ACTA ECOLOGICA SINICA
Volume 26, Issue 9, September 2006
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Cite this article as: Acta Ecologica Sinica, 2006, 26(9), 2783−2790.

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-

Received date: 2005-12-09; Accepted date: 2006-07-21
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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.

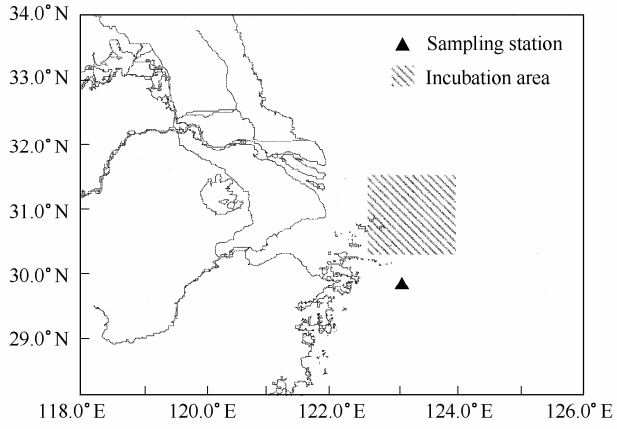

Fig.1 Sampling station (▲) and incubation area (shadow)

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

biomass at the beginning (t_1) and the end (t_0) of the experiment.

2 Results and discussion

2.1 Changes in phosphate uptake

Under high irradiance, the variations of phosphate uptake during the ship-board incubations had a similarity between those at the low and the intermediate levels, i.e. the phosphate concentration showed a decrease during the earlier stage of the experiments followed by a slow increase during the later stage of the experiments (Fig.2). But at high levels, the phosphate concentrations decreased throughout, which corresponded to an almost straight decline (the lineal regression formula was $y = -0.071x + 0.58$, $R^2 = 0.99$), and the values of uptake rates were obviously greater than those at low or intermediate level. The DIN: DIP ratio was about 46 in surface water of the sampling station, which indicated a strong limitation of photosynthesis, and the results described above, which stated that the phosphate uptake rates were considerably stimulated at high level, also proved the phosphate limitation in the study area.

Under low irradiance, the phosphate uptake at high level was not obviously stimulated in contrast to that under high irradiance, but was restrained. The initial DIN: DIP ratio at different phosphate levels was 46, 28 and 17, respectively. So under the condition of low irradiance, the uptake of nutrients was not in accordance with Redfield ratio, but showed a higher value.

Without irradiance, the phosphate concentrations kept increasing at three different levels during the incubations. Regression analysis showed that phosphate concentrations were linear greatly with the incubation time (Table 2), and the slopes of the three lineal formulae at different phosphate levels were 0.0096, 0.0109 and 0.0106, respectively, which showed that the values were very close to each other (standard deviation was only 0.0006); therefore, it can be concluded that the addition of phosphate did not affect the release rates of phosphate, i.e. the release rate of phosphate is a definite value in dark.

Table 2 The lineal regression formulae between phosphate concentrations and incubation time at the three phosphate levels without irradiance

Phosphat concentration levels	Lineal regression formulae	Value of R^2
Low phosphate	$y = 0.0096x + 0.2493$	$R^2 = 0.9483$
Intermediate phosphate	$y = 0.0109x + 0.4122$	$R^2 = 0.9577$
High phosphate	$y = 0.0106x + 0.5971$	$R^2 = 0.896$

It was necessary to point out that except nanopytoplankton and picophytoplankton, phytoplankton species in the incubations included some macrophytoplankton (>20 μm) and their phosphate uptake differed from others. The analysis of the factors leading to this difference needs further research.

2.2 Changes in growth patterns of nanophytoplankton and picophytoplankton

2.2.1 High irradiance

Corresponding to the changes in phosphate uptake, growth rate of nanophytoplankton also increased with phosphate concentration increasing (Fig. 3). Its initial growth rate was 0.98 d^{-1} at high phosphate and was 35.39% higher than that at low phosphate, which was 0.73 d^{-1}. The growth period of nanophytoplankton was prolonged, i.e. it required more time (2–3 days) to reach growth peak, and its peak value for cell density was 1.5262×10^4 cells/ml, but at intermediate and low phosphate levels, it was only 0.7407×10^4 and 0.5957×10^4 cells/ml, respectively, which was 48.53% and 39.03% of that at high phosphate level. So high phosphate concentration was suitable for nanophytoplankton under the condition of high irradiance.

The operational concept of picophytoplankton (<2μm) by definition includes the autotrophic cyanobacteria *Synechococcus* spp., *Prochlorococcus* spp., and small eukaryotic algal groups (picoeukaryote)[26]. From the data, the concentration of *Synechococcus* could reach 1.57469×10^5cells/m, which was one order of magnitude higher than those of nanophytoplankton and picoeukaryote. Under high irradiance, high phosphate concentration also stimulated the increase in *Synechococcus* population early in the incubations, whereas it decreased at

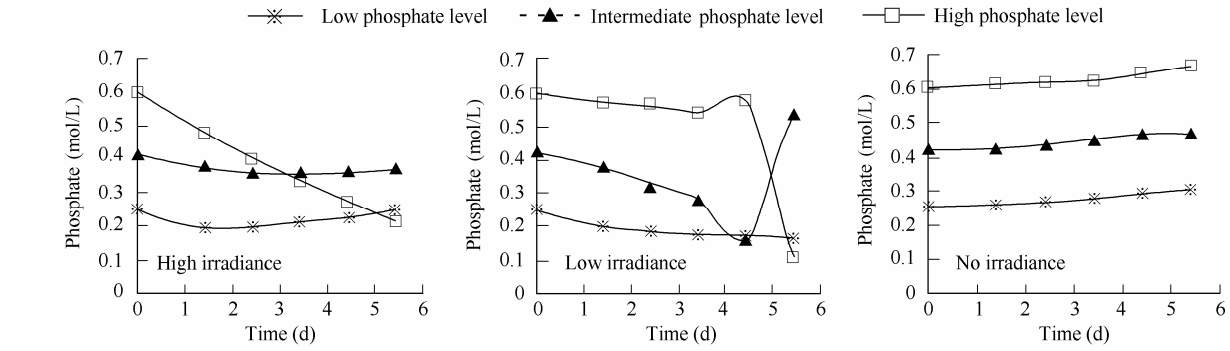

Fig.2 Temporal changes of phosphate uptake at the three phosphate levels under different irradiances

low or intermediate phosphate level. It should be noted that *Prochlorococcus* was almost absent near the Changjiang estuary, and hence was not detected using the FACScan flow cytometer. Picoeukaryote growth rate was highest (0.11 d^{-1}) at low phosphate level during the initial stage of the incubations, whereas it was lowest (0.03 d^{-1}) at high phosphate level, which revealed that picoeukaryote grew well at low phosphate level; therefore, picoeukaryote could exist in the low-nutrient ocean in abundance and was responsible for most of primary production[27,28]. It may also be the reason for the competition between picoeukaryote and nanophytoplankton, that was to say, when nanophytoplankton grew at a rapid rate and became the dominant species, it may lead to the suppression of picoeukaryote. So the phosphate added was mostly beneficial for the large-sized phytoplankton.

2.2.2 Low irradiance

Under low irradiance, phosphate uptake was restrained at high level as discussed above; similarly nanophytoplankton growth was also suppressed at high phosphate level (Fig. 4), and its initial growth rate was only 0.15 d^{-1}, which was 27.07% of that at intermediate phosphate level and 33.60% of that at low phosphate level. The cell density of nanophytoplankton was 0.8750×10^4 cells/ml at the peak of growth curve at intermediate phosphate level, which was 42.11% higher than that at low phosphate level and 2.34 times of that at high phosphate level. Therefore, under low irradiance, intermediate phosphate level was suitable for the growth of nanophytoplankton, which differed from the condition of high irradiance. This was an important finding. Red tide could not occur in high-turbidity zone, which was usually attributed to light limitation, as demonstrated in past research[29]; however, from the results of this article, the high concentration of nutrients in the high-turbidity zone may also be an important cause because high concentration of nutrients under low irradiance would limit growth of certain phytoplanktons.

The cell density of *Synechococcus* could reach 2.36728×10^5 cells/ml at the growth peak at the intermediate phosphate level, and it could reach 90.3% of the value at low phosphate level and only 77.1% at high phosphate level. Under high irradiance, the growth rate of *Synechococcus* kept decreasing at intermediate or low phosphate level, but showed a slight increase during the early incubation periods (the initial growth rate was only 0.10 d^{-1}) only at high phosphate level. However, under low irradiance, the initial growth rates at low, intermediate, and high phosphate levels were 0.22, 0.15 d^{-1} and 0.15 d^{-1}, respectively, which were all higher than those under high phosphate and high irradiance levels. So *Synechococcus* could survive under the condition of lower phosphate and lower irradiance levels. Picoeukaryote growth was also limited at high phosphate level, which was similar to that under

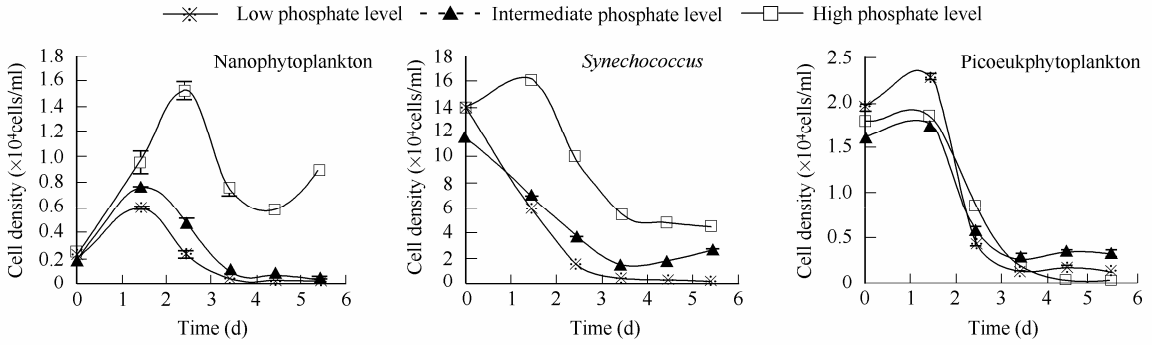

Fig.3 Alterations in growth patterns of nanophytoplankton and picophytoplankton at the three phosphate levels with high irradiance

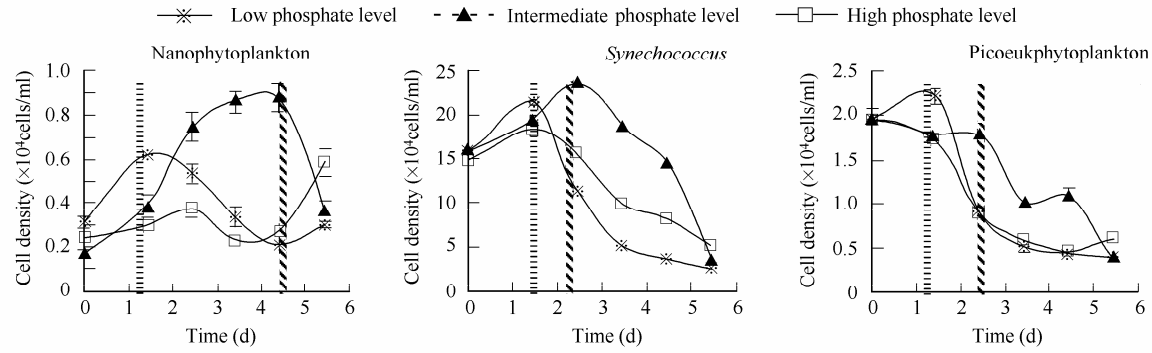

Fig.4 Changes in growth pattern of nanophytoplankton and picophytoplankton at the three phosphate levels under low irradiance

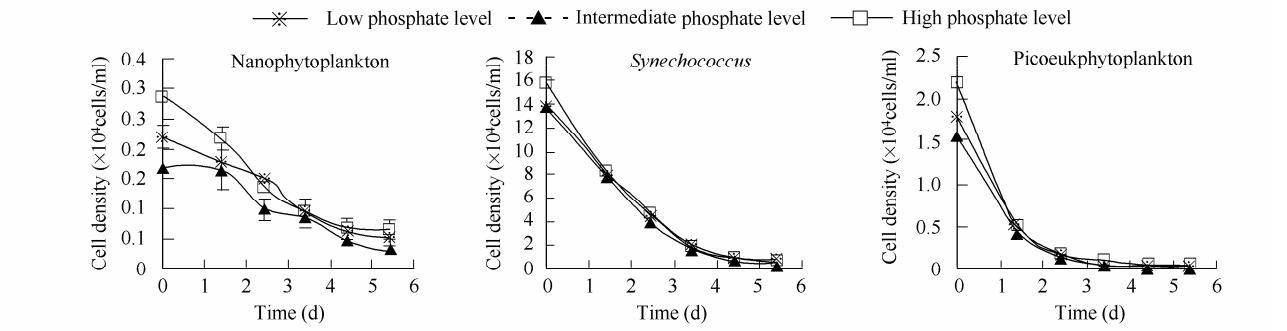

Fig.5 Changes in growth pattern of nanophytoplankton and picophytoplankton at the three phosphate levels without irradiance

the condition of high irradiance.

It should be noted that the growth periods of the three kinds of phytoplankton, especially nanophytoplankton, at intermediate phosphate level were all prolonged (Fig. 4, shown as bar graph) and the time required to reach the growth peak could be prolonged by about 3 days. So, under the condition of low irradiance, the nutrient limitation with respect to the phytoplankton was mainly reflected by its effects on their growth period.

2.2.3 No irradiance

From the results of incubation, any of the three kinds of phytoplankton could not grow in the absence of irradiance (Fig. 5). The death of phytoplankton was rapid during the early incubation period, and then became gradual. Regression analysis showed an exponential decrease in the phytoplankton cell density with the incubation time increasing (Table 3), and the regression curves at three different phosphate levels were very similar, which showed that the addition of phosphate did not affect the decrease rates in the phytoplankton cell density under no irradiance. That was to say, light was superior to all the other factors that were needed for the growth of phytoplankton. The addition of phosphate could stimulate phytoplankton growth only if there was sufficient irradiance.

Picoeukaryote growth curve had the sharpest inclination, followed by *Synechococcus*; therefore, the death of picoeukaryote was the most rapid, followed by that of *Synechococcus*, and then by that of nanophytoplankton, which was the slowest, and the coefficients of x in the exponential regression formulae of picoeukaryote were also obviously greater than those of nanophytoplankton and *Synechococcus*. The rapid death of phytoplankton was the main factor responsible for phosphate regeneration. It was remarkable that the coefficients of x among the formulae of the three kinds of phytoplankton had a specialty, i.e. it was -0.3329–-0.2836 for nanophytoplankton, -0.7575–-0.8389 for picoeukaryote, and -0.6947 –-0.5736 for *Synechococcus*, which indicated that the death rate was different among the three kinds of phytoplankton.

Table 3 Exponential regression formulae between nanophytoplankton and picophytoplankton cells and incubation time at the three phosphate levels without irradiance

	Phosphate concentration levels	Exponential regression formulae	Value of R^2
Nano	Low phosphate	$y = 0.2496e^{-0.2836x}$	$R^2 = 0.9608$
	Intermediate phosphate	$y = 0.2136e^{-0.3329x}$	$R^2 = 0.9255$
	High phosphate	$y = 0.2917e^{-0.2995x}$	$R^2 = 0.9655$
Euk	Low phosphate	$y = 1.2893e^{-0.7575x}$	$R^2 = 0.8745$
	Intermediate phosphate	$y = 1.265e^{-0.8245x}$	$R^2 = 0.9658$
	High phosphate	$y = 1.6755e^{-0.8389x}$	$R^2 = 0.9659$
Syn	Low phosphate	$y = 1.5859e^{-0.5736x}$	$R^2 = 0.9752$
	Intermediate phosphate	$y = 1.7339e^{-0.6947x}$	$R^2 = 0.9846$
	High phosphate	$y = 1.7934e^{-0.6367x}$	$R^2 = 0.9863$

3 Conclusions

(1) Under high irradiance by way of adding different volumes of phosphate, the tendency of the decrease in phosphate concentration was accelerated at high level, and cell density of nanophytoplankton was obviously increased, with *Synechococcus* exhibiting a slight increase, whereas picoeukaryote had a more rapid growth rate at low phosphate level, which indicated the adaptability between different kinds of phytoplankton and different phosphate levels. It may also be the factor responsible for the competition between nanophytoplankton and picoeukaryote. When nanophytoplankton grew rapidly and became the dominant species, it may lead to the suppression of picoeukaryote. So the phosphate added was beneficial mainly to the large-sized phytoplankton.

(2) Under low irradiance, phosphate uptake was restrained at high level, which differed from that under high irradiance, and nanophytoplankton growth and *Synechococcus* growth were also limited, which survived well at intermediate phosphate level, whereas picoeukaryote grew more rapidly at low phosphate level. However, the condition of low irradiance and low

phosphate did not exist in the high-turbidity zone; therefore, the reason for the nonoccurrence of red tide in high-turbidity zone should not be attributed only to light limitation. Moreover, growth periods of the three kinds of phytoplankton at intermediate phosphate level were all prolonged; therefore, under low irradiance, the limitation of nutrients with respect to phytoplankton was mainly reflected by its effects on their growth period.

(3) In dark, the release rates of phosphate differed, and were not suitable for the growth of phytoplankton. Phosphate concentrations kept linearly increasing with the incubation time, whereas phytoplankton cell density kept exponentially decreasing. The addition of phosphate did not affect the release rate of phosphate as well as the death rate of phytoplanktons. So the light was the most significant of all other factors, and the addition of phosphate could stimulate the occurrence of red tide only under the conditions of sufficient irradiance and appropriate temperature.

Acknowledgements

This study was funded by the Special Funds from the National Key Basic Research Program of the Ministry of Science and Technology, China (No. 2002CB412405).

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