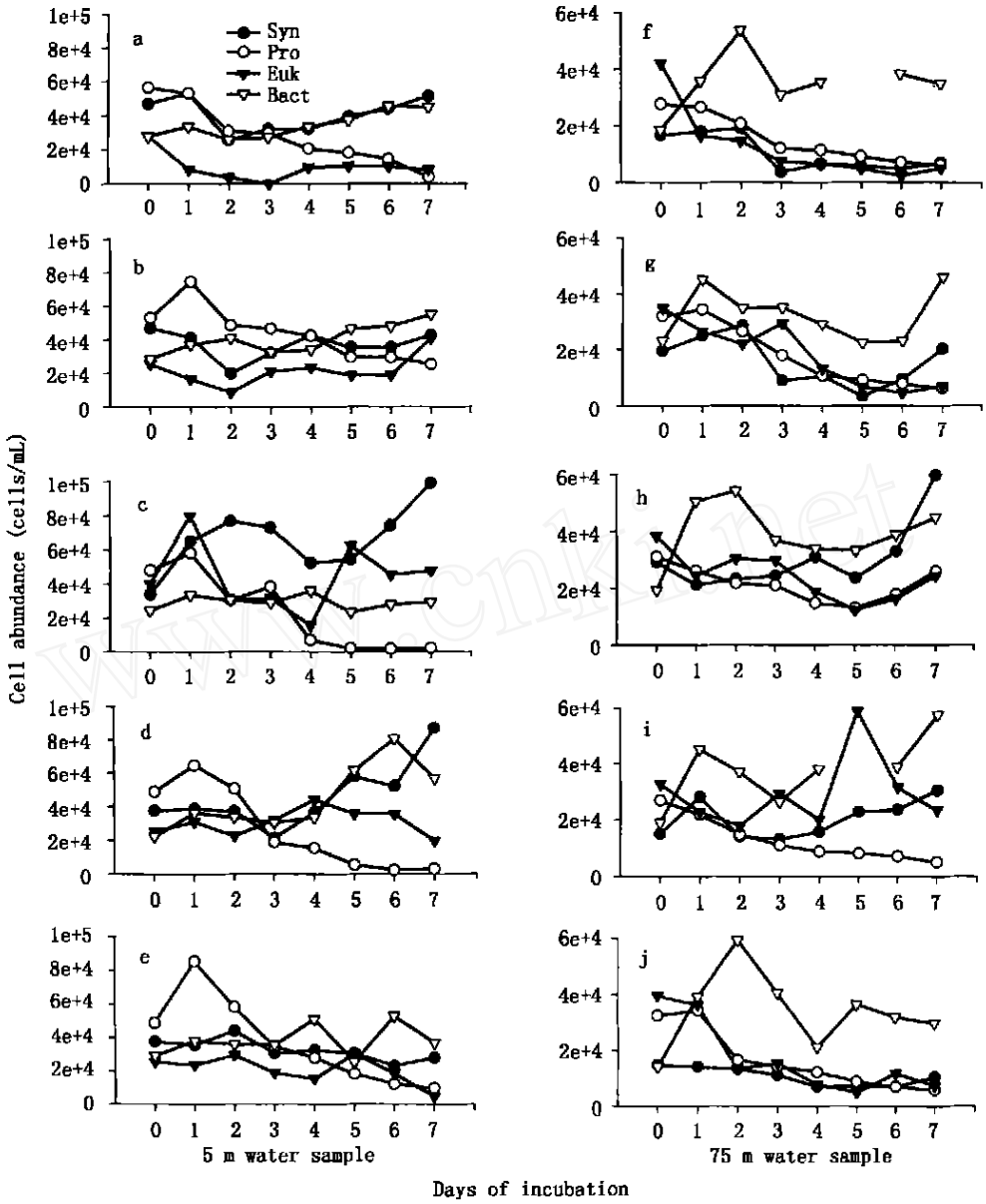

Fig. 1. Time course of cell abundance of picoautotrophs and heterotrophic bacteria in the nutrient enrichment experiments. 5 m (surface) water incubations: a. Control. b, c, d and e. Enriched with cobalt, iron, ammonia and phosphate respectively. Y axis units: Syn ×5, Pro ×1, Euk ×100, Bact/10 cells/mL. 75 m water incubations: f. Control. g, h, i and j. Enriched with cobalt, iron ammonia and phosphate respectively. Y axis units: Syn ×15, Pro ×1, Euk ×50, Bact/10 cells/mL.

For the Fe-enriched bottles (Fig. 1c), Syn had a significant response in the first three days and the last two days, reaching the highest abundance of 2×10^4 cells/mL at the end of the incubation. Euk responded rapidly to Fe addition in the first 24 h and then decreased. Then on the fifth day, there was another increase in Euk, to a concentration higher than the initial value, and which remained to the end of the incubation. The Pro population remained high for the first three days, but almost disappeared from the community on the fifth day. Unlike in the other treatments, the Bact population did not grow much, maintaining relatively stable throughout the incubation. Interestingly, the fast reproduction of Syn and Euk resulted in relatively lower cellular pigment contents in comparison with their pigment contents in other treatments (Fig. 2, a, c). The trend line of Pro cellular pigment was relatively low and straight, indicating that Pro cells were not in good condition (Fig. 2b).

When enriched with a high level of NH_4^+-N (Fig. 1d), Pro responded rapidly at first and then declined as Syn and Bact began to grow. Pro was actually removed from the community by the end of the experiment. Both Syn and Bact reached their highest abundance in the latter half of the incubation. The Euk population remained relatively stable during the first two days, then increased and maintained a higher level until the sixth day, and then decreased to the initial level at the end of the incubation. High cellular pigment content of Euk during the first four

days showed that these cells were very active initially before their numbers increased (Fig. 2c).

In P-enriched bottles (Fig. 1e), Pro responded rapidly to the enrichment; its population almost doubled in 24 h, reaching a concentration of 8.5×10^4 cells/mL, which was never equaled in any other treatments. From the third day, cell abundance of Pro decreased to a level lower than the initial and kept decreasing to the end of the incubation. However, its cellular pigment content kept increasing (Fig. 2b), distinguishing Pro from the other groups in terms of both cell numbers and cellular pigment content. Populations of the other two autotrophs, Syn and Euk, remained almost constant during the incubation, although their cellular pigment contents increased after the Pro population decreased (Fig. 2, a, c). Unlike in the Fe treatment where Syn and Euk were the dominant autotrophs, in this case, Pro was the one that remained dominant almost throughout the incubation.

2.2 Variations in cell abundance of the picoplankters and in cellular pigment content of the autotrophs in incubations of the 75 m water

The patterns of abundance variation of the four groups of organism in the incubation of the 75 m water (Fig. 1, f - j) were less complex than that in the surface-water experiments. Bact showed uniform patterns in both the nutrient-enrichment and the control bottles. Owing to the increase of temperature from 21 ℃ at the sampling depth to 29 ℃ of the cooling water from the surface layer, the Bact population increased dramatically to 6×10^5 cells/mL in the first two days, and after a little decline, increased slightly again. Syn, the most successful species in the surface-water incubations, was less successful in the deep-water incubations, basically due to its low initial abundance (about 1 000 cells/mL on average) and perhaps, to the limited remaining environmental carrying capacity after the Bact population thrived. Although cellular pigment content of Syn increased in all the bottles as a result of the enhanced temperature (Fig. 2d), the Syn population developed only in Fe-enriched incubations where Syn population reached 6 000 cells/mL by the end of the experiment. Euk populations grew only in Fe and N treatments. Pro population, in all but one treatment, gradually decreased to around 6 000 cells/mL, equivalent to one-fifth of their initial abundance. The exception was Fe enrichment, in which the Pro population decreased for the first five days and then increased to a level close to the initial abundance at the end of the incubation.

2.3 Relative responses of picoplankton to nutrient enrichments

Responses of Syn, Pro, Euk and Bact in cell

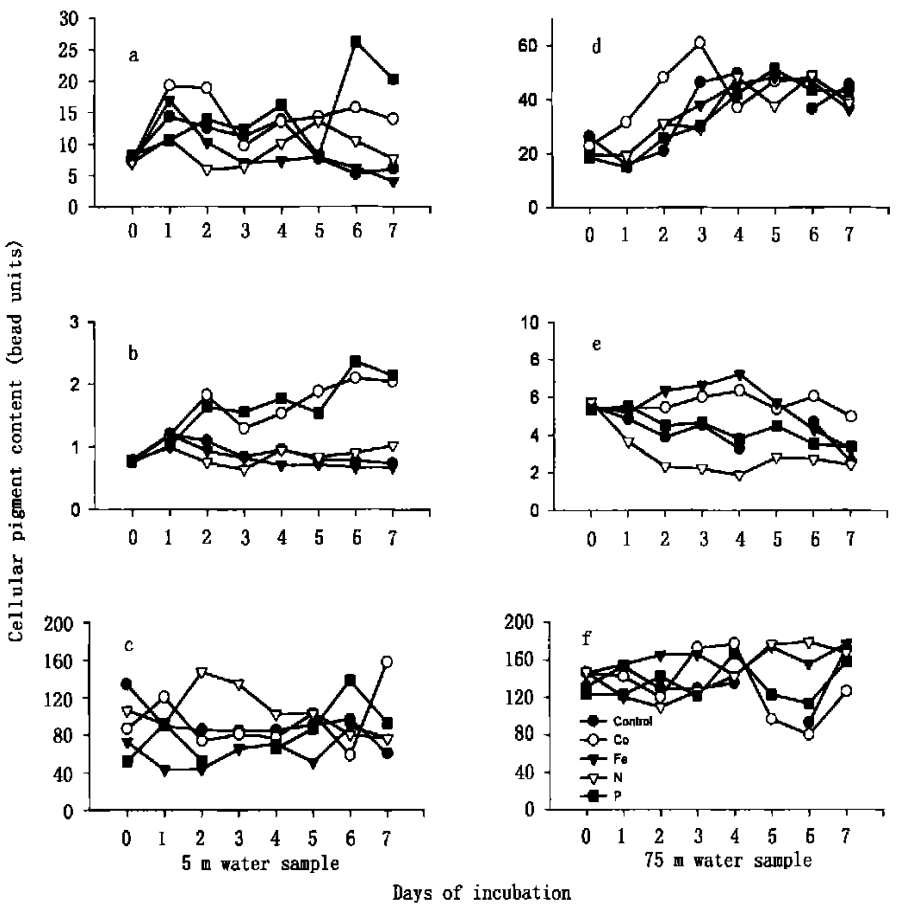

Fig. 2. Time course of autotrophic picoplankton cellular pigment content (in bead units) in the nutrient enrichment experiments. 5 m (surface) water incubation: a. Syn. b. Pro. c. Euk. 75 m water incubation: d. Syn. e. Pro. f. Euk.

abundance to the different treatments can be arranged in order. As seen from Fig. 1, in most cases, populations responded in the first 24 h of incubation and then underwent dramatic changes in the following two to three days, after that, relative stable trends can be seen from the later days. We thus took the ratio of the cell abundance after 24 h incubation (D1) to the initial abundance (D0) as an indicator of biological response to the treatments (Fig. 3). While took the cell abundance averaged over the last three days of the incubation as an indicator of the adaptability of each group of organism to the changed environments (Fig. 4).

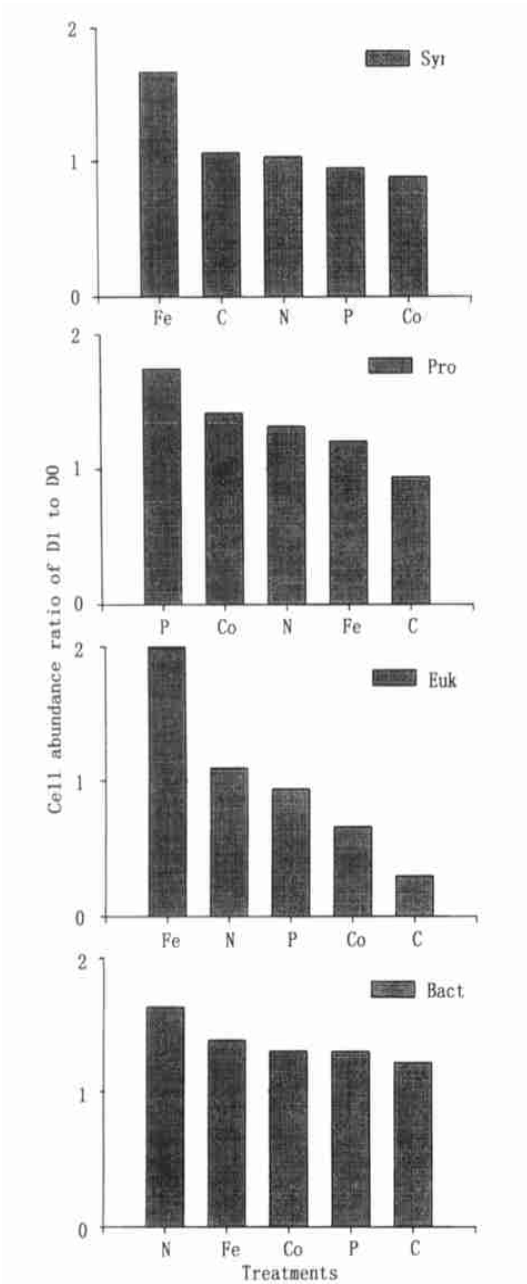

Fig. 3. Ratios of picoplankton cell abundance of D1 (after 24 h incubation) to D0 (the initial cell abundance) in different nutrient treatments of the surface water incubations.
Dashed line indicates D1/D0 = 1. C, control; Fe, enriched with iron; Co, enriched with cobalt; N, enriched with ammonia; P, enriched with phosphate.

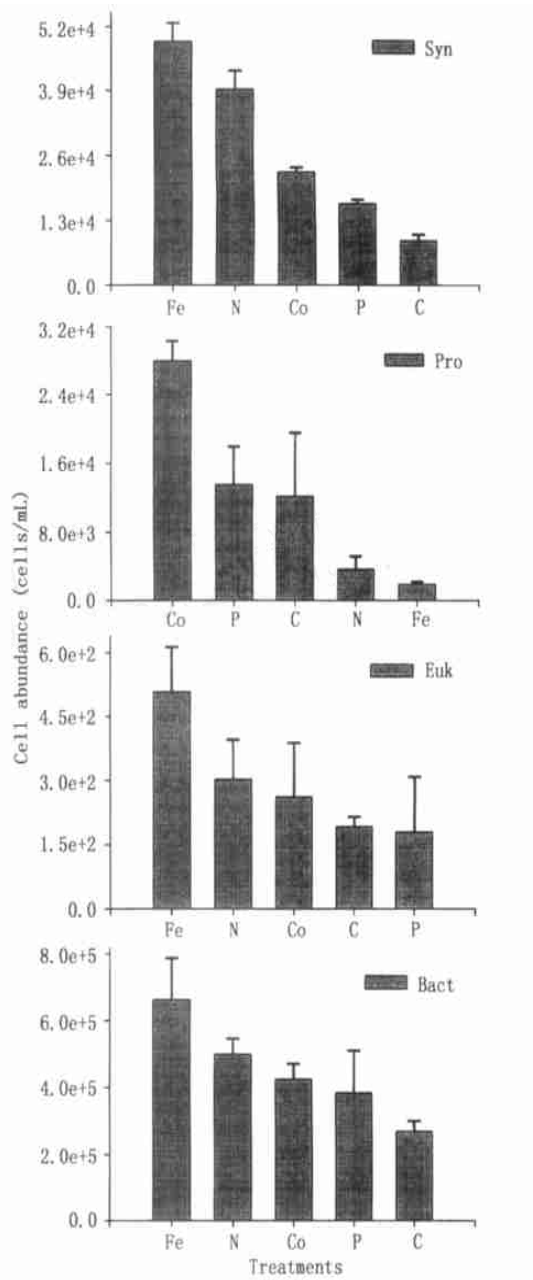

Fig. 4. Responses of picoplankton cell abundance to different nutrient in the surface water (5 m) incubations. The different treatments were arranged in order in light of cell abundance averaged over the 5th, 6th and 7th incubation days.
C, control; Fe, enriched with iron; Co, enriched with cobalt; N, enriched with ammonia; P, enriched with phosphate.

In the surface-water experiments, after 24 h incubation, Syn had a dramatic response to the Fe enrichment, its D1/D0 ratio was 1.67. Ratios in the other treatments and the control were around 1, indicating there was no significant stimulation of the addition of these nutrients to Syn or the responses of Syn to the treatments were slow (Fig. 3, Syn). While, in the case of Pro, except for the control (C), all the ratios were greater than 1, suggesting that Pro responded very quickly to all the nutrient addition with best to P treatment (Fig. 3, Pro). With the D1/D0 ratio in Fe treatment reaching 2, Euk's response to Fe

addition ranked No. 1 among all the organisms to all the treatments. While ratios of Euk in all the rest treatments were rather low. The response order of Fe > N > P > Co > C along with the sharp decreasing gradients also showed big differences in Euk's response to different treatments (Fig. 3, Euk). Unlike the autotrophs, D1/D0 ratios of Bact in all the treatments and the control incubation were greater than 1, indicating that the conditions in the incubation bottles somehow better suited for Bact to thrive (Fig. 3, Bact).

Cell abundance averaged on the last three days incubation showed great similarity in nutrient enrichment consequence of Syn, Euk and Bact (Fe > N > Co > P > C) and distinct difference from Pro (Co > P > C > N > Fe), indicating that Syn, Euk and even Bact were most favored by the addition of Fe, followed by N, while Pro was essentially pushed aside by them under such conditions. On the other hand, Co and P favored Pro better than the other picoplankters. Although Co and P were also effective to Syn, Bact and Euk (by comparison with Control treatments). Since abundance of Bact in the control bottles were the lowest either in the first day or the later days of incubation, all the nutrients added were likely to be favorable to Bact in competition (Fig. 4).

For the incubations of 75 m water, although an obvious response of Bact to enhanced temperature made the effects of nutrient addition vague, some trends were yet distinct. First, Fe was responded by all the picoplankters including Pro. Ammonia addition, again responded by Syn, Euk and Bact though ambient nitrate was abundant. Unlike the situation of the surface water incubation, in the 75 m water, Co was responded not only by Pro in the beginning but also by Syn and Euk at the end, and P was responded by Pro and Bact.

3 Discussion

3.1 Incubation conditions

Although long-time incubation entails the risk of failure to maintain the biota in healthy condition, five or more days are often employed in microplankton nutrient enrichment experiments[25]. In our experiment, except for the general decease in Pro population in the later days of the incubations, which is usually encountered during incubation due to Pro's somehow non-culturability, no severe inhibition occurred to the whole communities in the bottles within the seven days incubation period. Similar results were also recorded by previous authors[25]. Owing to "wall effects", small bottles may produce an artificial bias, especially in long-time incubation[26]. On the other hand, we did observe Pro population increases in the second day in most of the incubations which consistent with results of some short period (24 - 28 h) incubations in the Atlantic ocean[27]. Thus, the results from the latter days of our experiments should not be extended to natural ecosystems, but rather be used to explore the biological tolerance to environmental changes and the biological interactions of the organisms under conditions changing from their original environments.

3.2 Picoplankton nutrient requirements

Although there is no doubt that Fe limits diatoms growth in high-nutrient, low-chlorophyll (HNLC) ecosystems, there are different explanations about the effects of iron on picoplankton, especially prokaryotes. Experiments with deferriferrioxamine B demonstrated that growth of *Synechococcus* is not strongly limited by Fe in the HNLC equatorial Pacific Ocean[28]. Other studies on photosynthesis showed that growth of the dominant small phytoplankton is held below the physiological potential by iron deficiency[29]. From our incubation results, we speculate that Fe was a limiting factor for picoplankton in the study area; In the 75 m water, in particular, the naturally low Fe and light availability induced higher requirements for Fe for physiological activities[30] and created higher demand for Fe by the dominant picoplankton there. Relatively high cellular pigment content in most of the Fe-supplemented incubations (Fig. 2) is obviously the result of higher chlorophyll synthesis as a result of higher Fe availability[31 - 33]. Because nitrate reductase activity can be enhanced by Fe[33,34], the strong response to the addition of Fe by Syn and Euk in the surface water, and of all the autotrophs in the deeper water, can be easily understood. Even for Bact, Fe was shown to be essential in the 75 m water. This is consistent with results from Antarctic waters[35] and from the subarctic Pacific where bacteria are responsible for 20 % to 45 % of biological iron uptake[36].

Major nutrients such as N and P have drawn the interest of oceanographers for many years. N has been identified as the primary limiting nutrient for phytoplankton on a region-wide and year-round basis. P, although frequently reported to be more important than N in limiting phytoplankton productivity, is apparently less consistent, temporally and spatially, in potential regulatory importance than N[37 - 39]. These conclusions are basically for whole phytoplankton assemblages and applicable to general understanding. With respect to cell size, small cells prefer ammonia to nitrate, and the majority of nitrate uptake is accounted for by large cells[40,41]. Most recent work has revealed that only low-light ecotype Pro strain can take up nitrite and all Pro strains in culture do not utilize nitrate due to lack of nitrate reduction genes[42]. Thus high nitrate concentration favors others rather Pro. In the present study, although there was plenty of NO_3^- (15 μmol/L) in the 75 m water, addition of NH_4^+ induced in Euk a great increase in cell abundance with high cellular pigment content, indicating either that Euk preferred ammonium to nitrate or that there was inefficient nitrate uptake under conditions of iron deficiency as discussed above. Moreover, Syn and Euk had higher responses to N than to P, while Pro responded more to P than N.

Cobalt has been reported to be essential to phytoplankton metabolism, especially where zinc is depleted. Some species, e. g. *Synechococcus bacillaris*, needs Co but not Zn for growth[17]. However, Segatto *et al*[18] reported some neutral and negative effects of Co on growth

rate and biomass accumulation: the diatom *Ditylum brightwellii Bailey was not affected by cobalt addition, and the dinoflagellate Prorocentrum minimum* (Pavillard) Schiller was inhibited by cobalt addition. Our results showed significant response of Pro and weaker but still positive responses of other picoplankton to Co in terms of both cell abundance and cellular chlorophyll content. Even in the incubations of 75 m water, although Bact grew very well due to the temperature enhancement, all the autotrophs had higher cellular pigment content in Co than in the other treatments.

3.3 Implication of biological interactions

By plotting cell abundance over time in the different treatments for each group of the organisms, inverse trends between Pro and Bact, and Pro and Syn can be observed. To find the relationship among these organisms under relatively stable conditions, we removed the data from the first two days to avoid the influence of the instantaneous response to the nutrient pulses and averaged the data for the remaining five days for correlation analysis. The correlation coefficients were - 0.74 between Pro and Bact, and - 0.75 between Pro and Syn. Therefore, Bact and Syn would be the major competitors of Pro when the environmental conditions shifted away from typical oceanic conditions.

Large cells such as big diatoms, which we did not count in our incubations, might have had significant impact on Pro in some cases, especially in the Fe-enriched bottles. Although the natural abundance of large cells is lower compared with that of picoplankton in the study area (chlorophyll a in the less than 2 μm size fraction accounted for 84.3 % of the total), the populations of large cells might be able to grow very well in nutrient-enriched incubation bottles, especially in those enriched with Fe[43]. Thus, biological competition from large cells should be taken into consideration in interpreting picoplankton population dynamics under incubation conditions.

In HNLC regions and subtropical regions, grazing by microzooplankton is the dominant cause of phytoplankton mortality[26]. In both systems, the major small phytoplankton groups grow rapidly and are cropped to low stable levels by microzooplankton. Sustained high growth rates of the phytoplankton depend on remineralization of the by-products of grazing[44]. Assuming that the grazing mortality of Pro was balanced by its growth, as found in similar ecological conditions[4,45], Pro responded rapidly when pulsed with additional nutrients in the incubation bottles, but the grazing pressure remained unchanged or changed less proportionally in a relative short time. Thus, this balance was broken, resulting in a sudden increase in phytoplankton cell abundance. Pro actually increase to the maximum concentration at the first one or two days, and then decreased rapidly consequent upon the grazing pressure. However grazing loss might not be responsible for the major decrease in Pro population in the later days of the incubation course, as also can be seen from that the other groups, Syn, Euk and Bact, all reached their highest abundance in the latter days of incubation. Because Syn, Euk and Bact could also be cropped by graziers their populations would have dropped as Pro encountered in the later days of the incubation. Therefore we come to the point that grazing pressure was not the determining factor for the total loss of Pro cells during incubation.

We would therefore attribute the major loss of Pro, besides grazing mortality, to cells dying when stressed by biological competition and other environmental conditions. Typical cases in point are N and Fe enrichments in the surface water incubations, where nutrient enrichment induced a bloom of Syn and Euk and in turn inhibited growth of Pro. Indeed, we did notice a sub-population of Pro sinking down into noise in the FCM plots. Those cells actually had less and less cellular pigment content as incubation proceeded. On the other hand, Pro might also impacted upon the other organisms. An example is the P-enriched incubation in which Syn, Euk and even Bact were distinctly influenced. The later days of 75 m water incubation were typical cases of biological interaction in which temperature enhancement induced sharply increases in the populations of bacteria[46] and exerted severe stress on the autotrophs.

3.4 Factors of regulating Pro coast-wards distribution

Although temperature is crucial to Pro[5,6,11], it is not the sole factor controlling Pro distribution since the recorded temperature lower limits vary from place to place[5,7,8]. The difference between winter and summer in the East China Sea can be as large as 10 °C①. In consistent with other investigations that physical factors such as mixing and stratification influence Pro's distribution pattern[5,12], we also recognized, in another study, the great effects of ocean currents on distribution of Pro in the continental shelf of the East China Sea①. Beyond that, one basic concern is about nutrient which is easy to be thought of as one of the major differences between oceanic and coastal environments but not easy to test out due to their subtle effects and complicated consequences. Although Pro is most abundant in oligotrophic oceanic waters, it dose not necessarily mean Pro dislike high nutrients[13,43]. The quick response of Pro to nutrient addition at the first 24 h in the current study is also a piece of evidence for this point. However, since high nutrient levels may favor other picoplankters better than Pro, as demonstrated in the experiments, Pro would be finally pushed aside in the system. Situation of Pro's distribution in the coastal waters are most likely such cases. Beyond the direct effects of nutrients, the consequent biological influences are also not negligible. The relationship between Pro and other picoplankters during the incubation course are quite similar to what observed in the field along trophic gradients in the East China Sea[19]. Therefore, we speculate that Pro would encounter severe physical and

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chemical limitations as well as impacts from biological interactions in its coastwards distribution.

4 Summary

In our experiments on nutrient enrichment, Syn and Euk demonstrated high similarities in terms of nutrient requirements, especially their high demand for Fe and N. In contrast, Pro responded more to Co and P than to Fe and N.

Fe was inadequate for the autotrophs in the deep euphotic zone (75 m) in the study area. Although ambient nitrate concentration was high there, it could not be utilized efficiently by phytoplankton due to the deficiency of Fe. Co seemed to be particularly essential to Pro and Bact.

Biological interactions should be taken into consideration for analysis of shifts in microbial communities under changing environmental conditions. In the present study, addition of N seemed to be eventually unfavorable to Pro because high N favored Syn and Euk, which in turn inhibited the growth of Pro. The vice versus also appeared to apply in the P and Co treatments where Pro had impacts on the other groups of organisms. Bact had the least difference in response to different treatments. Bact growth in the enhanced temperature incubations of 75 m water inhibited all the autotrophs. Pro had different nutrient response orders from the other picoplankters and inversely correlated with Bact in the incubation course.

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从营养扰动实验看原绿球藻在近海分布的制约因素

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摘要: 地球上细胞最小、丰度最大的放氧光合自养原核生物原绿球藻(*Prochlorococcus*)发现于热带大洋,并被证实可在某些近海甚至近岸水域大量分布。但除温度之外,原绿球藻自然分布的控制因子尚不明了。从近海和大洋生态条件的主要差别考虑,在南海进行了主要营养盐——氮、磷和微量元素——铁、钴扰动的现场培养实验,并应用流式细胞技术监测原绿球藻及聚球藻(*Synechococcus*)、超微型真核浮游植物(pico-eukaryotes)的细胞丰度和单细胞色素含量的响应以及细菌的影响。结果表明,磷和钴的添加有利于原绿球藻,而氮和铁的添加更有利于聚球藻和超微型真核浮游植物。同时,由环境条件引起的生物响应又间接地导致超微型生物之间的相互作用。因而,原绿球藻在近海的分布,可能受到营养盐组成等环境因子以及生物之间的相互作用等多方面的限制和影响。

关键词: 原绿球藻; 超微型浮游生物; 营养盐; 铁; 钴; 南海
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