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Effect of nitrogen to phosphorus ratios on cell proliferation in marine micro algae*

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Abstract The ratio of nitrogen/phosphorus (N/P) is known to affect cell proliferation of some marine micro algae. We evaluated the effect of N/P ratios on the proliferation and succession of phytoplankton using five marine micro algae species. We used two sources of nitrogen, NH_4Cl (N_1) and urea (N_2), and a single source of phosphorous, NaH_2PO_4(P). The optimal N/P ratio that differed among the five species was affected by the source of nitrogen, being as follows (N_1/P, N_2/P in order): *Thalassiosira* sp. (30/1, 20/1), *Heterosigma akashiwo* (30/1, 30/1), *Chroomonas salina* (20/1, 30/1), *Chaetoceros gracilis* (40/1, 60/1), and *Alexandrium* sp. (10/1, 30/1). Thus, the source of nitrogen must be considered when analyzing the N/P ratio. Our results provide insight for predicting phytoplankton succession in coastal waters and may be used to forecast the potential risk of harmful algal blooms.

Keyword: nitrogen; phosphorus; ratio; proliferation; micro alga

1 INTRODUCTION

It is well known that high levels of nitrogen and phosphorus are closely linked with harmful algae blooms (HABs) (Smith, 1984; Chen, 1993; Tang et al., 1993; Shen et al., 1996; Smayda, 2008). The magnitude of these blooms is influenced by both the concentration of nitrogen and phosphorus and the ratio of their occurrence (Antia, 1975; Hodgkiss and Ho, 1997; Sun et al., 2004). The C:N:P stoichiometry of phytoplankton varies with growth rate, nutrient and light limitation, species and phylum in large parts of the world ocean (Lenton and Klausmeier, 2007). The use of artificial and/or natural feeds during the intensive culture of aquatic organisms can result in rapid changes to the concentration of nutrients, particularly nitrogen and phosphorus, in the holding area. Such changes are likely to affect the proliferation and succession of phytoplankton in the water. Phytoplankton is an important part of the ecosystem, both as a source of natural feed and because of their effects on water quality (Lu et al., 1996, 1997; Wang et al., 2005).

Redfield (1934; 1958) noted the similarity between the average nitrogen-to-phosphorus ratio in plankton (N/P=16 by atoms) and in deep oceanic waters (N/P=15) (Klausmeier et al., 2004). However, many classic studies suggest that the N/P atom ratio can range between 17.4:1 and 5.5:1. For example, Antia et al. (1963) recorded ratios of between 16.5:1 and 13.5:1 in a study of coastal phytoplankton. McAllister et al. (1960) reported a ratio of 17:1 for an ocean micro alga population. *Phaeodactylum tricornutum* assimilates nitrogen and phosphorus at a ratio of 15:1 although this ratio varies if one of the nutrients becomes limiting. The result of model study (Klausmeier et al., 2004; 2008) predicts that optimal N/P ratios will vary from 8.2 to 45.0, depending on the ecological conditions. And the canonical Redfield N/P ratio of 16 is not a universal biochemical optimum, but instead represents an average of species-specific N/P ratios. Thus, ratios that deviate from 16:1 may represent a transition state, whereas over a longer period phytoplankton

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assimilate nitrogen and phosphorus at a ratio of 16:1. When considering nutrient reserves, the effect on micro algae growth is often of more interest than the instantaneous relative quantity of these two elements (Liu, 1982).

Because the assimilation of nitrogen and phosphorus reflects the Redfield ratio when both nutrients are abundant, the N/P ratio is often used as a standard to judge nutrient limitation (Li et al., 1993; Hong et al., 1994; Xu and Wu, 1995). For example, Darley (1982) suggested that a higher N/P ratio (e.g. >30) implied limitation of phosphorus, and lower N/P ratios (e.g. <5) implied limitation of nitrogen. Rhee (1978) found that the limiting nutrient changed temporally in micro algae that were cultured in a chemostat. N/P ratios of >30 reflected a limitation of phosphorus whereas ratios <30 reflected a limitation of nitrogen. In addition, a number of studies in China have suggested that the N/P ratio is correlated with the rate of cell proliferation in marine micro algae. The value of cellular N/P that corresponds to colimitation by N and P, the critical or optimum ratio, has been used to infer the competitive advantage of phytoplankton growing in P-impoverished systems. Increases as residual concentrations of nutrients increase to saturate transport kinetics oligotrophic waters require a lower nutrient N/P to avoid P limitation than do eutrophic waters (Flynn, 2002; 2010). On the other hand, the appropriate description of the control of the transport of the non-limiting nutrient is also important; in particular, a fixed algal N/P should not be assumed. And P-limitation may not develop even in high resource N/P situations due to light limitation (Flynn, 2008, 2010).

The preference for nitrogen and phosphorus varies among marine micro algae (Clark et al., 2002; Flynn, 2002). For example, dinoflagellates prefer lower N/P ratios whereas diatoms prefer a higher ratio (Hu et al., 2008). Thus, N/P ratios may also be used to study competition among red tide algae and the succession of phytoplankton. The N/P ratio may also be used to predict algal blooms and the associated risk. Wen et al. (2009a) constructed a relational model of the N/P ratio and of the time to HAB outbreak based on the growth velocity and cell density of micro algae. The danger levels were also set in this model for predicting and analyzing the magnitude of the HAB.

Despite the experimental evidence, it is often difficult to quantify nutrient limitation based on a fixed N/P ratio due to the complexity of phytoplankton community structure and the variation in N/P ratio optima among the different species of micro algae. In some instances, the N/P ratio reflects nitrogen limitation, however, the pattern of fluctuation of nitrogen and phosphorus suggests that phosphorus is often more directly limiting. This may be due to the replacement velocity of these two elements in water (Hong et al., 1993). Sun et al. (2004) concluded that most statistical analyses ignored the differences in the nutrient demands of micro algae during their growth period. However, these differences shape the outcome of competition among micro algae and maintain the basis of biodiversity.

We evaluated the effect of N/P ratios and nitrogen compounds on the cell proliferation of five marine micro algae. Our results may be used to predict the succession of phytoplankton in both natural and intensive culture settings.

2 MATERIAL AND METHOD

2.1 Algal species and culture conditions

We used the following strains of marine micro algae: *Chaetoceros gracilis*, *Heterosigma akashiwo*, *Thalassiosira* sp., *Alexandrium* sp., and *Chroomonas salina*. *Thalassiosira* sp. were purchased from the Institute of Oceanology, Chinese Academy of Sciences. The remaining species were obtained from the Ocean University of China.

The micro algae were cultured in f/2 culture medium with the addition of sodium silicate for the diatom species (Chen, 1995). The laboratory temperature was controlled using air-conditioning. The water temperature was maintained at 22±1°C; pH 7.8–8.2; salinity 29–30; illumination 34–40 μmol photons/(m^2·s); and the photoperiod was 14 h:10 h (light: dark). The experimental instruments and drugs were sterilized following standard methods (Chen, 1995).

We used sea water from the coastal area of Qingdao. The concentrations of nitrogen and phosphorus in the sea water were reduced using bio-methods (Li et al., 1998). The sea water was precipitated, filtered, and boiled then stored in glass bottles. Water that was stored for a long period of time was re-boiled prior to the experiment.

2.2 Experimental design

We used two nitrogen compounds, ammonium chloride (NH_4Cl) and urea ($CO(NH_2)_2$), and a single form of phosphorus NaH_2PO_4. The initial concentrations of these nutrients in each batch

Table 1 Initial concentrations of nutrients in each experiment

Nutrient salt	Levels					Note
	1	2	3	4	5	
N (μmol/L)	5	50	100	150	250	*Thalassiosira*
	50	100	200	300	400	Other algae
P (μmol/L)	0.5	1	5	10	15	All algae

culture are listed in Table 1. The concentrations were calculated based on the number of nitrogen or phosphorus atoms. We tested five concentrations of each nutrient, however, the initial concentrations of nitrogen differed for the five micro algae species and were based on preliminary tests and previously published reports. We used an orthogonal experimental table $L_{25}(5^6)$ to study the effects of nitrogen, phosphorus, and the interaction among these two elements. Nitrogen was laid in the first column of the orthogonal layout and phosphorus was laid in column 2. The remaining four columns were used to analyze their interaction. There were twenty-five controls in each experiment and two repetitions for each control.

We cultured the micro alga to the logarithmic phase in modified f/2 culture medium that contained no nitrogen and phosphorus. After 5 d culture, we transferred the appropriate amount of micro alga into a 5 L triangular flask and added f/2 culture media (without nitrogen and phosphorus). The mixture was then shaken gently to a uniform density and 100 mL was removed and placed in a 250 mL triangular flask. The nitrogen (NH_4Cl or urea) and phosphorus were transferred from their original solutions to the 250 mL triangular flask to serve as the control in the orthogonal layout (two repetitions for each control). The mixtures were then gently shaken prior to the start of the experiment.

The densities of the micro algae were counted microscopically every 2 d using a blood counting chamber. The experiments were conducted over ~15 d until the growth of the micro algae grew reached the stationary phase. The flasks were shaken twice each day during the early experimental period. The frequency of shaking increased as the density increased during the experiment. The experimental conditions were similar to the culture conditions. The growth velocities (μ) of micro algae were calculated using the follow formula:

$$\mu = (\lg N_t - \lg N_0)/(t \times \lg 2)$$

where t represents the number of days, N_t represents the density at t, and N_0 represents the initial density.

3 RESULT AND DISCUSSION

3.1 Optimal N/P ratios

The growth curves of micro algae were consistent with the growth mode of batch culture. Nitrogen and phosphorus had significant effects on the proliferation of all five micro algae species, and interaction existed between the nitrogen and phosphorus. The optimal N/P ratios are listed in Table 2. In addition, the optimal ratios derived from other studies are provided for comparison.

The majority of the optimal N/P ratios differed from the Redfield ratio (16:1) and covered a broad range, from 4:1 to 160:1. In general, the ratios derived in our study were somewhat higher than those in previous studies. These differences likely reflect the contribution of many factors, including both intrinsic and extrinsic. Regardless, our results may be divided into two key findings: 1) the optimal N/P ratio is species specific. Thus, a single ratio cannot be used to assess all micro algae species. Furthermore, nutrient limitation cannot be assessed using a single N/P ratio (Flynn, 2008). 2) The optimal N/P ratio is dependant on the form of nitrogen, a fact that has largely been overlooked. Thus, the Redfield ratio may only reflect a general state or the ultimate result of biochemical reactions. The ability to transform and utilize nitrogen varies among micro algae resulting in differences in algal demands for N/P ratio in coastal waters (Zhang and Zou, 1997; Atsushi, 1999; Zhang et al., 2002).

3.2 Relationship between micro algae species, nitrogen compounds, and the N/P ratio

The relationship between the N/P ratio, the species of micro algae, and the different nitrogen compounds is illustrated in Fig.1.

Pyrrophyta and Bacillariophyta species are the primary contributors to HABs in coastal waters, so are represented more often in the data than other species. Pyrrophyta species typically preferred lower N/P ratios (<20:1) whereas Bacillariophyta species preferred a higher N/P ratio (>20:1). The optimal N/P ratios of the remaining micro algae were generally between 16:1 and 30:1. These ratios are consistent with previous studies (Chen, 1990). In situ surveys have revealed fluctuations in the N/P ratio that affect phytoplankton density and succession (Hong et al., 1993; Tang et al., 1993). Thus, we may

Table 2 Optimal ratio of N to P for marine micro algae proliferation

Algae species	N/P ratio	Nitrogen source*	Reference
Phaeocystis globosa	11:1, 33:1	NO_3	Cai et al., 2009
Alexandrium catenella	15–30:1	DN	Hodgkiss and Ho, 1997
Ceratium furca	12–22:1	DN	Hodgkiss and Ho, 1997
Gonyaulax polygramma	4–8:1	DN	Hodgkiss and Ho, 1997
Gymnodinium nagasakiense	11–16:1	DN	Hodgkiss and Ho, 1997
Noctiluca scintillans	8–14:1	DN	Hodgkiss and Ho, 1997
Prorocentrum dentatum	6–13:1	DN	Hodgkiss and Ho, 1997
Prorocentrum minimum	4–13:1	DN	Hodgkiss and Ho, 1997
Prorocentrum sigmoides	4–15:1	DN	Hodgkiss and Ho, 1997
Prorocentrum triestinum	8–15:1	DN	Hodgkiss and Ho, 1997
Scrippsiella trochoidea	6–13:1	DN	Hodgkiss and Ho, 1997
Skeletonema costatum	15–30:1	DN	Hodgkiss and Ho, 1997
Phaeocystis globosa	30:1	NO_3	Hu et al., 2008
Pseudo-nitzschia pungens	10:1	NO_3	Hu et al., 2008
Prorocentrum donghaiense	30:1, 40:1	NO_3	Hu et al., 2008; Zhou, 2009
Heterosigma akashiwo	25:1, 50:1	NO_3	Jiang et al., 2006; Zhou, 2009
Gymnodinium sp.	6:1	NO_3	Kang et al., 2006
Thalassiosira pseudonana	16:1	NO_3	Kang et al., 2006
Chlorella pyrenoidosa	24:1	NH_4NO_3	Liang et al., 2008
Cryptomonaserosa	24:1	NH_4NO_3	Liang et al., 2008
Niztzschia closterium	24:1	NH_4NO_3	Liang et al., 2008
nnichloropsis oculata	24:1	NH_4NO_3	Liang et al., 2008
Isochrysis galbana	16:1	NO_3	Liu et al., 2002a
Skeletonema costatum	16:1	NO_3	Liu et al., 2002b
Prorocentrum donghaiense	12:1	NO_3	Lü et al., 2006
Prorocentrum donghaiense	8:1	NH_4Cl	Lü et al., 2006
Prorocentrum donghaiense	16:1	Urea	Lü et al., 2006; Zhou, 2009
Cylindrotheca closterium	160:1	NO_3	Sun et al., 2004
Karenia mikimotoi	80:1	NO_3	Sun et al., 2004
Platymonas helgolandica	4:1	NO_3	Sun et al., 2004
Skeletonema costatum	31.7:1, 37.4:1	NO_3	Wen et al., 2009b
Isochrysis sp.	15:1	NH_4	Zhang et al., 2002
Isochrysis sp.	50:1	Urea	Zhang et al., 2002
Alexandrium sp.	10:1	NH_4	This study
Alexandrium sp.	30:1	Urea	This study
Chaetoceros gracilis	40:1	NH_4	This study
Chaetoceros gracilis	60:1	Urea	This study
Chroomonas salina	20:1	NH_4	This study
Chroomonas salina	30:1	Urea	This study
Heterosigma akashiwo	30:1	NH_4	This study
Heterosigma akashiwo	30:1	Urea	This study
Thalassiosira sp.	20:1	Urea	This study
Thalassiosira sp.	30:1	NH_4	This study

*DN: dissolved nitrogen

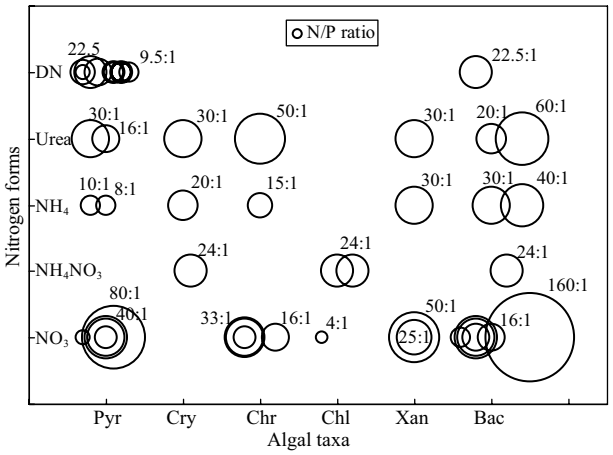

Fig.1 Relationship analysis of algae, nitrogen forms, and N/P ratios

Pyr: Pyrrophyta; Cry: Cryptophyta; Chr: Chrysophyta; Chl: Chlorophyta; Xan: Xanthophyta; and Bac: Bacillariophyta. DN: dissolved nitrogen. The diameter of the circle represents the magnitude of the N/P ratio

be able to predict bloom types and risk levels using real time monitoring of nitrogen and phosphorus.

The majority of previous studies have used nitrate nitrogen. We found that the form of nitrogen had a significant effect on the optimal ratio of N/P. The N/P ratios were low when using ammonium chloride or dissolved nitrogen but high when using organic nitrogen. This difference was especially obvious among the Cryptophyta, Chrysophyta, and Bacillariophyta species. We hypothesize that this is due to differences in the ability of each species to assimilate the varying forms of nitrogen. The optimal N/P ratios for NH_4NO_3 were typically intermediate between those for NO_3 and NH_4.

Several studies have shown that ammonia nitrogen is most suitable for micro algae proliferation as it requires less energy for assimilation. Conversely, organic nitrogen must be transformed before it can be even be assimilated by some micro algae (McCarthy, 1972; Chen, 1987; Bricelj and Lonsdale, 1997). This is consistent with our analysis of the relationship between the different forms of nitrogen and the optimal N/P ratio. The N/P ratios were typically higher for urea than for ammonium chloride suggesting that these algae were less able to assimilate urea. In contrast, little is known about the influence of different forms of phosphorus on marine micro algae proliferation. We did not investigate this in the current study; however, in a previous study the phosphorus source had a significant effect on the optimal N/P ratio for cyanobacteria in fresh water. Thus, the sources of nitrogen and phosphorus must be paid noted when analyzing the N/P ratios.

A number of studies support the hypothesis that ammonia nitrogen is more easily assimilated by phytoplankton than other forms of nitrogen. However, the actual utilization of ammonia is affected by environmental factors (Hong et al., 1992; Li et al., 1993; Qi et al., 1993; Jiao, 1995; Zhang and Zou, 1997). After surveying the proportion of inorganic nitrogen in the water during the red tide period, Hong et al. (1993) concluded that the red tide phytoplankton were relying primarily on nitrate nitrogen for their proliferation. Interestingly, Lin (1988) reported that although micro algae used ammonia nitrogen during the summer, nitrate nitrogen was more important during the spring. These fluctuations in the availability of nitrogen compounds in situ are almost certain to have affected previous analyses of optimal N/P ratios. Thus, it is imperative that attention be paid to measuring the ratio of the different forms of nitrogen, not just total nitrogen and phosphorus.

3.3 Use of the N/P ratio

Our study results and the Redfield ratio are not necessarily in conflict. The former represents the results of surveys of the water environment whereas the latter represents the cell components. Many authors have agreed that, over a long period, phytoplankton assimilate nitrogen and phosphorus at a ratio that is close to 16:1. Deviation from this ratio in coastal waters indicates nitrogen limitation or phosphorus limitation. Hodgkiss and Ho (1997) reported that the effects of nutrient ratios (e.g. N/P and N/Si) were much higher than that of Liebig's law of minimum, particularly for algal selection. Hence, many researchers use the N/P ratio to analyze the nutrient limitation or predict the occurrence of HABs. Given this, we did not expect that the standard used to judge nutrient limitation would be equal to the Redfield ratio.

Our results suggest that the N/P ratio may be used for predicting algal succession based on differences in the optimal N/P ratio among micro algae species. Despite the influence of N/P ratios on the occurrence of HABs, the influence of multiple factors means that it is likely not practical to prevent their occurrence by artificially manipulating the ratio of N/P. For example, Hu et al. (2008) reported that N/P ratios may only be used to judge nutrient limitation in certain environmental conditions. The influence of the N/P ratio was lower than that of the different salts and was also affected by a range of other factors. Furthermore, manipulating the N/P ratio of coastal waters by adding nitrogen or phosphorus

is likely to increase water pollution and it is not currently economical to remove nitrogen or phosphorus from such large areas.

4 CONCLUSION

1) The optimal N/P ratio ranged from 10:1 to 60:1 and was dependent on the species and form of nitrogen. Thus, monitoring programs for phytoplankton abundance should also account for the different forms of nitrogen when measuring total nitrogen concentrations.

2) In general, Pyrrophyta species preferred lower N/P ratios (<20:1) whereas Bacillariophyta species preferred higher N/P ratio (>20:1). The optimal N/P ratios of the remaining micro algae species generally ranged between 16:1 and 30:1. The optimal N/P ratio was lowest for ammonium chloride or dissolved nitrogen and highest for organic nitrogen. This may reflect the ability of these algae species to assimilate the different forms of nitrogen. The factors affecting assimilation and the differences among a wider range of species deserves further attention.

3) N/P ratios are useful for predicting algal succession and may be used. We hypothesize that it may be possible to control succession by controlling the input of nitrogen and phosphorous in closed or semi-closed culture systems, thus promoting the proliferation of advantageous algae while inhibiting disadvantageous algae.

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References

Antia N J. 1975. Comparative evaluation of certain organic and inorganic sources of nitrogen for phototrophic growth of marine microalgae. *J. Mar. Biol. Ass. U.K.*, **55**: 519-539.

Antia N J, McAllister C D, Parsons T R, Stephens K, Strickland J D H. 1963. Further measurements of primary production using a large-volume plastic sphere. *Limnol. Oceanogr.*, **8**(2): 166-183.

Atsushi M. 1999. Effects of nitrogenous and phosphorus nutrients on the growth of toxic dinoflagellate *Alexandrium catenella*. *Nippon Suisan Gakkaishi*, **65**(5): 847-885.

Bricelj V M, Lonsdale D J. 1997. *Aureococcus anophagefference*: Cause and ecological consequences of brown tides in U.S. mid-Atlantic coastal waters. *Limnol. Oceanogr.*, **42**(5.Part 2): 1 023-1 038.

Cai Z P, Huang W, Duan S. 2009. Cell proliferation of *Phaeocystis globasa*, a red tide causative marine micro alga in various phosphorus (P)-and nitrogen (N)-replete conditions. *Ecol. and Environ. Sci.*, **18**(2): 447-452. (in Chinese with English abstract)

Chen C M, Bao J J, Wu Y D. 1990. Relations between nutrition form and content level and phytoplankton proliferation competition in sea area accepting sewage (I) effects of phosphorus. *Mar. Environ. Sci.*, **9**(1): 6-12. (in Chinese with English abstract)

Chen M Y. 1995. Culture of Biological food. Chinese Agriculture Press, Beijing, China. p.25-92. (in Chinese)

Chen S T. 1993. Relation between inorganic nitrogen and phosphorus in Jiulongjiang estuary and western Xiamen harbour. *Mar. Sci. Bull.*, **12**(5): 26-32. (in Chinese with English abstract)

Chen Y W, Chen C M, Huang J. 1987. Comprehensive effect of nutrients and environmental factors on the growth of three kinds of micro algae. *Mar. Environ. Sci.*, **6**(4): 20-26. (in Chinese with English abstract)

Clark D R, Flynn K J, Owens N J P. 2002. The large capacity for dark nitrate-assimilation in diatoms may overcome nitrate limitation of growth. *New Phytologist*, **155**(1): 101-108.

Darley W M. 1982. Algal biology: a physiological approach. Black-well Scientific Publications, Oxford London. p.168.

Flynn K J. 2002. How critical is the critical N:P ratio? *J. Phycology*, **38**(5): 961-970.

Flynn K J. 2008. The importance of the form of the quota curve and control of non-limiting nutrient transport in phytoplankton models. *J. Plankton Res.*, **30**(4): 423-438.

Flynn K J. 2010. Do external resource ratios matter? Implications for modelling eutrophication events and controlling harmful algal blooms. *J. Mar. Systems*, **83** (3-4): 170-180.

Hodgkiss I J, Ho K C, 1997. Are changes in N:P ratios in coastal waters the key to increased red tide blooms? *Hydrobiologia*, **352**(1-3): 141-147.

Hong J C, Huang X Q, Jiang X S. 1992. Red tides phenomenon of *Skeletonema costaum* in Yangtze estuary. *Mar. Environ. Sci.*, **11**(3): 75-79. (in Chinese with English abstract)

Hong J C, Huang X Q, Jiang X S. 1993. The dominant factor analysis of red tides of *Skeletonema costaum* occurring process in Chengsi sea area. *Acta Oceanologica Sinica*, **15**(6): 135-141. (in Chinese with English abstract)

Hong J C, Huang X Q, Jiang XS, Wang G L. 1994. Analysis of environmental factors during occurrence of *Skeletonema costaum* red tide in Changjiang river estuary—the nutrient state. *Oceanologia et Limnologia Sinica*, **25**(2): 179-184. (in Chinese with English abstract)

Hu Z X, Xu N, Li A F, Duan S S. 2008. Effects of different N:P ratios on the growth of *Pseudo-nitzschia pungens*, *Prorocentrum donghaiense* and *Phaeocystis globosa*. *Acta Hydrobiologica Sinica*, **32**(4): 482-487. (in Chinese with English abstract)

Jiang Y, Gan X H, Tang Y X, Chen X L, Chen Y R. 2006. Effects of nutrients nitrogen and phosphorus on *Heterosigma akashiwo* growth. *Chinese J. of Appl. Ecol.*, **17**(3): 557-559. (in Chinese with English abstract)

Jiao N Z. 1995. Marine microplankton nitrogen uptake kinetics with special reference to size-fractionation.

Oceanologia et Limnologia Sinica, **26**(2): 191-198. (in Chinese with English abstract)

Kang Y Y, Liang J R, Gao Y H, Lin R C, Gao H. 2006. Influence of N/P ratio on the growth and competition of two red tide algae. *Acta Oceanologica Sinica*, **28**(5): 117-122. (in Chinese with English abstract)

Klausmeier C A, Litchman E, Daufresne T, Levin S A. 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature*, **429**(6 988): 171-174.

Klausmeier C A, Litchman E, Daufresne T, Levin S A. 2008. Phytoplankton stoichiometry. *Ecol. Res*., **23**(3): 479-485.

Lenton T M, Klausmeier C A. 2007. Biotic stoichiometric controls on the deep ocean N:P ratio. *Biogeosciences*, **4**(3): 353-367.

Li W Q, Zheng A R, Li S Y. 1993. Studies on the relationship between seawater culture and ecological environment (I) the effect of inorganic nitrogen on the growth of phytoplankton. *J. of Tropical Oceanog*., **12**(3): 46-51. (in Chinese with English abstract)

Li X C, Zhang G C, Cui Y A, Lei Y Z. 1998. Static purification of *Ulva pertusa* kjellman for abalone effluents. *Transactions of the Chinese Society of Agricultural Engineering*, **14**(1): 173-176. (in Chinese with English abstract)

Liang W F, Li Z J, Chen S W, Wen G L, Cao Y C. 2008. Niche characteristics of microalgae community in N/P ratio and silicon concentration. *J. of Agro-environment Science*, **27**(2): 773-777. (in Chinese with English abstract)

Lin Q F. 1988. Red tides investigation and study abroad. *Mar. Environ. Sci*., **7**(1): 26-33. (in Chinese with English abstract)

Liu D Y, Sun J, Chen Z T, Wei T D. 2002b. Effect of N/P ratio on the growth of a red tide diatom skeletonema costatum. *Transaction of Oceanol. and Limnol*., **2**: 39-44. (in Chinese with English abstract)

Liu D Y, Sun J, Gong J, Qian S B. 2002a. The effects of different N/P ratios on the growth of *Isochrysis galbana*. *Mar. Fisherries Res*., **23**(1): 29-33. (in Chinese with English abstract)

Liu G. (trans.). 1982. Riley J P, Skirrow G eds. Chemical oceanography (2nd edition). Ocean Press, Beijing, China. p.283-320. (in Chinese)

Lu J R, Li D S, Xu N et al. 1997. Effects of different fertilizer on the phytoplankton community. *App. Env. Bio. Tran*., **3**: 172-176. (in Chinese with English abstract)

Lu J R, Li D S, Yang H S, Xu N, Zhang H Y. 1996. Prelimnary studies of phytoplankton in enclosed Tilapia-rearing ecosystems in seawater ponds in Laizhou Bay. *J. of Fishery Sci. of China*, **3**(1): 176-183. (in Chinese with English abstract)

Lü S H, Ou M S. 2006. Effects of different nitrogen sources and N/P ratios on the growth of a marine dinoflagellate *Prorocentrum donghaiense*. *Mar. Environ. Sci*., **25**(2): 33-36. (in Chinese with English abstract)

McAllister C D, Parsons T R, Strickland J D H. 1960. Primary productivity and fertility at station "P" in the North-East Pacific Ocean. *J. Cons. int. Explor. Mer*., **25**: 240-259.

McCarthy J J. 1972. The uptake of urea by natural population of marine phytoplankton. *Limnol. Oceanogr*., **17**: 738-748.

Qi Y Z, Chu J H, Huang Y H. 1993. Environmental element impact on triggering off chattonella marina bloom. *Mar. Sci. Bull*., **12**(2): 30-34. (in Chinese with English abstract)

Redfield A C. 1934. *In*: Daniel R J ed. "James Johnston Memorial Volume". University of Liverpool Press, Liverpool, U.K. p.176-192.

Redfield A C. 1958. The biological control of chemical factors in the environment. *Am. Sci*., **46**: 205-222.

Rhee G Y. 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition and nitrate uptake. *Limnol. Oceanogr*., **23**: 10-25.

Shen G Y, Shi B Z. 1996. Marine Ecology (2nd edn). Xiamen University Press, Xiamen, China. p.237-247. (in Chinese)

Smayda T J. 2008. Complexity in the eutrophication-harmful algal bloom relationship, with comment on the importance of grazing. *Harmful Algae*, **8**(1): 140-151.

Smith S V. 1984. Phosphorus versus nitrogen limitation in the marine environment. *Limnol. Oceanogr*., **29**(6): 1 149-1 160.

Sun J, Liu D Y, Chen Z T, Wei T D. 2004. Growth of *Platymonas helgolandica var. tsingtaoensis*, *Cylindrotheca closterium* and *Karenia mikimotoi* and their survival strategies under different N/P ratios. *Chinese J. of App. Ecol*., **15**(11): 2 122-2 126. (in Chinese with English abstract)

Tang S M, Zhuang D F, Lin Y. 1993. Disturbance of eutrophication to the nutrient structure in enclosed ecosystem. *Acta Oceanologica Sinica*, **15**(2): 135-139. (in Chinese with English abstract)

Wang H L, ZhaoW, Li X D, Liu X. 2005. Effect of different N/P ratios of fertilizer on phytoplankton community succession in polyhaline water ponds. *J. of Dalian Fisheries University*, **20**(3): 238-243. (in Chinese with English abstract)

Wen S Y, Zhao D Z, Zhao L et al. 2009a. Study on hazard degree assessment method of harmful algal blooms disaster based on N/P ratios. *Acta Scientiarum Naturalium Universitatis Sunyatseni*, **48**(2): 84-89. (in Chinese with English abstract)

Wen S Y, Zhao D Z, Zhao L, Yang J H, Zhang F, Wang L, Gao S G, Sun D. 2009b. Tolerance response model of N/P ratios for red tide algae. *J. of Dalian Maritime University*, **35**(1): 118-122. (in Chinese with English abstract)

Xu L, Wu Y D. 1995. Effect of organic nitrogen on the growth of marine phytoplankton. *J. of Xiamen University* (*Natural Science Edition*), **34**(5): 824-828. (in Chinese with English abstract)

Zhang C, Zou J Z. 1997. Nutrient uptake kinetics and growth under nutrient limitation of pseudonitzschia. *Oceanologia et Limnologia Sinica*, **28**(6): 599-603. (in Chinese with English abstract)

Zhang Q T, Zhang Z Q, Dong S L. 2002. Effects of nitrogen and phosphorus on the growth of microalgae *Isochrysis*. *Transaction of Oceano. and Limnol*., **2**: 45-51. (in Chinese with English abstract)

Zhou G. 2009. Influence of N/P ratio on the growth of two red tide algae. *J. of West Anhui University*, **25**(2): 79-81. (in Chinese with English abstract)