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The relationship between light intensity and nutrient uptake kinetics in six freshwater diatoms

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ABSTRACT

In order to find effective measures to control diatom blooms, a better understanding of the physiological characteristics of nutrient uptake in diatoms is needed. A study of P and Si-uptake kinetics for diatom species from two light regimes was conducted at low (LL), moderate (ML) and high light intensities (HL) (2, 25 and 80 μmol photons/(m^2·sec)), respectively. The results showed that P uptake of diatoms was heavily influenced by historic light regimes. P affinity changed with growth and photosynthetic activity. The lowest half saturation constant for P uptake ($K_{m(P)}$) was under HL for high-light adapted diatoms while the lowest half-saturation constant for low-light adapted diatoms was observed under LL. The Si half-saturation constant ($K_{m(Si)}$) increased with increasing light intensities for pennate diatoms but decreased for centric diatoms. Diatom volumes were correlated with the maximum Si uptake rates ($V_{m(Si)}$) at HL and $K_{m(Si)}$ at ML and HL for six diatom species. Our results imply that when we assess the development of diatom blooms we should consider light intensity and cell volume in addition to ambient Si or P concentration. The relationship between light intensity and P-uptake suggests that we can find suitable methods to control diatom blooms on the basis of reducing phytoplankton activity of P-uptake and photosynthesis simultaneously.

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Introduction

Building dams on the rivers not only impact on the hydrological conditions but also bring out the trapping effect of nutrient retention in reservoirs. In Australia, the retention of phosphorus by Chaffey Dam was in the range of 16%–95% (Sherman et al., 2001). The dissolved silicon (DSi) concentration in the lower reaches of the Danube River dropped more than 50% since the IornGate Dam had been built in 1970 (Jickells, 1998). The DSi concentration dropped from 210 to 10 μmol/L in the Nile River after the construction of the Aswan High Dam (Wahby and Bishara, 1980). Zeng (2006) pointed out that the large amounts of phosphate and silicate were trapped after building the Three Gorges Dam, which causes the chain reactions, especially decrease of nutrient concentration, occurred in the lower Yangtze River. After the water storage of the Three Gorges Reservoir, the decrease of DSi concentration in the Yangtze River estuary was about six times higher than before and the blocking effect of the Three Gorges Dam led to the retention rate of total phosphorus up to 80% (Yu et

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al., 2006). Although Si and P load decreased dramatically in the lower reaches, diatom blooms still broke out frequently in the middle–lower reaches of the Yangtze River after building the Three Gorges Dam (*e.g.*, Wang et al., 2011; Liang et al., 2012).

Nutrient uptake in algae can be influenced by light (Chisholm and Stross, 1976), temperature (Agawin et al., 2000), cell size (Stolte and Riegman, 1995), nutritional status of algal cells (Ritchie et al., 2001), pH (Thoreson et al., 1984) and other factors. In oligotrophic ecosystems, some species with highly efficient Si or P-uptake systems can develop diatom biomass with optimum conditions (Krause et al., 2012). Light intensity might be considered the important factor because photosynthesis provides energy for active absorption of nutrients (Deane and O'Brien, 1981).

Diatoms from distinct light regimes can have dramatic differences in light adaptation (Gameiro et al., 2011; Kropuenske et al., 2009). In this study, we hypothesized that light intensity has different effects on uptake of Si and P in diatoms isolated from various light regimes. We conducted nutrient kinetics experiments to explore: (1) whether light intensity has an obvious effect on Si or P uptake in diatoms isolated from two different light regimes; (2) if the optimal light intensity for nutrient uptake is the same as that for growth of diatoms and (3) which factors are most critical in controlling nutrient uptake rates in diatoms? The results will help to assess and predict the growth and development of phytoplankton blooms and select suitable measures for controlling diatom blooms.

1. Materials and methods

1.1. Diatoms isolation and culture

Six species of diatoms were isolated from two freshwater ecosystems: Lake Taihu (30–31°N, 119–120°E) and Hangjiang River (30–34°N, 106–114°E). According to the underwater light intensities in these ecosystems and chlorophyll *a* fluorescence in the six diatoms species measured in preliminary experiment, 3 species (*Cyclotella meneghiniana*, *Stephanodiscus parvus* and *Synedra acus*) from Lake Taihu were defined as high-light adapted (HLA) species and 3 species (*Stephanodiscus hantzschii*, *Fragilaria crotonensis* and *Nitzschia palea*) from Hanjiang River were defined as low-light adapted (LLA) species. *C. meneghiniana* was the dominant species in Lake Taihu while *S. hantzschii* is the dominant species in the Hanjiang River during spring. Sub-dominant species found in the Lake Taihu were *S. parvus* and *S. acus* while in the Hanjiang River were *F. crotonensis* and *N. palea*. The information of diatom stains and two field sites are listed in Table 1.

The six species of diatoms were cultured at eight light intensities (from 2 to 300 μmol photons/(m^2·sec)) in the preliminary experiment. Diatoms from the Hanjiang River showed high photosynthetic activities and growth rates at light intensities less than 25 μmol photons/(m^2·sec) but showed photoinhibition at light intensity of 80 μmol photons/(m^2·sec). Growth rates of diatoms from Lake Taihu increased with higher light intensities but showed light-limited growth rates at an intensity of 2 μmol photons/(m^2·sec).

The isolation and purification of diatoms were conducted according to standard phycological methods (Stein and Hellebust, 1980). Concentrated diatom cells were observed with a scanning electron microscope (JSM-6390LV, NTC, Japan) after sulfuric acid treatment (Zhang and Huang, 1991). Identification of algal species was based on the pattern and shape of the shell of the diatom (Hu and Wei, 2006). Monoclonal diatoms of six strains were cultured in D1 medium (Zhang and Huang, 1991).

Stock cultures were transferred weekly to fresh medium to maintain exponential growth. All glassware was soaked in 5% HCl for 24 hr and rinsed with double-distilled water before use. Flasks and D1 medium were sterilized at 121°C for 30 min. All treatments were kept at identical conditions and were conducted in triplicate. Each step was carried out under axenic conditions.

1.2. Growth experiment

Growth experiments were conducted in illumination incubators with 20°C/18°C temperature regimes and 14/10 light/dark cycle. The light treatment was set at 2, 12.5, 25, 60 and 80 μmol photons/(m^2·sec) with cool-white fluorescent light. We used 80 μmol photons/(m^2·sec)[1] as the highest light treatment because the light treatment of 80 μmol photons/(m^2·sec) was enough to cause photoinhibition to LLA diatoms from the results of the preliminary experiment.

Table 1 – Dominant species volume and environmental concentration of total phosphorus (TP–P) and dissolved silicon (DSi–Si) in sampling sites where diatom isolated.

Dominate species	Average volume (μm^3)	Aquatic ecosystems	Light intensity (μmol photons/(m^2·sec))	TP–P (μmol/L)	DSi–Si (μmol/L)
Cyclotella meneghiniana	144.32	Lake Taihu	564.3	3.37 ± 1.31	88.01 ± 5.52
Stephanodiscus parvus	320.713	Lake Taihu	564.3	3.37 ± 1.31	88.01 ± 5.52
Synedra acus	2161.03	Lake Taihu	564.3	3.37 ± 1.31	88.01 ± 5.52
Stephanodiscus hantzschii	930.14	Hanjiang River	85.7	2.49 ± 0.23	71.97 ± 9.34
Fragilaria crotonensis	1558.59	Hanjiang River	85.7	2.49 ± 0.23	71.97 ± 9.34
Nitzschia palea	1058.28	Hanjiang River	85.7	2.49 ± 0.23	71.97 ± 9.34

Light intensity indicated underwater light intensity at 0.5 m depth. Light intensity of Lake Taihu was cited from Zhang et al., 2004. Light intensity of Hanjiang River was measured in spring of 2010 using illuminometer (wi97178, Ruida, China).

The specific growth rates of diatoms were calculated from cell count using counting chambers under an Olympus CX21 light microscope. The specific growth rate (μ, day^{-1}) of each species was calculated by Eq. (1).

$$\mu = [\ln B_{t2} - \ln B_{t1}]/\Delta t \quad (1)$$

where, B_{t1} (cells/L) is the initial cell density, and B_{t2} (cell/L) is the cell density on the last day of the logarithmic growth phase.

The effective quantum yield of PS II ($\Delta F/F'_m$) was determined by the quenching method (Schreiber et al., 1994). $\Delta F/F'_m$ values were calculated based on Eq. (2).

$$\Delta F/F'_m = [F'_m - F_s]/F'_m \quad (2)$$

where, F_s (dimensionless) is the minimal fluorescence at actinic light, and F'_m (dimensionless) is the maximum fluorescence after saturating light pulses. The effective quantum yield ($\Delta F/F'_m$) is closely related to the CO_2 assimilation rate that can affect the capacity to convert solar energy into dry material (Krause et al., 1995).

1.3. Nutrient uptake

Si or P-depleted cultures were transferred to darkness for 1 day before the experiment. Then they were transferred into three preconditioning cultures at three different light intensities (LL: 2 μmol photons/(m^2·sec); ML: 25 μmol photons/(m^2·sec); HL: 80 μmol photons/(m^2·sec)) for 5 hr. Aliquots of the concentrated cells were then dispensed into ten flasks containing 80 mL of D1 medium with rising concentrations of nutrient addition (Si: 0.5, 1, 2, 4, 8, 16, 32, 48, 64 and 80 mol/L; P: 1.3, 3.2, 6.4, 9.6, 19.2, 32.0, 64.0, 96.0, 128.0 and 160.0 mol/L) with corresponding light treatments. 5 mL samples were collected and filtered through GF/C filter membranes (Whatman, Maidstone, UK) to measure the decrease in concentration of Si or P in the modified D1 medium. Sample measurements were obtained at 0, 1, 2 and 3 hr during the experiments (Shen and Song, 2007). P concentrations were determined using the colorimetric method of Murphy and Riley (1962). Si concentrations were determined according to Strickland and Parsons (1968).

The maximal uptake rate (V_m) and the half saturation constant (K_m) were determined by fitting the Michaelis–Menten equation (De La Rocha et al., 2000; Bhattacharyya and Volcani, 1980; Leynaert et al., 2009) to the experimental results. The uptake rate (V) was determined by the disappearance of nutrient concentration in each subculture (Eq. (3)).

$$V = \frac{V_m[S]}{K_m + [S]} \quad (3)$$

where, V_m represents the maximum uptake rate and K_m represents the half-saturation constant for uptake defined as the substrate concentration where $V = 1/2\ V_m$. S is concentration of substrate (P/Si).

1.4. Statistical analysis

Origin 8.0 (Microcal Software, Northampton, MA, USA) was used for graphical analyses and SPSS 16.0 (Windows software, Inc., Chicago, IL, USA) was used for statistical analyses. Differences in specific growth rate between LLA and HLA diatoms at five light intensities and differences in uptake kinetics parameters between centric and pennate diatoms among three light intensities in each species were examined by one-way ANOVA followed by Tukey post-hoc tests. Probability values <0.05 were considered statistically significant. The data are presented as mean ± standard deviation. Correlation analysis was used to quantify relationships between algal uptake kinetics parameters and cell characteristics (volume and cell quota).

2. Results

2.1. Specific growth rate

The specific growth rates of all HLA diatoms (*C. meneghiniana*, *S. parvus* and *S. acus*) increased with higher light intensities but a significant decrease was found for LLA diatoms (*S. hantzschii*, *F. crotonensis* and *N. palea*) at light intensity above 60 μmol photons/(m^2·sec) (Fig. 1). The average growth rates of LLA diatoms were significantly higher than that of HLA diatoms at light intensities of 12.5 and 25 μmol photons/(m^2·sec) ($P < 0.05$). The maximum average growth rates of HLA diatoms at 80 μmol photons/(m^2·sec) was 1.69 times ($F = 8.181$, $P = 0.029$) greater than the average growth rates for LLA diatoms. The optimum light intensity for growth in LLA diatoms was approximately 25 μmol photons/(m^2·sec) and for HLA diatoms the optimum was approximately 80 μmol photons/(m^2·sec).

2.2. Phosphorus uptake

We found significant differences among light treatments in $K_{m(P)}$ and $V_{m(P)}$ values ($P < 0.05$) for all diatoms except *N. palea* and *S. parvus* ($P > 0.05$). Moreover, some differences depending on habitat light regime were found (Table 2). HLA diatoms

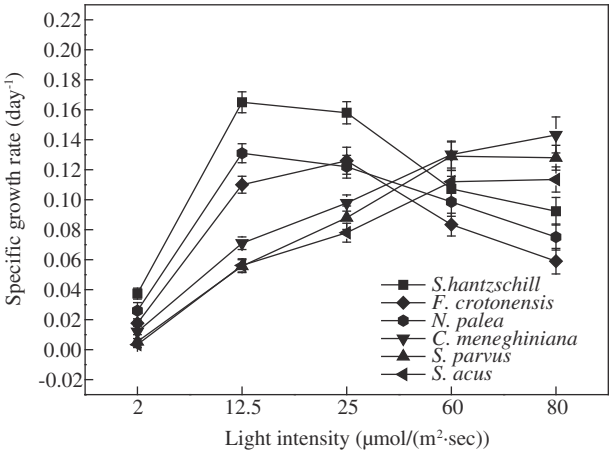

Fig. 1 – Specific growth rates of 3 LLA diatoms and 3 HLA diatoms under five light intensities. LLA diatoms: *S. hantzschii*, *F. crotonensis* and *N. palea*; HLA diatoms: *C. meneghiniana*, *S. parvus* and *S. acus*.

showed a decreasing trend in K_m values with higher light intensity. HLA diatoms had the highest P uptake affinity along with the highest activity of growth and photosynthetic at HL but the lowest P uptake affinity along with the lowest activity of growth and photosynthetic at LL (Figs. 1 and 2a). In contrast, $K_{m(P)}$ values of LLA diatoms were higher with increasing light intensities. The specific growth rates and photosynthetic activities of LLA diatoms were significantly lower at HL (Figs. 1 and 2a).

The P cell quotas ($Q_{(P)}$) for the six species were calculated at the beginning of the uptake experiment (Table 2). $Q_{(P)}$ was significantly correlated with $V_{m(P)}$ at LL ($r = 0.918$, $P < 0.05$), ML ($r = 0.946$, $P < 0.05$) and HL ($r = 0.963$, $P < 0.001$). Diatom biovolume was not correlated with $V_{m(P)}$ and $K_{m(P)}$.

2.3. Silicon uptake

There were considerable differences among light treatments in $K_{m(Si)}$ and $V_{m(Si)}$ values in all six diatoms ($P < 0.05$). All centric diatoms in the present study showed a uniform trend in $K_{m(Si)}$ values which decreased with higher light intensity while $K_{m(Si)}$ values for all pennate diatoms increased with higher light intensity (Table 3). Centric diatoms had their highest values of $V_{m(Si)}$ at ML except *C. meneghiniana* whose highest $V_{m(Si)}$ occurred at HL. For pennate diatoms, $V_{m(Si)}$ increased with the higher light intensities and the highest values of V_m were found at HL. Si cell quotas ($Q_{(Si)}$) were also calculated at the beginning of the uptake experiment (Table 3). $Q_{(Si)}$ was not correlated with $V_{m(Si)}$ and $K_{m(Si)}$.

The results from the correlation analysis showed that the biovolume of diatoms was significantly correlated with $K_{m(Si)}$ at ML ($r = 0.795$, $P < 0.05$) and HL ($r = 0.827$, $P < 0.05$). The biovolumes of the diatoms were significantly correlated with $V_{m(Si)}$ at HL ($r = 0.829$, $P < 0.05$). All $K_{m(Si)}$ values of all species at three light intensities were significantly different between centric diatoms and pennate diatoms (Fig. 3). The $K_{m(Si)}$ values of centric diatoms were significantly lower than that of pennate diatoms at ML ($F = 36.85$, $P < 0.05$) and HL ($F = 101.743$, $P < 0.001$). The $V_{m(Si)}$ values of centric diatoms were significantly lower than that of pennate diatoms at all three light treatments ($P < 0.05$) (Table 3).

3. Discussion

3.1. Phosphorus uptake

K_m values reflect nutrient uptake affinity (Leynaert et al., 2004). The differences in $K_{m(P)}$ values among three light intensities and the opposite tendencies of $K_{m(P)}$ values between HLA and LLA diatoms indicated that historic light regimes play an important role in P uptake. The effects of light intensity on P uptake were also studied in other algae (*e.g.*, Feijoó et al., 2008; Hessen et al., 2002). The P uptake rate of zooxanthellae increased with light intensity (from 0 to 360 μmol photons/(m^2·sec)), suggesting a positive correlation between P uptake and photosynthetic activity in zooxanthellae (Godinot et al., 2009). The alkaline phosphatase activity and P uptake kinetics of algae (*Dunaliella tertiolecta*, *Thalassiosira pseudonana*,

Table 2 – Comparison of P-uptake kinetic parameters in high-light adapted (HLA) and low-light adapted (LLA) diatoms (20°C).

	Species	$K_{m(P)}$ (μmol/L P)			$V_{m(P)}$ ((pmol/L P)/(cell·hr))			$V_{m(P)}/K_{m(P)}$			$Q_{(P)}$ ((pmol/L P)/cell)		
		LL	ML	HL	LL	ML	HL	LL	ML	HL	LL	ML	HL
HLA diatoms	*C. meneghiniana*	2.385 ± 0.101	1.613 ± 0.101	1.233 ± 0.064	0.522 ± 0.031	0.479 ± 0.032	0.47 ± 0.062	0.219 ± 0.018	0.298 ± 0.041	0.381 ± 0.042	0.243 ± 0.018	0.244 ± 0.007	0.241 ± 0.014
	S. parvus	2.661 ± 0.126	1.127 ± 0.085	1.122 ± 0.065	0.134 ± 0.009	0.114 ± 0.012	0.115 ± 0.019	0.053 ± 0.009	0.101 ± 0.008	0.102 ± 0.013	0.116 ± 0.013	0.115 ± 0.022	0.112 ± 0.021
	S. acus	3.268 ± 0.235	3.172 ± 0.263	2.148 ± 0.079	1.193 ± 0.095	1.163 ± 0.095	1.088 ± 0.212	0.365 ± 0.042	0.367 ± 0.025	0.507 ± 0.062	0.586 ± 0.021	0.583 ± 0.021	0.581 ± 0.018
LLA diatoms	*S. hantzschii*	2.347 ± 0.193	2.796 ± 0.325	3.571 ± 0.302	0.123 ± 0.019	0.133 ± 0.042	0.158 ± 0.021	0.052 ± 0.008	0.048 ± 0.007	0.044 ± 0.006	0.116 ± 0.008	0.122 ± 0.018	0.124 ± 0.013
	F. crotonensis	1.601 ± 0.089	1.679 ± 0.206	2.699 ± 0.198	0.417 ± 0.062	0.476 ± 0.065	0.531 ± 0.033	0.261 ± 0.032	0.283 ± 0.043	0.197 ± 0.026	0.411 ± 0.023	0.414 ± 0.026	0.417 ± 0.036
	N. palea	1.016 ± 0.076	1.076 ± 0.112	1.421 ± 0.083	0.087 ± 0.011	0.089 ± 0.011	0.099 ± 0.014	0.086 ± 0.008	0.083 ± 0.009	0.071 ± 0.008	0.153 ± 0.036	0.157 ± 0.031	0.162 ± 0.025

$V_{m(P)}$: the maximum uptake rate of P; $K_{m(P)}$: the half saturation constant for P uptake; $Q_{(P)}$: P cell quota.

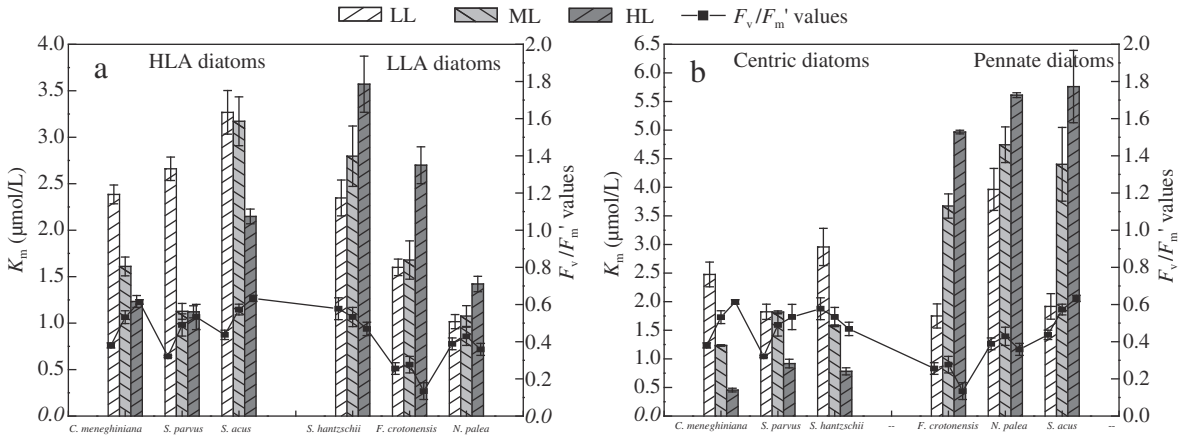

Fig. 2 – K_m values and $\Delta F/F'_m$ values in six diatoms. (a) $K_{m(P)}$ and $\Delta F/F'_m$ values (b) $K_{m(Si)}$ and $\Delta F/F'_m$ values (20°C). $K_{m(P)}$: the half saturation constant for P uptake; $K_{m(Si)}$: the half saturation constant for Si uptake.

Phaeodactylum tricornumtum and *Prymnesium parvum*) were influenced by the prevailing light conditions (Wynne and Rhee, 1988). Litchman et al. (2004) indicated that P uptake rate of phytoplankton in the nighttime was lower than that in the daytime and presumed that the difference in P uptake rates between the nighttime and daytime might be caused by the growth rate difference in nighttime and daytime. P uptake in phytoplankton depends on irradiance (*e.g.*, Davis, 1976; Nalewajko et al., 1981) because photosynthesis requires phosphate to synthesize adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Clark and Flynn, 2002).

The consist results for $K_{m(P)}$ and $\Delta F/F'_m$ indicate the optimum light intensities for both P uptake and cell growth of diatoms for these diatom species (Fig. 2a). The role of light fluctuations on nutrient competition is similar to that for competition for light in *Nitzschia* sp. (Litchman and Klausmeier, 2001). In study of Valenzuela-Espinoza et al. (2011), the best response to light intensity both in the growth and P uptake of *Amphidinium carteri* was found at the same light intensity (300 μmol photons/(m^2·sec)). High P demand of HLA diatoms at LL and LLA diatoms at HL reflected one kind of compensation effect which can relieve discomfort of diatoms under light stress temporarily (Cloern et al., 1995; Hessen et al., 2002). The growth rate of *Cryptomonas* sp. at 10 μmol photons/(m^2·sec) is only 10% of that at 150 μmol photons/(m^2·sec), whereas P uptake increases by 100% (Weng et al., 2009). Increase of P demand was considered as one kind of light adaptation to prevailing light condition (Hessen et al., 2002).

The calculated V_m/K_m provides some information about competitive ability, especially in oligotrophic water column (Grover, 1989; Healey, 1980). Increase in V_m or decrease in K_m or both of them can enhance the competitiveness for limiting nutrients (Button, 1991; Zou et al., 2001). In HLA diatoms, $V_{m(P)}$ values at HL were not the highest ones. However, the highest V_m/K_m values of HLA diatoms are obtained at HL (Table 2). It suggested that HLA diatoms were good competitors for P at HL while LLA diatoms owned better competitive ability for limiting P at LL. In summary, we concluded that dynamic light conditions can modify nutrient competition among phytoplankton by affecting nutrient uptake.

In natural phytoplankton community, low $Q_{(P)}$ values can be considered as an indicator for P limited (Van Donk et al., 1989). The decrease of V_m from starved to steady state of cells was closely related to the increase of $Q_{(P)}$ (Riegman and Mur, 1984). The correlation of $Q_{(P)}$ and V_m indicated that $Q_{(P)}$ was species specific in the 6 diatoms.

3.2. Silicon uptake

Si uptake kinetics of the present experiment belongs to one of the three classic models of Si uptake, surge uptake (Martin-Jézéquel et al., 2000). Surge uptake occurred when intracellular Si level was very low and the maximum uptake rate could be obtained in this process (Conway and Harrison, 1977; Conway et al., 1976). Energy for Si uptake came from respiration (Coombs et al., 1967). Krause et al. (2012) pointed that the kinetics parameters of Si uptake (values of $V_{m(Si)}$ and $V_{m(Si)}$:$K_{m(Si)}$) in phytoplankton were coupled with ambient light availability and they increased with the increasing of irradiance at two of the three field stations. Some studies also found that the light stress led to high Si uptake rates in diatoms (Claquin et al., 2002; Davis, 1976; Pondaven et al., 2000). However, other studies showed that Si uptake (Mongin et al., 2003) and Si metabolisms (Brzezinski, 1992) were independent of the ambient light intensity and the rate of Si transport (Blank and Sullivan, 1979) during the day and night was similar in field studies.

Previous studies have found that $K_{m(Si)}$ are generally lower in centrics than pennates (*e.g.*, Irwin et al., 2006; Moloney and Field, 1991). The affinity differences in Si uptake can be influenced by various types of silicic acid transporter (SIT) genes and the synthesis of higher affinity transporters (Martin-Jézéquel et al., 2000). Thamatrakoln et al. (2006) pointed that there were structural differences of SITs between centrics and pennates. The incorporation of Si is mainly linked to cell wall synthesis phase (Martin-Jézéquel et al.,

Table 3 – Comparison of Si-uptake kinetic parameters in centric and pennate diatoms (20°C).

Species	$K_{m(Si)}$ (μmol/L Si)			$V_{m(Si)}$ ((pmol/L Si)/(cell·hr))			$V_{m(Si)}/K_{m(Si)}$			$Q_{(Si)}$ ((pmol/L Si)/cell)		
	LL	ML	HL	LL	ML	HL	LL	ML	HL	LL	ML	HL
Centric diatoms												
C. meneghiniana	2.476 ± 0.216	1.234 ± 0.013	0.454 ± 0.032	0.297 ± 0.021	0.369 ± 0.025	0.395 ± 0.054	0.122 ± 0.007	0.299 ± 0.035	0.871 ± 0.098	0.248 ± 0.052	0.243 ± 0.032	0.236 ± 0.026
S. parvus	1.823 ± 0.133	1.818 ± 0.021	0.918 ± 0.075	0.812 ± 0.071	0.866 ± 0.035	0.692 ± 0.046	0.445 ± 0.216	0.476 ± 0.051	0.754 ± 0.083	0.512 ± 0.034	0.508 ± 0.032	0.501 ± 0.028
S. hantzschii	2.957 ± 0.325	1.582 ± 0.011	0.782 ± 0.0621	0.329 ± 0.021	0.397 ± 0.021	0.262 ± 0.019	0.111 ± 0.017	0.251 ± 0.036	0.335 ± 0.024	1.048 ± 0.072	1.042 ± 0.063	1.038 ± 0.084
Pennate diatoms												
F. crotonensis	1.751 ± 0.211	3.672 ± 0.212	4.966 ± 0.032	1.231 ± 0.086	2.278 ± 0.312	2.801 ± 0.312	0.703 ± 0.062	0.621 ± 0.045	0.564 ± 0.043	3.212 ± 0.163	3.209 ± 0.233	3.201 ± 0.245
N. palea	3.963 ± 0.367	4.744 ± 0.312	5.611 ± 0.042	2.045 ± 0.132	2.712 ± 0.256	2.817 ± 0.257	0.516 ± 0.032	0.572 ± 0.023	0.502 ± 0.056	0.216 ± 0.015	0.229 ± 0.012	0.221 ± 0.020
S. acus	1.917 ± 0.223	4.402 ± 0.645	5.761 ± 0.632	1.517 ± 0.113	2.545 ± 0.316	3.021 ± 0.243	0.792 ± 0.061	0.578 ± 0.038	0.524 ± 0.035	2.234 ± 0.021	2.232 ± 0.018	0.239 ± 0.019

$V_{m(Si)}$: the maximum uptake rate of Si; $K_{m(Si)}$: the half saturation constant for Si; $Q_{(Si)}$: Si cell quota.

2000). The silica concentration of diatom cells depended on the cell size and larger cells have more silica than smaller ones (Falasco et al., 2009). Leynaert et al. (2009) observed that small size phytoplankton tended to dominate in nutrient-poor water columns, whereas large size phytoplankton dominated in nutrient-rich regions. Martin-Jézéquel et al. (2000) found that silicification of diatoms can be influenced by light which improves Si uptake by promoting phytoplankton growth (Xu et al., 2009). In Monterey Bay, Si uptake of algae in upwelled waters increased with the growing light intensity (Kudela, 2008). In our study, pennate diatoms reached their highest K_m values at HL which might be due to their high demands of Si for fast growth. There were no correlations among $K_{m(Si)}$, growth rates and photosynthetic activities in HLA and LLA diatoms (Fig. 2b). However, the negative relationship between $K_{m(Si)}$ and light intensity was found in centric diatoms and the positive relationship between them was found in pennate diatoms, which implied that Si uptake, unlike P uptake characteristic, correlates with cell volume more closely than historic light regime.

3.3. Implication for diatom bloom control and diatom survey

Previous studies indicated that acclimation to a given irradiance of phytoplankton depends on the intrinsic capacity of the algae species concerned and nutrient availability (Behrenfeld et al., 2004; Falkowski and Raven, 2007). Light intensity and the efficiency of nutrient uptake were strongly coupled at lower substrate availability (Krause et al., 2012). In the present study, correlation analysis showed that uptake kinetic parameters of Si and P were not correlated with ambient Si and P concentration of historic regimes ($r < 0.3$, $P > 0.05$). Decrease of P and Si load induced by building dam did not reduce the occurrence frequency of diatom bloom, which implied that the diatoms can grow well under suitable light intensity; even the ambient nutrient concentration is low because they can reach their optimal affinity of nutrient under proper light conditions. Physiological regulation in diatoms might be unlikely to be achieved when both P concentration and the light intensities are not suitable for diatom growth. Thus, it would be effective to control diatom bloom by altering light intensity in small-scale eutrophic water columns. On the one hand, it is difficult to meet the P requirement of diatoms under unfavorable light conditions; on the other hand, adverse light conditions itself can lead to a decrease of diatom growth and photosynthesis activity. Light control measures can be considered as follows.

Creating a physical cover, such as planting macrophytes and using riparian vegetation to reduce light intensity might be effective ways to reduce the photosynthetic activity of HLA spices. Planting floating plants and mixing vertically have dual effects on reducing light intensity and nutrient concentration in the water column. For LLA diatoms, we can plant trees in the upstream of fast-flowing rivers to reduce soil erosion for light intensity increase. Planting submerged plants at proper sites can reduce nutrient concentration and increase light penetration in the water column.

Although historic light conditions did not affect Si uptake significantly as P uptake, the different light treatments cause reverse tendency of Si uptake between centric and pennate

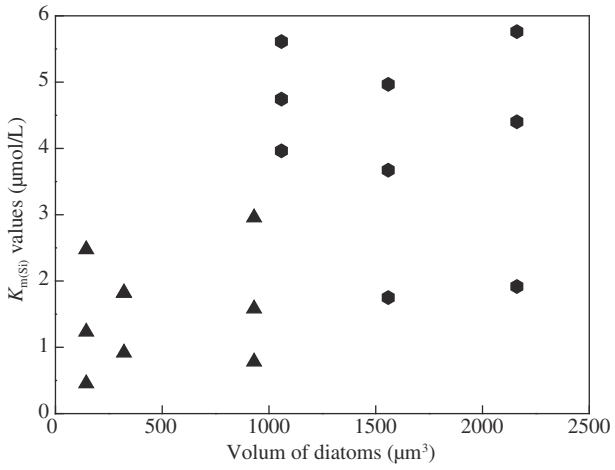

Fig. 3 – Relationship between $K_{m(Si)}$ values and volume of diatoms at three light intensities (20°C). $K_{m(Si)}$: the half saturation constant for Si uptake.

diatoms. The differences in $V_{m(Si)}/K_{m(Si)}$ values among light intensities were mainly induced by $K_{m(Si)}$ values. Results of $V_{m(Si)}/K_{m(Si)}$ values suggested high competition for Si of centric diatoms which occurred at HL and of pennate diatoms which occurred at LL. Thus, we can take light-control measures according to dominate diatom in the local ecosystem. If dominate species of diatom bloom appeared as low-light adapted, we can take measures which could cause photo-inhibition to reduce photosynthetic and P uptake activity of the dominate species simultaneously. Increase in Si:P can transfer centric-dominate ecosystem to pennate-dominate ecosystem (Shatwell et al., 2008). Martin-Jézéquel et al. (2000) pointed that *Navicula pelliculosa* could transport enough silicic acid to form a new cell wall in no more than 3 hr at maximum Si-uptake rate. It suggested that pennate diatoms which V_{max} were much higher than centric diatoms were of more advantages under Si sufficient environment. But in the middle or final stage of spring diatom blooms, Si always became limiting factor for diatom growth (Laugaste et al., 2010) and transformation of dominate species may occur in aquatic ecosystem (Reynolds et al., 2006). Si uptake is diffusion-controlled at high silicic acid concentrations but SIT-determined at low silicic acid concentrations (Thamatrakoln and Hildebrand, 2008). Thus, we should understand the related factors for Si uptake when assessing growth and development of diatom blooms in aquatic ecosystem.

4. Conclusions

Kinetics parameters for P-uptake and Si-uptake can be dramatically influenced by light intensities. P-uptake affinity of HLA and LLA diatoms and Si-uptake affinity of centric and pennate diatoms differed. Diatoms can response to light stress by nutrient compensation temporarily. Since there is the close relationship between nutrient-uptake (P and Si) and light conditions in diatoms, we propose that light conditions should be considered as a key factor when we assess and predict the development of diatom blooms and light control measures might be useful in diatom bloom control.

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REFERENCES

Agawin, N.S.R., Duarte, C.M., Agustí, S., 2000. Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnol. Oceanogr. 45 (3), 591–600.

Behrenfeld, M.J., Prasil, O., Babin, M., Bruyant, F., 2004. In search of a physiological basis for covariations in light-limited and light-saturated photosynthesis. J. Phycol. 40 (1), 4–25.

Bhattacharyya, P., Volcani, B.E., 1980. Sodium-dependent silicate transport in the apochlorotic marine diatom *Nitzschia alba*. Proc. Natl. Acad. Sci. U. S. A. 77 (11), 6386–6390.

Blank, G.S., Sullivan, C.W., 1979. Diatom mineralization of silicic acid. Arch. Microbiol. 123 (2), 157–164.

Brzezinski, M.A., 1992. Cell-cycle effects on the kinetics of silicic acid uptake and resource competition among diatoms. J. Plankton Res. 14 (11), 1511–1539.

Button, D.K., 1991. Biochemical basis for whole-cell uptake kinetics: specific affinity, oligotrophic capacity, and the meaning of the Michaelis constant. Appl. Environ. Microbiol. 57 (7), 2033–2038.

Chisholm, S.W., Stross, R.G., 1976. Phosphate uptake kinetics in *Euglena gracilis* (Z) (Euglenophyceae) grown in light/dark cycles. II. Phased PO_4-limited cultures. J. Phycol. 12 (2), 217–222.

Claquin, P., Martin-Jézéquel, V., Kromkamp, J.C., Veldhuis, M.J.W., Kraay, G.W., 2002. Uncoupling of silicon compared with carbon and nitrogen metabolisms and the role of the cell cycle in continuous cultures of *Thalassiosira pseudonana* (Bacillariophyceae) under light, nitrogen, and phosphorus control. J. Phycol. 38 (5), 922–930.

Clark, D.R., Flynn, K.J., 2002. N-assimilation in the noxious flagellate *Heterosigma carterae* (Raphidophyceae): dependence on light, N-source, and physiological state. J. Phycol. 38 (3), 503–512.

Cloern, J.E., Grenz, C., Vidergar-Lucas, L., 1995. An empirical model of the phytoplankton chlorophyll: carbon ratio—the conservation factor between productivity and growth rate. Limnol. Oceanogr. 40 (7), 1313–1321.

Conway, H.L., Harrison, P.J., 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation IV. Transient response of *Chaetoceros debilis*, *Skeletonema costatum*, and *Thalassiosira gravida* to a single addition of the limiting nutrient. Mar. Biol. 43 (1), 33–43.

Conway, H.L., Harrison, P.J., Davis, C.O., 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. Mar. Biol. 35 (2), 187–199.

Coombs, J., Darley, W.M., Holmhans, O., Volcani, B.E., 1967. Studies on the biochemistry and fine structure of silica shell formation in diatoms. Chemical composition of *Navicula pelliculosa* during silicon-starvation synchrony. Plant Physiol. 42 (11), 1601–1609.

Davis, C.O., 1976. Continuous culture of marine diatoms under silicate limitation. II. Effect of light intensity on growth and nutrient uptake of *Skeletonema costatum*. J. Phycol. 12 (3), 291–300.

De La Rocha, C.L., Hutchins, D.A., Brzezinski, M.A., Zhang, Y., 2000. Effects of iron and zinc deficiency on elemental composition and silica production by diatoms. Mar. Ecol. Prog. Ser. 195, 71–79.

Deane, E.M., O'Brien, R.W., 1981. Uptake of phosphate by symbiotic and free-living dinoflagellates. Arch. Microbiol. 128 (3), 307–310.

Falasco, E., Bona, F., Badino, G., Hoffmann, L., Ector, L., 2009. Diatom teratological forms and environmental alterations: a review. Hydrobiologia 623 (1), 1–35.

Falkowski, P.G., Raven, J.A., 2007. Aquatic Photosynthesis. Princeton University Press, Princeton, USA.

Feijoó, C., Comerma, M., Marce, R., Garcia, J.C., Balayla, D., Navarro, E., et al., 2008. Influence of phosphorus and irradiance on phytoplanktonic chlorophyll-a concentration and phosphorus contents at a diel scale in a Mediterranean reservoir. Limnetica 27 (2), 329–341.

Gameiro, C., Zwolinski, J., Brotas, V., 2011. Light control on phytoplankton production in a shallow and turbid estuarine system. Hydrobiologia 669 (1), 249–263.

Godinot, C., Ferrier-Pagés, C., Grover, R., 2009. Control of phosphate uptake by zooxanthellae and host cells in the scleractinian coral *Stylophora pistillata*. Limnol. Oceanogr. 54 (5), 1627–1633.

Grover, J.P., 1989. Phosphorus-dependent growth kinetics of 11 species of freshwater algae. Limnol. Oceanogr. 34 (2), 341–348.

Healey, F.P., 1980. Slope of the Monod equation as an indicator of advantage in nutrient competition. Microb. Ecol. 5 (4), 281–286.

Hessen, D.O., Færøvig, P.J., Andersen, T., 2002. Light, nutrients, and P:C ratios in algae:grazer performance related to food quality and quantity. Ecology 83 (7), 1886–1898.

Hu, H.J., Wei, Y.X., 2006. The Freshwater Algae of China: Systematic, Taxonomy and Ecology. Science Press, Beijing, China.

Irwin, A.J., Finkel, Z.V., Schofield, O.M.E., Falkowski, P.G., 2006. Scaling-up from nutrient physiology to the size-structure of phytoplankton communities. J. Plankton Res. 28 (5), 459–471.

Jickells, T.D., 1998. Nutrient biogeochemistry of the coastal zone. Science 281 (5374), 217–222.

Krause, G.H., Virgo, A., Winter, K., 1995. High susceptibility to photoinhibition of young leaves of tropical forest trees. Planta 197 (4), 583–591.

Krause, J.W., Brzezinski, M.A., Villareal, T.A., Wilson, C., 2012. Increased kinetic efficiency for silicic acid uptake as a driver of summer diatom blooms in the North Pacific subtropical gyre. Limnol. Oceanogr. 57 (4), 1084–1098.

Kropuenske, L.R., Mills, M.M., van Dijken, G.L., Bailey, S., Robinson, D.H., Welschmeyer, N.A., et al., 2009. Photophysiology in two major Southern Ocean phytoplankton taxa: photoprotection in *Phaeocystis antarctica* and *Fragilariopsis cylindrus*. Limnol. Oceanogr. 54 (4), 1176–1196.

Kudela, R.M., 2008. Silicon: Nitrogen Interactions in the Marine Environment. Elsevier Academic Press, San Diego, USA.

Laugaste, R., Haberman, J., Blank, K., 2010. Cool winters versus mild winters: effects on spring plankton in Lake Peipsi. Eston. J. Ecol. 59 (3), 163–183.

Leynaert, A., Bucciarelli, E., Claquin, P., Dugdale, R.C., Martin-Jézéquel, V., Pondaven, P., et al., 2004. Effect of iron deficiency on diatom cell size and silicic acid uptake kinetics. Limnol. Oceanogr. 49 (4), 1134–1143.

Leynaert, A., Longphuirt, S.N., Claquin, P., Chauvaud, L., Ragueneau, O., 2009. No limit?: the multiphasic uptake of silicic acid by benthic diatoms. Limnol. Oceanogr. 54 (2), 571–576.

Liang, K.X., Wang, X.Y., Zhang, D.B., Zhou, Y.H., 2012. Ecological conditions of diatom water bloom formulation in the middle and lower reach of the Hanjiang River and strategy for water bloom control. Environ. Sci. Technol. 35 (S2), 113–116.

Litchman, E., Klausmeier, C.A., 2001. Competition of phytoplankton under fluctuating light. Am. Nat. 157 (2), 170–187.

Litchman, E., Klausmeier, C.A., Bossard, P., 2004. Phytoplankton nutrient competition under dynamic light regimes. Limnol. Oceanogr. 49 (4), 1457–1462.

Martin-Jézéquel, V., Hildebrand, M., Brzezinski, M.A., 2000. Silicon metabolism in diatoms: implications for growth. J. Phycol. 36 (5), 821–840.

Moloney, C.L., Field, J.G., 1991. The size-based dynamics of plankton food webs. I. A simulation model of carbon and nitrogen flows. J. Plankton Res. 13 (5), 1003–1038.

Mongin, M., Nelson, D.M., Pondaven, P., Brzezinski, M.A., Treguer, P., 2003. Simulation of upper-ocean biogeochemistry with a flexible-composition phytoplankton model: C, N and Si cycling in the western Sargasso Sea. Deep-Sea Res. I Oceanogr. Res. Pap. 50 (12), 1445–1480.

Murphy, J., Riley, J., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.

Nalewajko, C., Lee, K., Shear, H., 1981. Phosphorus kinetics in Lake Superior: light intensity and phosphate uptake in algae. Can. J. Fish. Aquat. Sci. 38 (2), 224–232.

Pondaven, P., Ruiz-Pino, D., Fravalo, C., Treguer, P., Jeandel, C., 2000. Interannual variability of Si and N cycles at the time-series station KERFIX between 1990 and 1995 — a 1-D modelling study. Deep-Sea Res. I Oceanogr. Res. Pap. 47 (2), 223–257.

Reynolds, C.S., Usher, M., Saunders, D., Dobson, A., Peet, R., Adam, P., et al., 2006. The Ecology of Phytoplankton. Cambridge University Press, Cambridge.

Riegman, R., Mur, L.R., 1984. Regulation of phosphate uptake kinetics in *Oscillatoria agardhii*. Arch. Microbiol. 139 (1), 28–32.

Ritchie, R., Donelle, A., Larkum, A., 2001. Phosphate limited cultures of the cyanobacterium Synechococcus are capable of very rapid, opportunistic uptake of phosphate. New Phytol. 152 (13), 189–201.

Schreiber, U., Bilger, W., Neubauer, C., 1994. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E.D., Caldwell, M.M. (Eds.), Ecophysiology of Photosynthesis. Springer Berlin Heidelberg, Berlin, pp. 49–70.

Shatwell, T., Koehler, J., Nicklisch, A., 2008. Warming promotes cold-adapted phytoplankton in temperate lakes and opens a loophole for Oscillatoriales in spring. Glob. Chang. Biol. 14 (9), 2194–2200.

Shen, H., Song, L.R., 2007. Comparative studies on physiological responses to phosphorus in two phenotypes of bloom-forming *Microcystis*. Hydrobiologia 592 (1), 475–486.

Sherman, B., Ford, P., Grey, L., Maher, B., Hatton, P., Whittington, J., et al., 2001. The Chaffey Dam Story. Murray-Darling Freshwater Research Centre, Victoria, Australia.

Stein, J.R., Hellebust, J.A., 1980. Handbook of Phycological Methods: Culture Methods and Growth Measurements. Cambridge University Press, New York.

Stolte, W., Riegman, R., 1995. Effect of phytoplankton cell size on transient-state nitrate and ammonium uptake kinetics. Microbiology 141 (5), 1221–1229.

Strickland, J.D.H., Parsons, T.R., 1968. A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Ottawa.

Thamatrakoln, K., Hildebrand, M., 2008. Silicon uptake in diatoms revisited: a model for saturable and nonsaturable uptake kinetics and the role of silicon transporters. Plant Physiol. 146 (3), 1397–1407.

Thamatrakoln, K., Alverson, A.J., Hildebrand, M., 2006. Comparative sequence analysis of diatom silicon transporters: toward a mechanistic model of silicon transport. J. Phycol. 42 (4), 822–834.

Thoreson, S.S., Clayton Jr., J.R., Ahmed, S.I., 1984. The effect of short-term fluctuation in pH on NO_3^- uptake and intracellular constituents in *Skeletonema costatum* (Grev.) Cleve. J. Exp. Mar. Biol. Ecol. 83 (2), 149–157.

Valenzuela-Espinoza, E., Millan-Nunez, R., Santamaria-del-Angel, E., Trees, C.C., 2011. Macronutrient uptake and carotenoid/chlorophyll a ratio in the dinoflagellate *Amphidinium carteri* Hulburt, cultured under different nutrient and light conditions. Hydrobiologica 21 (1), 34–48.

Van Donk, E., Mur, L.R., Ringelberg, J., 1989. A study of phosphate limitation in Lake Maarsseveen: phosphate uptake kinetics versus bioassays. In: Munawar, M., Dixon, G., Mayfield, C.J., Reynoldson, T., Sadar, M.H. (Eds.), Environmental Bioassay Techniques and their Application. Springer, Berlin, pp. 201–209.

Wahby, S.D., Bishara, N.F., 1980. The Effect of the River Nile on Mediterranean Water, Before and After the Construction of the High Damat Aswan. UNEP, IOC, SCOR, United Nations, New York.

Wang, M., Zhang, Z., Guo, W.H., Zeng, X.L., Yuan, S.C., 2011. Occurrence regularity of diatom bloom in the confluence section of Jialing River. Res. Environ. Sci. 24 (2), 191–198.

Weng, H.X., Qin, Y.C., Sun, X.W., Chen, X.H., Chen, J.F., 2009. Effects of light intensity on the growth of *Cryptomonas* sp. (Cryptophyceae). Environ. Geol. 57 (1), 9–15.

Wynne, D., Rhee, G.Y., 1988. Changes in alkaline phosphatase activity and phosphate uptake in P-limited phytoplankton, induced by light intensity and spectral quality. Hydrobiologia 160 (2), 173–178.

Xu, B., Liu, M., Hou, L.J., Lu, M., Yan, H.M., Xu, S.Y., 2009. Influence of simulative light on silicon and nitrogen diffusion fluxes across sediment–water interface in tidal flats of the Yangtze Estuary. Res. Environ. Sci. 22 (3), 327–331.

Yu, L.H., Fang, D.J., Fang, T., Li, Y., Gao, L., 2006. Distributions of DSi, DIN and changes of Si: N ratio on summer in Changjiang Estuary before and after storage of Three Gorges Reservoir. Acta Ecol. Sin. 26 (9), 2817–2826.

Zeng, H., 2006. PhytoPlankton in Yangtze and Three Gorges Reservoir: Dynamics and Relationship with Nutrient Sand Hydrological Conditions. (PhD thesis). University of Chinese Academy of Sciences, Beijing, China.

Zhang, Z.S., Huang, X.F., 1991. Research Methods of Freshwater Plankton. Science Press, Beijing, China.

Zhang, Y., Qin, B., Chen, W., Gao, G., Chen, Y., 2004. Experimental study on underwater light intensity and primary productivity caused by variation of total suspended matter. Adv. Water Resour. 15 (5), 615–620.

Zou, L., Zhang, J., Pan, W.X., Zhan, Y.P., 2001. *In situ* nutrient enrichment experiment the Bohai and Yellow Sea. J. Plankton Res. 23 (10), 1111–1119.