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RESEARCH ON RED TIDE OCCURRENCES USING ENCLOSED EXPERIMENTAL ECOSYSTEM IN WEST XIAMEN HARBOR, CHINA —— RELATIONSHIP BETWEEN NUTRIENTS AND RED TIDE OCCURRENCE*

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Abstract This study on the distribution of phosphate and its relation to phytoplankton biomass in Western Xiamen Harbor using marine ecosystem enclosures to isolate the culture water from the tidal currents and salinity changes outside indicated that the phytoplankton biomass variation closely related to dissolved inorganic phosphorus (DIP) in the seawater as described by the equation: $[\text{Chl-a}] = A \times e^{-B[PO4]}$. The biomass changes lagged by about two days the corresponding DIP. The research also dealt with the minimal DIP concentration for stopping diatom bloom and the possible maximal diatom biomass was estimated from the DIP external concentration in the seawater. The threshold of DIP initiating *Skeletonema costatum* red tide was calculated for use as an index to forecast its red tides. In addition, the relationships between a dinoflagellate red tide and nutrients are discussed. The results showed that the multiplication of dinoflagellate was not entirely dependent on the nutrients in the seawater.

Key words: red tide, phosphate, nutrients, DIP

INTRODUCTION

Phosphorus is a key nutritive element for the growth of marine phytoplankton. Recently, because of environmental pollution, the eutrophication problems in coastal and oceanic waters have become more and more serious. The environment ecological response in the long-term to the flux of phosphorus has become an important research subject of marine scientists of the world (Harrison, 1983; Smith, et al., 1985). Vast areas along the coast of China are P-limited for primary production (Harrson et al., 1990). There is some eutrophication in Western Xiamen Harbor where N was found to be high and P low, and where several red tides occurred in recent years. The N:P ratio monthly average was 61:1 in 1987 (Zhuang, 1991; Cai, 1988). Normally, the nutrients peaked in March or April every year, but with the spring bloom of phytoplankton the phosphate always decreased to about 0.2 μmol/L. However the nitrate concentration still remained at 5 μmol/L when the bloom started to decline, probably because of the shortage of inorganic phosphorus. Red tide occurred in Western Xiamen Harbor in June 1986, and in May 1987, just after an abnormal peak of phosphate concentration (Du, 1989; Zhuang, 1991). The growth and decline of the spring bloom in normal years and red tides caused by excessive phytoplankton growth in Western Xiamen Harbor might be related to the changes of phosphate concentration there.

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We used marine experimental ecosystem enclosures to study the relationships between the changes of phosphate and the development of a phytoplankton community under controlled conditions. To gain further understanding of how red tides occur in seawater with different nutrient concentrations, and especially in N-deficient aquacultural areas, a case of shortage of nitrogen supply was studied.

MATERIALS AND METHODS

Table 1 shows the experimental conditions for three marine ecosystems enclosed experiments. The experimental equipment and methods for sample treatment and analysis were the same as described in Lin et al. (2000).

Table 1 Experimental conditions in the enclosure experiments conducted at 3 different times

Experiment	Date of exp.	Enclosed vol.	Experimental conditions
R1	May - July, 1990	7.6 m^3	L1: 1 - 22 d at low phosphate conc., from day 23 nutrient conc. like that in H1, stirring. H1: 1 - 34 d at nutrient conc. of about NO_3:30, PO_4:3, SiO_4:20 μmol/L respectively, stirring.
R5	Sept. 19 - Dec. 4, 1992	1.5 m^3	C5: control, 1 - 51 d at continuously rich nutrient conc. $NO_3 > 25$ μmol/L, $PO_4 > 0.5$ μmol/L, $SiO_4 > 20$ μmol/L., no stirring. N: intermittent NO_3 supply, phosphate and silicate conc. same as C5, no stirring.
R6	Oct. 30 - Nov. 12, 1995	1.5 m^3	O: no nutrients added. (control) P: NO_3:50 μmol/L, PO_4:1.8 μmol/L. Q: same as in P.

Experiment R1

Two enclosures (L1, H1) with different nutrient concentrations were used in the earlier stage of R1 (0 - 22 d). A low phosphate concentration was used in L1. The concentration was similar to that in the sea after a spring bloom, when nitrate and phosphate were 15 and 0.2 μmol/L respectively, and were kept continuously at this level by adding nutrients and monitoring the result. In H1, nitrate and phosphate concentrations were also kept continuously above 30 and 3 μmol/L respectively by nutrient additions. In the second stage (23 - 34 d), the nutrient concentration in L1 was raised to the same level as that in H1 in order to observe the variation in phytoplankton biomass due to nutrient enrichment (Lin et al., 1992). All enclosures were stirred by bubbling for two hours per day.

Experiment R6

To gain understanding of the relationship between biomass and the concentration of phosphorus, in experiment R6, P and Q were duplicate enclosures with the same concentration of nitrate (50 μmol/L) and phosphate (1.8 μmol/L). Enclosure O served as control, with nutrient concentration the same as that of the captured enclosure water; nitrate: 22.6 μmol/L; phosphate: 0.89 μmol/L.

The enclosures were stirred twice a day at 8:30 and 18:00 and sampled at 8:00 every day.

Experiment R5

This was an experiment on intermittent nitrate supply. Enclosure C5 was used as control and maintained as a nutrient enriched enclosure with > 25 μmol/L nitrate, > 0.5 μmol/L phosphate, and > 20 μmol/L silicate for 51 days, after which nutrient additions were stopped. In enclosure N, the concentrations of phosphate and silicate were the same as that in C5, but the nitrate supply was intermittent, so high nitrate concentration was not continuously maintained (Fig. 1). Nitrate was added after a short period of time when it had been exhausted (Lin et al., 1994c).

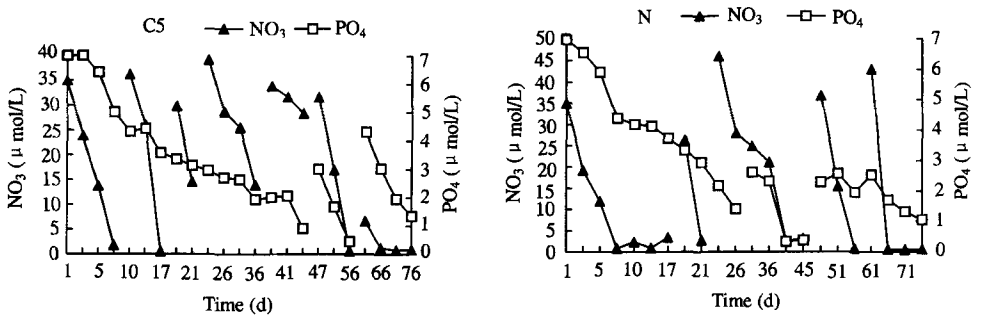

Fig. 1 Change in concentrations of nitrate and phosphate in enclosures C5 and N during Experiment R5

RESULTS

Phytoplankton community changes in response to phosphate concentration in R1

Fig. 2 shows the changes in chlorophyll-a concentration under different supply of phosphate in L1 and H1 during Experiment R1. No bloom occurred under the low phosphate level (< 0.2 μmol/L) in L1, but a bloom occurred after nutrients at the same concentration as that in H1 were added to the enclosure after day 22. As mentioned by Lin Yu and Lin Rongcheng (1999), a phytoplankton bloom or red tide occurrence was also observed after enough nutrients were added to the control enclosures C2, C3 and C5 in the experiments R2, R3 and R5 respectively (Lin et al., 1993; 1994a, b, c). Those results indicated that changes in nutrient concentration, especially that of phosphate were closely related to the spring bloom and red tides occurrence. Phosphate is one of the main factors causing and controlling phytoplankton bloom or red tides in Western Xiamen Harbor (Lin et al., 1992).

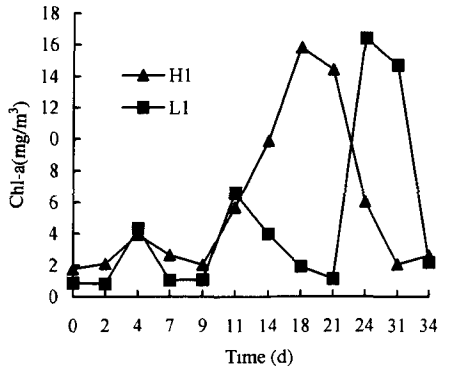

Fig. 2 Changes in chlorophyll-a concentration in L1 and H1 during Experiment R1

Relationship between diatom biomass and phosphate concentration (R6)

A *Skeletonema costatum* dominated diatom bloom with algae density peaks of 9.1 and 4.6×10^6 cells/L respectively occurred in the enclosures P and Q (Fig.3). In enclosure O, no diatom bloom occurred because no additional nutrients were added to the water. The phytoplankton communities consisted mainly of dinoflagellates and microflagellates but diatoms were scarce. The chlorophyll-a concentration was 1.7 – 1.9 mg/m^3 in the first 3 days, and then decreased to < 1 mg/m^3.

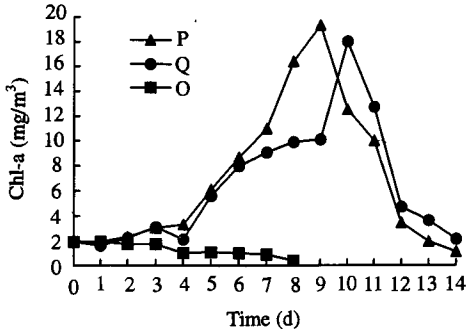

Fig.3 Changes in chlorophyll-a values in barrels P, Q and O during Experiment R6

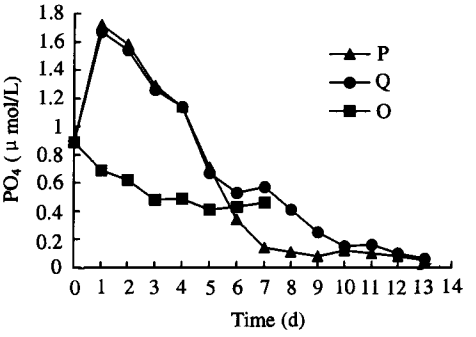

Fig.4 Changes in phosphate concentration in barrels P, Q and O during Experiment R6

Analysis of the interrelation of phosphate concentration (Fig. 4) with chlorophyll-a taken from every sampling day during the phytoplankton multiplication in P and Q (P:1 – 9 d, Q:1 – 10 d), yielded the regression equation:

$$[\text{chl-a}] = 16.6e^{-1.37[\text{DIP}]} \quad (n = 19,\ r = 0.971) \qquad (1)$$

Our linear regression analysis for DIP and chlorophyll-a showed that phytoplankton biomass variation lagged the corresponding DIP concentration values by about two days.

Changes in the phytoplankton community in Experiment R5

Phytoplankton in C5 started to grow a few days after the beginning of the experiment. Algae formed a bloom and reached a peak of 3.6×10^6 cells/L on day 21 (Fig.5). The dominant species was *Nitzschia closterium*. In enclosure N, a diatom bloom without obvious dominant species occurred, but the bloom stopped developing because of shortage of inorganic nitrogen supplied. The peak of 2.1×10^6 cells/L in the bloom appeared on day 9, started to decline afterward (Fig.6).

The phytoplankton of C5 grew rapidly after addition of nutrients while inorganic nitrogen was kept at > 25 μmol/L during the first 51 days. With the diatom bloom, dinoflagellates decreased slightly in number, but remained at the same order of magnitude. Dinoflagellates started to increase rapidly on day 26 and formed a red tide on day 41. Even though nitrate declined to < 0.5 μmol/L after day 56, the dinoflagellate red tide dominated by *Prorocentrum micans* still developed and reached a peak of 5.0×10^7 cells/L on day 66. The red tide was maintained for more than 20 days until the end of the experiment.

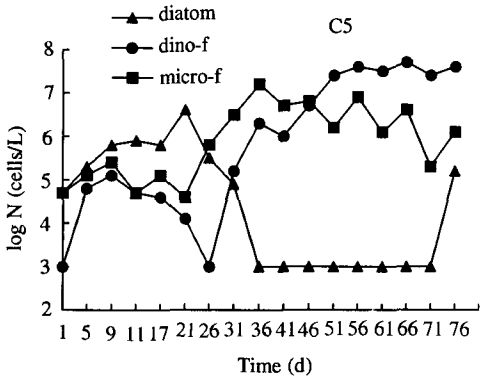

Fig. 5 Temporal distribution of diatoms, dinoflagellates and microflagellates in barrel C5 of Experiment R5

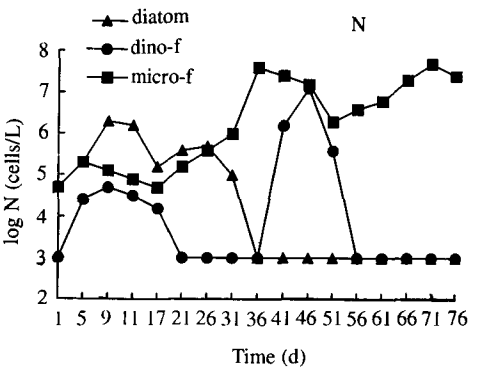

Fig. 6 Temporal distribution of diatoms, dinoflagellates and microflagellates in barrel N of Experiment R5

In the enclosure N, the dinoflagellates started to bloom on day 36 when the diatom bloom declined as in C5. *Gymnodinium* sp. dominated the red tide formed on day 46 of the experiment. Our results indicated that the frequency and amount of nutrient supply had no effect on the successional order of phytoplankton in the marine ecosystem enclosures. In this experiment, the peak of *Gymnodinium* sp. was 1.4×10^7 cells/L on day 46, but the *Gymnodinium* red tide declined quickly and reached a minimum by day 56. The duration of the dinoflagellate red tide that occurred in the enclosure N was shorter than that in enclosure C5. In the 10 days (26 – 35 d) before the dinoflagellates began to bloom, the concentration of inorganic nitrogen was 21 – 28 μmol/L, while nitrate was < 20 μmol/L during the dinoflagellate bloom (36 – 45 d).

DISCUSSION

1. The DIP minimal concentration for stopping diatom bloom

In enclosure O of experiment R6, after day 3 the DIP decreased to < 0.5 μmol/L and the phytoplankton community was dominated by dinoflagellates. The DIP concentrations calculated from Eq. (1) was 0.19 μmol/L in P and Q, when the diatom bloom started to decline. This result showed that the phytoplankton community dominated by diatoms stopped growth when DIP decreased to < 0.2 μmol/L in the seawater. This agreed with the results of experiment R1 (Lin et al., 1992; 1994a). For an about two day delay of the phytoplankton growth, the minimal DIP concentration for stopping diatom bloom should be about 0.4 μmol/L. Because varying multiplication rates existed in different algal species, the exact time of the delay and the exact DIP minimum could not be known. Therefore, 0.2 μmol/L could only be referred to as the minimum DIP external concentration for stopping diatom bloom.

2. The DIP threshold for causing diatom red tides

Skeletonema costatum, the dominant species in Experiment R6 was taken as an example here. Based on our experimental data, the chlorophyll-a content of this alga was about $(3.4 - 10.0) \times 10^{-7}$ μg/cell. Taking the upper limit, if the density of *S. costatum* was more than 10^7 cells/L when red tide occurred, then the content of chlorophyll-a should be more than 10 mg/m^3. According to

our experimental results, an increase in chlorophyll-a by 10.6 μg was accompanied by DIP consumption of 1 μmol/L. Therefore, the concentration of DIP should have decreased by 0.94 μmol/L if the number of *S. costatum* reached to red tide level from the beginning of the multiplication. Thus, the concentration of DIP that might form *S. costatum* red tide should be more than 1.2 μmol/L, if 0.2 μmol/L DIP stopped diatom bloom. Zhou et al. (1983) and Cheng et al. (1994) also mentioned that the threshold of DIP for eutrophication was 1.45 μmol/L.

In fact, the quantity of phosphorus required by phytoplankton for multiplication was also influenced by the composition of the phytoplankton community and the ecological environmental conditions. This paper only dealt with data available from existing routine methods to try to find the threshold for diatom red tide formation. Our experimental method was suitable for a closed or semi-closed harbor where the hydrodynamic conditions are very close to those in our experiments. The obtained parameter values after revision can be used as reference for forecasting harmful diatom bloom.

3. Dinoflagellate red tides and nutrients

The effects of nutrients on the phytoplankton succession in the enclosed ecosystems will first be discussed (Lin et al., 1994c). According to results of Experiment R5, the amount of nutrients did not hamper the process of succession. When dinoflagellates dominated, a red tide of a certain species of dinoflagellate may possibly be formed even under nutrients shortage conditions. For example, the *P. micans* red tide still lasted for over 20 days after inorganic nitrogen in the water of enclosure C5 was exhausted during the last stage of Experiment R5. The inorganic nitrogen concentration was < 0.5 μmol/L at that time. *Gymnodinium* sp. red tide also occurred under the condition of inorganic nitrogen supply shortage in the N enclosure. These observations indicate that the amount of nutrients in the water may not be the decisive factor causing the formation of dinoflagellate red tides. The nutrient resource for dinoflagellates may be different from that for diatoms, because the former may migrate vertically (Taylor, 1987). The two red tides of two different dinoflagellate species in C5 and N during Experiment R5 may be due to the fact that different species of dinoflagellates have different requirements. Takahashi et al. (1982) put red tide organisms into three categories, micro-nutrient, macro-nutrient and intermediate type. *Gymnodinium* sp. belongs to the intermediate type. The results might be related to the migration of dinoflagellates as described by Taylor (1987). He pointed out dinoflagellates can migrate vertically and move down to a water layer where the nutrient concentration is suitable for growth, so that they can take up nutrients and may form a red tide.

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