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RESEARCH PAPER

Effects of irradiance and phosphate on growth of nanophytoplankton and picophytoplankton

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Abstract: *In situ* incubation experiments were conducted to investigate the phosphate uptake and the growth variations of nano- and picophytoplankton under controlled phosphorus concentrations and irradiances in Changjiang estuary and its adjacent sea in China. The results showed that the rates of phosphate uptake were accelerated at high levels (0.60 μmol/L) under the condition of 100% natural irradiance, and the cell densities of nanophytoplankton and *Synechococcus* spp. obviously increased, whereas picoeukaryote was adapted to low phosphate levels (0.25 μmol/L). Under low irradiance (50% of natural irradiance), uptake of phosphate was restrained at high levels, and the growth of both nanophytoplankton and *Synechococcus* spp. was also limited. Morerover, nanophytoplankton and *Synechococcus* spp. grew well at intermediate phosphate levels (0.41 μmol/L), whereas picoeukaryote grew well at low phosphate levels. In addition, the growth period of phytoplankton at intermediate phosphate levels was obviously prolonged, suggesting that the limitation of phytoplankton growth mainly reflected the changes during its growth period. In the absence of irradiance, the addition of phosphate did not affect the release rates of phosphate with a linear increase in the phytoplankton, whereas the growth rates of the phytoplankton showed an exponential decrease, which showed that phosphate regeneration was faster during day than during night; therefore, the irradiance was a significant factor that affected phosphorous biogeochemical cycle in the Changjiang estuary in China.

Key Words: Changjiang estuary; phytoplankton; irradiance; phosphate

Riverine materials are transported from the Changjiang river to the sea, and these materials form the main source of nutrients for both the estuary and its adjacent sea. Variations in the nutrient levels and their ratios can affect estuarine ecosystems, for instance, the production rate of phytoplankton[1]. The DIN: DIP ratios were more than twice the Redfield ratio[2,3] whose value is about 16 in the Changjiang estuary, which showed that the growth of phytoplankton was limited by phosphate[4−8]. In addition to the role of nutrients, light availability also plays an important role in the growth of phytoplankton and therefore needs to be considered. Suspended sediments and resuspended sediments, which are formed as a result of tidal disturbance, are transported from the Changjiang river to the Changjiang estuary, and these form a high-turbidity area in the Changjiang estuary, with a transparency of lower than 3 m. Light limitation resulting from the suspended sediments has a stronger influence on the release of nutrients to the estuarine ecosystems, which lowers primary production, biodiversity, and density rates in that area compared with those in the adjacent sea[9]. Pu *et al.*[10,11] proposed that the Changjiang estuary may be divided into three areas: irradiance-limited inshore, light- and phosphate-limited transitional area, and nitrogen-limited offshore. This concept of transition from limitation of light to that of nutrient along the salinity gradients has been reported in several studies on other estuarine systems[12−14]. However, the means by which the gradients of light and nutrients interact to influence the growth of phytoplankton in estuarine ecosystems, especially that of the nanophytoplankton and picophytoplakton, which form 60%–70% of the components with respect to primary production in many oceans of the world, is not clear [15−17]. The correlative researches mostly focused on their biomass, primary production in marine ecosystem, and their contributions to the nutrient regeneration, carbon cycles, etc[18−24]. However, very little data of *in situ* incubation experiments have been published to illuminate their physiological and ecological proc-

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esses. In this article, a series of *in situ* incubation experiments was carried out to investigate the effects of irradiance and phosphate on the growth of phytoplankton and the biogeochemistry processes of the high-turbidity estuary by altering the phosphate concentrations of the incubation water and the natural irradiances.

1 Materials and methods

1.1 Sampling station

Ship-board incubations were conducted on board R/V "Hai-Jian No. 47" on September, 23–29, 2004. The sampling station was located at 123°00′E, 30°05′N (Fig. 1) at a depth of about 46 m. The concentrations of ammonium, nitrate, nitrite, phosphate, and silicate were 10.95, 3.01, 0.08, 0.25 μmol/L, and 8.09 μmol/L , respectively, in surface water of the station. Temperature, salinity, and suspended sediment concentration in surface water were also recorded, which was found to be 24.84℃, 28.68 and 0.033 kg/m^3, respectively.

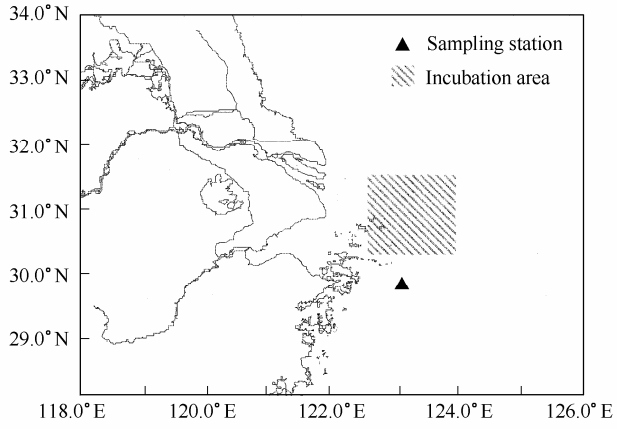

Fig.1 Sampling station (▲) and incubation area (shadow)

Table 1 The design of experimental groups

Incubation bottles	Volumes (ml) of 5mmol/L NaH_2PO_4added	Phosphate concentration levels in incubation water	Level ofirradiance
C1	0	Low phosphate	High irradiance
C2	0.5	Intermediate phosphate	High irradiance
C3	1	High phosphate	High irradiance
C4	0	Low phosphate	Low irradiance
C5	0.5	Intermediate phosphate	Low irradiance
C6	1	High phosphate	Low irradiance
C7	0	Low phosphate	No irradiance
C8	0.5	Intermediate phosphate	No irradiance
C9	1	High phosphate	No irradiance

1.2 Ship-board incubations

The surface water was filtered through a 50μm nylon mesh to remove large-sized zooplankton, and then transferred into 5-liter transparent polyethylene incubation bottles in series. According to the variations in the range of phosphate concentration in Changjiang estuary[25], 5 mmol/L of sodium dihydrogen phosphate of three different volumes were added to these bottles, which represented three phosphate levels, i.e. low phosphate level (0.25 μmol/L), intermediate phosphate level (0.41 μmol/L), and high phosphate level (0.60 μmol/L) (Table 1). These incubation bottles were also under the control of three irradiances: high irradiance (100% *in situ* irradiance), which simulated the surface-water irradiance; low irradiance (about 50% *in situ* irradiance), which simulated the maximum turbidity zone (MTZ) irradiance; no irradiance, which simulated the dark environment of the bottom layers. The seawater was incubated at about 16℃ by placing the bottles into a large tank on board, and the incubation bottles were regularly shaken about once for every 6 hours to avoid phytoplankton aggregation and settling.

All the nylon meshes, incubation bottles, filters for the experiment were previously soaked in HCl (pH=4.0) for 24 hours, washed using distilled water, and then using Mili-Q water.

1.3 Nutrients

Water samples for nutrient analysis were filtered through precleaned cellulose-acetate filters with a pore size of 0.45 μm, and saturated mercury chloride (1%) was added to the filtrates and the solution was stored in dark until analysis. The determinations of nutrient species were spectrofluorimetrically performed on a Continuous Flow Analyzer (Skalar^{plus} System, Skalar Analytical B.V., the Netherlands).

1.4 Abundance of nanophytoplankton and picophytoplankton

To prevent plugging of the sample injection port, samples for nanophytoplankton and picophytoplankton determinations were filtered through pre-cleaned cellulose-acetate filters with a pore size of 20 μm pore size, to remove large particles. Paraformaldehyde (final concentration: 1%) was added to the filtrates and then kept frozen in liquid nitrogen until the samples are taken back to the laboratory. Nanophytoplankton and picophytoplankton groups were measured on an FACS can flow cytometer (Bection Dicknson, USA).

1.5 Calculation of growth rate

Growth rate of the phytoplankton is calculated as:

$$\mu = \ln(\frac{C_1}{C_0}) / (t_1 - t_0)$$

Where μ is called specific growth rate, C_1 and C_0 are the