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# Can Hydrodynamics Change Phosphorus Strategies of Diatoms?—Nutrient Levels and Diatom Blooms in Lotic and Lentic Ecosystems

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**Abstract** Diatom blooms occur in many water bodies worldwide, causing significant ecological and social concerns. In order to understand the mechanisms of diatom blooms formation, the effects of varying phosphorus (P) concentration and hydrodynamics on the growth of diatoms were studied by combining results from field observations and laboratory experiments. The field investigation showed that spring diatom blooms (*Cyclotella meneghiniana* and *Stephanodiscus hantzschii*) occurred in Lake Taihu and Hanjiang River with similar environmental factors such as water temperature, pH, and dissolved oxygen in 2008. Concentrations of total phosphorus (TP), total nitrogen, and ammonia nitrogen ($NH_4$-N) in Lake Taihu were significantly higher than the concentrations in the Hanjiang River. Laboratory experiments were conducted to evaluate growth and physiological responses of four lotic diatoms (*Cyclotella atomus*, *Fragilaria crotonensis*, *Nitzschia palea*, and *S. hantzschii*, isolated from the Hanjiang River) and three lentic diatoms (*C. meneghiniana*, *Melosira varians*, and *Stephanodiscus minutulus*, isolated from Lake Taihu, Lake Donghu, and Guanqiao Pond, respectively) to various P concentrations under small-scale turbulent and standing conditions. Our results showed that, with turbulence, lotic diatoms *C. atomus*, *F. crotonensis*, *N. palea*, and *S. hantzschii* demonstrated a significant increase in affinity for P compared with lentic diatoms *C. meneghiniana*, *M. varians*, and *S. minutulus*. Moreover, lotic diatoms *C. atomus*, *F. crotonensis*, and *N. palea* had higher growth rates and photosynthetic efficiencies with small-scale turbulence than with standing conditions both in P-limited and P-replete conditions. Lotic species *S. hantzschii* and three lentic diatoms (*C. meneghiniana*, *M. varians*, and *S. minutulus*) grew well under standing conditions. Our results may explain our field observation that the occurrence of diatom blooms in lakes is often associated with higher TP concentrations whereas in rivers, diatom blooms occur at a wide range of TP concentrations under flows. Therefore, different hydrodynamics and nutrient concentrations determined the dominant diatom species, according to their habitat-dependent physiological characteristics.

Peili Wang and Hong Shen contributed equally to this work.

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## Introduction

Diatom blooms are observed worldwide in both lotic (e.g., rivers) [20, 35, 41] and lentic ecosystems (e.g., lakes and ponds) [31, 47, 79]. Many studies have found differences in the dominant diatom species among different types of freshwater ecosystems. Centric diatom blooms (such as *Stephanodiscus* spp. and *Cyclotella* spp.) and pennate diatoms blooms (such as *Nitzschia* spp. and *Asterionella*

spp.) have been both frequently reported in various rivers all over the world. For example, *Stephanodiscus* spp. blooms occur in the Meuse River (Belgium), Nakdong River (South Korea), and Hanjiang River (China) nearly every year [9, 17, 20]. Dominance by *Nitzschia* spp. has been reported for some sections of Spree River (Germany), Hunter River (Australia), and the Meuse River (Belgium) [31, 41, 73]. However, the great majority of reports regarding the dominant species of diatom in lakes and ponds have focused on centric diatoms, *Cyclotella* spp. and *Melosira* spp. [13, 31, 35, 38, 68, 78, 79].

These studies have indicated that the fate of diatom populations in fluvial flow is not consistent within phylogenetic groups [3]. Some diatoms are able to grow (*Nitzschia*, *Diatoma*, *Stephanodiscus*) or, at least, to maintain their abundance (*Synedra*, *Asterionella*, *Limnothrix*) along the river courses. In contrast, some dominant diatoms in calm periods experience a decrease in biomass (*Cyclotella*) in these river stretches [31]. Even small centric diatoms with similar appearance attain completely different rates of biomass change both along the river course and within the lake [31]. Obviously, closely related species with similar morphometry are not necessarily adapted to the same environmental conditions. With respect to their habitat specificity, diatom communities may vary among different water bodies owing to difference in relative importance of habitat-dependent physiological and ecological processes under different prevailing hydrodynamic regimes.

Hydrodynamic conditions affect the competitive interactions among the different components of phytoplankton communities [52, 53, 82,]. Two similar mesocosm experiments in lakes [13, 38] both showed that artificial destabilization could trigger a diatom bloom during the summer to levels usually seen only during the spring. Furthermore, Mitrovic et al. [41] found that *Cyclotella* biomass was negatively related to flow rates in the rivers. There are several studies indicating that a decreasing flow rate directly contributes to the spring diatom biomass [20, 30, 35, 70]. Thus, the mechanisms behind diatom dominance in lentic and lotic water might not be identical because hydrodynamic conditions may differ between these types of systems. Thus, the flow regime should be considered as a prerequisite inducement for diatom blooms in natural water bodies [13, 28].

Hydrodynamics may affect the growth of phytoplankton by changing their nutrient acquisition strategies [12, 42, 80]. Phosphorus is considered to be the principal nutrient that limits algal growth in most freshwater habitats [34, 37, 40, 62, 65]. In order to cope with P-limited conditions, algae have developed different nutrient adaptive strategies. Nutrient uptake kinetics of phytoplankton is important for understanding competition, succession, and dominance in natural phytoplankton communities [11]. Many studies have reported that environmental factors such as turbulence [52], temperature [46], light intensity, and nutrient concentration [14] affect rates of P uptake in algae. Appropriate turbulence may be beneficial for phytoplankton by increasing the diffusion rate of nutrients to the cell surface [29]. Different algae exhibit different nutrient uptake traits in terms of their responses to turbulent conditions. Peters et al. [52] showed that the phosphate uptake rate in the marine diatom *Coscinodiscus* sp. was increased by small-scale turbulence while the uptake rate in the diatom *Thalassiosira pseudonana* was unaffected. They attributed this difference to be cell size dependent. Moreover, synthesis of extracellular alkaline phosphatase (AP), which liberates orthophosphate ($P_i$) from dissolved organic phosphorus, is another adaptive strategy of algae to cope with P-deficient conditions. Moreover, several studies have shown that alkaline phosphatase activity (APA) is affected by environmental factors like light, temperature, pH, and salinity [16, 24, 25]. In addition, turbulence is another factor that can influence APA in diatoms, and the response to turbulence differed markedly between *Coscinodiscus* sp. and *T. pseudonana* [52]. This implies that nutrient strategies play an important role in phytoplankton dominance. Compared to our knowledge about effects of other environmental factors on species dominance and nutrient strategies, our understanding of relationships between turbulence and nutrient strategies in diatoms is limited.

$P_i$ always plays an important role in diatom growth [18], and changes in TP concentration are significantly related to the stability of diatom community [67]. Habitat-related differences in P uptake and growth likely reflect the environmental habitats from which the species or strain is isolated [14]. Considering the wide presence of diatom blooms in both lentic and lotic systems, we asked the following questions: (1) whether nutrient levels for diatom blooms are identical for lentic and lotic ecosystems, (2) whether physiological responses of diatoms to hydrodynamics are habitat dependent, and (3) whether bloom-forming diatoms in lentic and lotic systems have developed different adaptive strategies to cope with P-limitation.

Our field study was conducted in the Hanjiang River and Lake Taihu to compare and contrast among a range of nutrient conditions and flow regimes during diatom dominance. We also performed a series of laboratory experiments to evaluate the effects of small-scale turbulence on the growth rate, photosynthesis, P uptake, and alkaline phosphatase activity in bloom-forming diatoms isolated from flowing and standing waters under both P-replete and P-limited conditions. The diatoms *Cyclotella atomus*, *Fragilaria crotonensis*, *Nitzschia*

*palea*, and *Stephanodiscus hantzschii* were isolated from the Hanjiang River, and *Cyclotella meneghiniana*, *Melosira varians*, and *Stephanodiscus minutulus* were isolated from a eutrophic pond, Lake Donghu, and Lake Taihu, respectively. The goal of this study was to examine relationships among concentration level (P), hydrodynamics, and diatom species dominance to provide a tool for a better prediction of diatom bloom occurrence in different flow regimes.

## Material and Methods

### Field Study

#### *Study Site*

The field portion of this study utilized one lentic and one lotic ecosystem representing typical eutrophic conditions in many freshwater ecosystems in China. The lotic system, the Hanjiang River (1567 km in length), is the longest tributary of the Yangtze River (Changjiang) (Fig. 1a). Annually, recurrent diatom blooms have been observed in winter and early spring in the middle and lower reaches of Hanjiang River since 1992. Our field sites were at the lower reach of the Hanjiang River (Zekou, Yuekou, Shidou, and Zongguan; 30°24′–30°34′ N, 112°29′–114°29′ E). The lentic system we studied was in Meiliang Bay, located in the northern part of Lake Taihu (30°56′–31°33′ N, 119°54′–120°36′ E), one of the five largest freshwater lakes in China (Fig. 1b). Serious *Microcystis* blooms are often observed in summer and diatoms dominate in spring [68, 78].

#### *Sample Collection*

Four sites were sampled in the Hanjiang River and six sites in Meiliang Bay. The sampling sites were defined using a global positioning system. Samples were collected monthly during early spring from January to March 2008. Water and phytoplankton samples were collected using a 5-L Schindler sampler at the surface (0–0.5 m). Water samples for identification and counts of phytoplankton were preserved with 1% Lugol's iodine solution. Water temperature, pH, and dissolved oxygen (DO) were measured using a YSI6600 Multi-Parameter Water Quality Sonde.

#### *Water Quality Measurements*

Physicochemical and biological water quality-related parameters, including water temperature, flow rates, pH, DO, chemical oxygen demand (COD), total nitrogen (TN), ammonia nitrogen ($NH_4$-N), total phosphorus (TP), diatom densities, and dominate species were measured for each sample, according to the methods described by Niu et al. and Zheng et al. [47, 81].

### Laboratory Experiment

#### *Species and Culture Conditions*

The diatom species examined in this study were isolated from the field samples (Table 1). The volume of each species was calculated by measuring morphometric parameters (diameter or length and width) [43]. Axenic strains were established by streak plate isolation on D1 medium (Table S1) and then grown in batch culture in D1 medium (Table S1) at 20±1°C under 35 μmol $m^{-2}$ $s^{-1}$ of cool-white fluorescent illumination on a 12:12-h light/dark cycle. Stock cultures were transferred weekly to new medium and were maintained in the exponential phase.

#### *Growth Experiment*

Diatom cells were collected by centrifugation and washed three times in P-free D1 medium ($K_2HPO_4$ and $KH_2PO_4$ were replaced by equimolar KCl) and thereafter transferred into P-free medium for 2 weeks in order to deplete intracellular phosphorus stores. According to Oh et al. [48], ambient orthophosphate concentration below the detection limit of 0.01 mM in the medium did not support any algal cell growth; therefore, phosphorus-deficient cells were prepared. The experiment was carried out at standing and turbulent conditions under P-limited (0.2 mg $L^{-1}$ P) and P-replete (2 mg $L^{-1}$ P) conditions. Phosphate was added in the form of orthophosphate ($K_2HPO_4$). Each species was inoculated into 250-mL flasks containing 200 mL of autoclaved medium at an initial algal concentration of $4\times10^7$ cells $L^{-1}$. Four different treatments were compared: two standing treatments with initial P concentrations of 0.2 and 2 mg $L^{-1}$ and two turbulent treatments with initial P concentrations of 0.2 mg and 2 mg $L^{-1}$. The turbulent treatments were put on a shaker (Guohua Company, Jiangsu, China) at 120 rpm. The theoretical speed was 0.38 m $s^{-1}$, which was estimated according to the shaking diameter [51, 60]. The flasks of the standing treatments were agitated manually once every 6 h in order to avoid sedimentation of the cells in the unstirred cultures. A preliminary examination showed that the growth rate of most diatoms was inhibited by a P concentration of 0.2 mg $L^{-1}$, but all diatom species grew normally at a P concentration of 2 mg $L^{-1}$. The average flow rate during diatom blooms in the Hanjiang River was approximately 0.38 m $s^{-1}$ (unpublished data). The experiment lasted

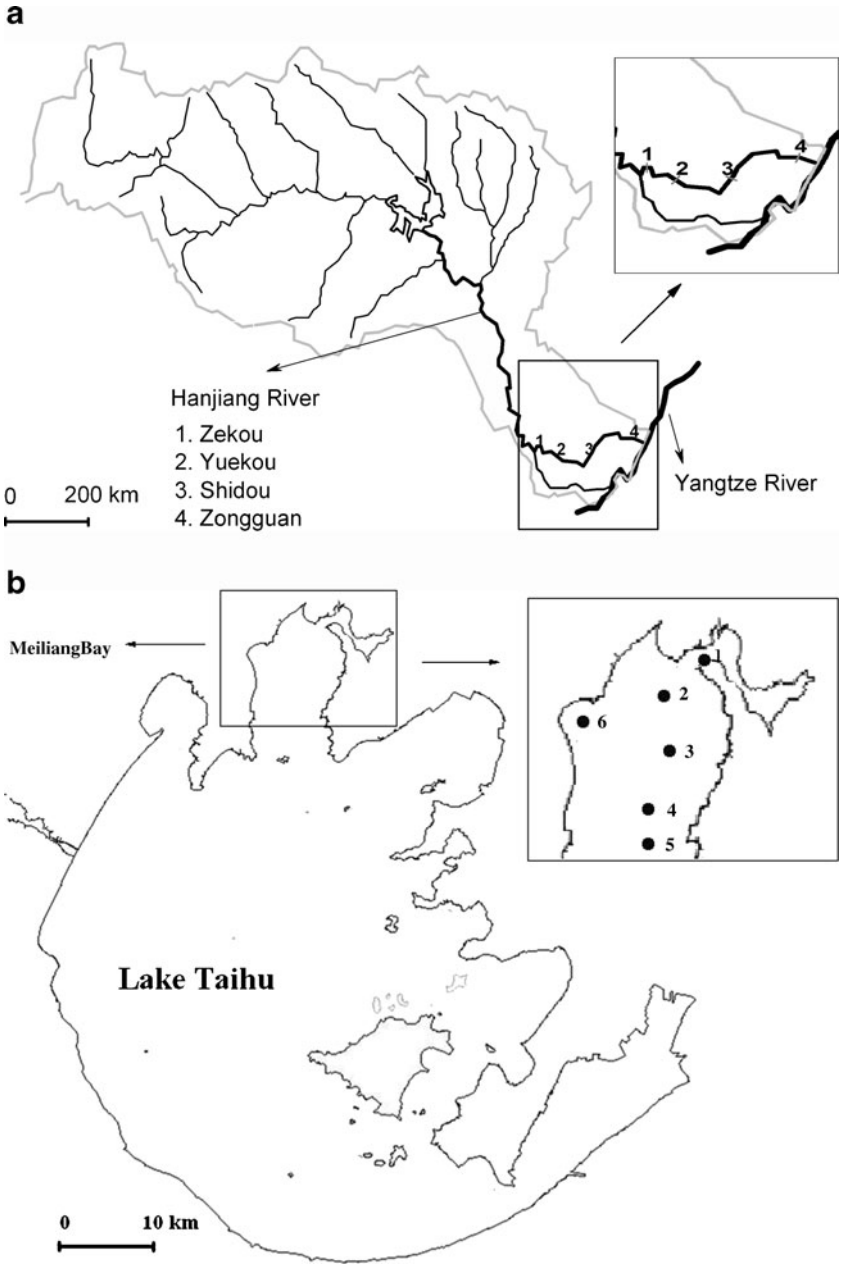


**Figure 1** **a** Map of the Hanjiang River, with the location of the study sites shown. **b** Map of Lake Taihu basins, with the location of the study sites shown

21 days and the cultures were harvested every second day to monitor changes in growth and physiological parameters. All treatments were kept at identical conditions and were conducted in triplicate. All glassware was soaked in 0.1 M HCl for 24 h and then rinsed with ultrapure water before use in order to prevent P contamination. The medium and

**Table 1** Diatom species in this study

| Species | Sampling stations | Sampling date | Average volume ($\mu m^3$) |
|---|---|---|---|
| *Cyclotella atomus* | Hanjiang River | 2006 | 276.96 |
| *Fragilaria crotonensis* | Hanjiang River | 2008 | 2,350.78 |
| *Nitzschia palea* | Hanjiang River | 2008 | 788.20 |
| *Stephanodiscus hantzschii* | Hanjiang River | 2010 | 1,554.97 |
| *Cyclotella meneghiniana* | Guanqiao pool | 2007 | 850.74 |
| *Melosira varians* | Lake Donghu | 2008 | 1,102.68 |
| *Stephanodiscus minutulus* | Lake Taihu | 2010 | 593.34 |

flasks were sterilized at 121°C for 30 min, and each step of the culturing procedure was carried out under sterile conditions.

*P Uptake Experiment*

P uptake experiments were conducted as follows: P-starved cells were inoculated into 50-mL flasks containing 30 mL modified D1 medium. $K_2HPO_4$ was added to give final P concentrations of 0.02, 0.2, 1, 2, and 5 mg $L^{-1}$ and incubated at standing (0 m $s^{-1}$) and turbulent (0.38 m $s^{-1}$) conditions as described above. An initial algal concentration of $1\times10^8$ cells $L^{-1}$ was added to each flask. Algal cells were collected by filtering through GF/C filters (Whatman, Maidstone, UK) at regular intervals during the experiment in order to estimate the decrease in inorganic P concentration in the modified D1 medium. Samples were measured at 1, 2, and 3 h for the P uptake experiment. All treatments were conducted in triplicate. P concentrations were measured colorimetrically according to Murphy and Riley [45].

The P uptake rate ($\rho$; micromoles of P per milligram of C per hour) was calculated from the difference between the initial concentration and the final concentration. The carbon biomass of each strain was estimated using cell volume [71]: $\log_{10} C=0.758 \log_{10} V-0.422$ (for diatoms), where $C$ is cell carbon in picograms and $V$ is cell volume in cubic micrometers. The results were fitted to the Michaelis–Menten equation [46].

$$\rho = \rho_{\max} \times \frac{S}{(K_m + S)}$$

where $\rho_{\max}$ is the maximum uptake rate (micromoles of P per milligram of C per hour), $S$ is the ambient P concentration (micromoles), and $K_m$ is the half-saturation constant of P uptake (micromoles).

Growth Parameters

*Growth Rates*

The specific growth rate of each strain under different treatments was determined by using the exponential growth equation [54]:

$$\mu = \frac{\ln B_{t2} - \ln B_{t1}}{\Delta t}$$

where $\mu$ is the specific growth rate (per day), $B$ the cell concentration (cell per liter), and $t$ is time in days. The cell concentrations were counted using counting chambers under a microscope.

*Alkaline Phosphatase Activity*

Under P-limited conditions, phytoplankton synthesize the enzyme AP, which hydrolyzes a phosphate group from organic molecules [6]. The expression of APA is commonly used as an indicator of P deficiency in algae [8, 22]. Extracellular APA was determined using the colorimetric method with *p*-nitrophenyl phosphate (*p*NPP; Sigma) as the substrate [56]. We added 200 μL 3.6 mM *p*NPP with 2 mM $MgCl_2$ in 0.2 M Tris–HCl buffer (pH 8.5) to 1 mL of culture. The final volume was 3 mL. Vials were incubated at 37°C for 2 h, and the incubation was then terminated by the addition of 300 μL 4 M NaOH. The samples were centrifuged (10,000 rpm, 10 min), and the supernatant was used to determine the extracellular APA. Buffer without a sample was used as the control. Absorbance was read at 410 nm in a spectrophotometer and compared to the standard absorbance curve for *p*-nitrophenol (*p*NP) [65]. The increase in optical density (OD) with time was converted to activity units using a standard curve prepared with the end product of the reaction. Enzyme activity was expressed in terms of micromoles of *p*NP released per milligram of C per hour.

*Chlorophyll a Fluorescence*

The maximum photochemical efficiency of PS II was measured using a pulse-amplitude-modulated fluorescence monitoring system (PAM; Walz, Effeltrich, Germany).

$$\frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m}$$

where $F_0$ and $F_m$ are the minimal and maximal fluorescence yields of a dark-adapted sample, with all PS II reaction centers fully open or closed, respectively. $F_v$ is the variable fluorescence. Prior to fluorescence measurements, cells were dark-adapted for 15 min. The rapid light curves, which are based on measurements of the relative electron transport rates (ETRs), were derived from estimates of $\triangle F/F_m'$ (the operational quantum yield of PSII, $\Phi_{PSIIe}$). The numerical values of chlorophyll fluorescence of samples exposed to 12 intensities of actinic light ranging from 0 to 1265 μmol photons PAR $m^{-2}$ $s^{-1}$ were recorded during a 3-min time series. Photosynthetic ETR was calculated using the following formula:

$$\mathrm{ETR} = \frac{(F_m' - F_t)}{F_m} \times 0.84 \times 0.5 \times \mathrm{PAR}(\mathrm{m}^{-2}\ \mathrm{s}^{-1})$$

where $F_m'$ and $F_t$ denote the maximum and steady-state fluorescence in light, respectively. The nominal amount of incident light absorbed by the algae was 0.84, of which approximately 0.5 was transferred to PSII [76].

*Pigment Analysis*

A 5-mL cell suspension was centrifuged at 10,000 rpm for 10 min. The supernatant was decanted and the pellet was resuspended in 5 mL 90% acetone. After extraction, samples were kept overnight in the dark at 4°C. Afterward, the sample was centrifuged for 10 min at 10,000 rpm. The supernatant was collected and read at 665, 645, 635, and 450 nm. The concentration of chlorophyll *a* (Chl *a*) was calculated using the following equation [69]:

$$\text{Chl}\,a(\text{mg/L}) = 11.6\text{OD}_{665} - 1.31\text{OD}_{645} - 0.14\text{OD}_{630}.$$

Statistics

Statistica 6.0 (Statsoft, Tulsa, OK, USA) was used for statistical analyses, and Origin 7.5 (Microcal Software, Northampton, MA, USA) was used for graphical plotting. Growth rate, APA, $F_v/F_m$, and $\text{ETR}_{max}$ of the species were compared using two-way analysis of variance (factorial ANOVA) to evaluate any significant differences among the treatments. ANOVA effects and treatment differences were considered significant at $P<0.05$. Regression analyses were used to quantify relationships between algal phosphate uptake rates and the external phosphate concentration.

## Results

Field Study

Spring diatom blooms occurred in Lake Taihu and the Hanjiang River during the study period (Table 2). Diatom density in the Hanjiang River was greater than that in Lake Taihu, especially in January, when the density reached $1.4\times 10^7$ cells $L^{-1}$. Only minor differences in environmental factors such as water temperature, pH, and DO between Lake Taihu and Hanjiang River were found during our monitoring. The dominant species were *C. meneghiniana* and *S. hantzschii* in Lake Taihu and Hanjiang River, respectively. Concentrations of COD, TN, TP, and $NH_4$-N differed between Lake Taihu and the Hanjiang River ($P<0.1$). The average flow volume (500–600 $m^3\ s^{-1}$) or velocity (0.3–0.4 m $s^{-1}$) during spring in 2008 was much lower than the normal flow (above 1,000 $m^3\ s^{-1}$ or 0.6 m $s^{-1}$) in the corresponding period in the Hanjiang River.

Laboratory Experiments

*P Uptake*

In order to examine the phosphate affinity of the different species, P uptake rates were analyzed as a

**Table 2** Physical, chemical, and biological parameters for Meiliang Bay, Lake Taihu, and Lower reach of Hanjiang River

| | Water temperature (°C) | Flow rate ($m^3\ s^{-1}$/m $s^{-1}$) | pH | DO (mg $L^{-1}$) | COD (mg $L^{-1}$) | TP (mg $L^{-1}$) | TN (mg $L^{-1}$) | $NH_4$-N (mg $L^{-1}$) | Diatom density ($10^7$ cells $L^{-1}$) | Dominant species |
|---|---|---|---|---|---|---|---|---|---|---|
| Meiliang Bay, Lake Taihu | | | | | | | | | | |
| January | 4.05 | Data not shown | 8.09 | 11.66 | 4.97 | 0.13 | 4.61 | 1.47 | 0.28 | *Cyclotella meneghiniana* |
| February | 6.50 | | 7.98 | 9.75 | 6.41 | 0.31 | 6.80 | 3.11 | 0.34 | |
| March | 14.10 | | 7.98 | 9.90 | 5.47 | 0.16 | 6.14 | 1.37 | 0.02 | |
| Lower reach of Hanjiang River | | | | | | | | | | |
| January | 5.65 | 500–600 $m^3\ s^{-1}$ | 7.92 | 9.77 | 2.24 | 0.10 | 2.35 | 0.24 | 1.41 | *Stephanodiscus hantzschii* |
| February | 6.25 | or 0.3 m $s^{-1}$[a] | 7.87 | 9.77 | 3.17 | 0.11 | 2.40 | 0.29 | 3.60 | |
| March | 11.33 | | 8.02 | 10.72 | 3.28 | 0.08 | 3.55 | 0.21 | 2.31 | |

[a] Flow rates reach above 1,000 $m^3\ s^{-1}$ or 0.6 m $s^{-1}$ during the normal-flow year

function of external phosphate concentrations. The kinetic parameters for P uptake ($K_m$ and $\rho_{max}$) are shown in Table 3. We found much higher $K_m$ for lentic diatoms compared with lotic diatoms including *S. hantzschii*. The $K_m$ values for lotic diatoms *C. atomus* and *N. palea* were decreased more significantly (nearly 1/2) by small-scale turbulence compared to standing conditions, except for *C. meneghiniana*, which showed an increase in $K_m$ values (Table 3). The dominant diatom in the Hanjiang River during spring, *S. hantzschii*, showed a small increase in $K_m$ in turbulent conditions compared to that under standing condition.

### *Specific Growth Rates*

The growth rates are shown in Fig. 2, and the statistical results are shown in Table 4. Under identical turbulent condition, the specific growth rates in lentic diatoms were lower under P-limited than under P-replete conditions ($P<0.05$). However, except for *N. palea*, the other three lotic diatoms showed no significant difference between P-limited and P-replete conditions under standing condition. With small-scale turbulence, the growth rates of the lotic diatoms *C. atomus*, *F. crotonensis*, and *N. palea* significantly increased ($P<0.05$). The growth rate of lotic diatom *S. hantzschii* and the lentic diatoms (*C. meneghiniana*, *M. varians*, and *S. minutulus*) declined strongly with turbulence ($P<0.05$). The interaction analysis results (Table 4) indicated that P concentration ($P<0.05$) and turbulent condition ($P<0.001$) both can impact on the growth rates of three lentic diatoms (*C. meneghiniana*, *M. varians*, and *S. minutulus*) and the growth rates in *C. meneghiniana* and *S. minutulus* determined by interactive effect of P concentration and turbulence. In contrast, the growth rates of three lotic diatoms (*C. atomus*, *F. crotonensis*, and *S. hantzschii*) were strongly dependent on turbulent conditions ($P<0.001$) rather than P concentrations. Only the growth of *N. palea* was related with interactive effect of P concentration and turbulence.

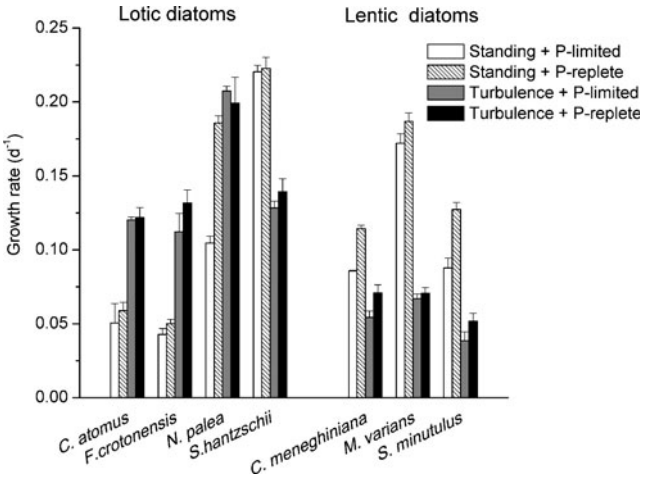


**Figure 2** Specific growth rate of four lotic diatoms and three lentic diatoms in the logarithmic growth phase at varying P concentrations and hydrodynamic regimes

**Table 3** Comparison of uptake kinetic parameters for phosphate in lotic and lentic diatoms (20°C)

| Algae (Exp conditions) | $K_m$ (μM P) | $\rho_{max}$ (μM P mg $C^{-1}$ $h^{-1}$) | $Q_0$ (μM P mg $C^{-1}$) | $V_{max}$ ($h^{-1}$) | $V_{max}/K_m$ (μM $P^{-1}$ $h^{-1}$) |
|---|---|---|---|---|---|
| Lotic diatoms | | | | | |
| *C. atomus* Sta | 10.98 | 6 | 4.79 | 1.25 | 0.11 |
| *C. atomus* Tur | 4.21 | 6.64 | 4.79 | 1.39 | 0.33 |
| *F. crotonensis* Sta | 3.25 | 0.24 | 0.77 | 0.31 | 0.10 |
| *F. crotonensis* Tur | 2.19 | 0.11 | 0.77 | 0.14 | 0.07 |
| *N. palea* Sta | 14.64 | 0.87 | 0.21 | 4.14 | 0.28 |
| *N. palea* Tur | 4.65 | 0.48 | 0.21 | 2.29 | 0.49 |
| *S. hantzschii* Sta | 4.3 | 1.24 | 0.52 | 2.38 | 0.55 |
| *S. hantzschii* Tur | 5.86 | 0.86 | 0.52 | 1.65 | 0.28 |
| Lentic diatoms | | | | | |
| *C. meneghiniana* Sta | 9.42 | 2.24 | 0.34 | 6.59 | 0.70 |
| *C. meneghiniana* Tur | 17.25 | 1.91 | 0.34 | 5.62 | 0.33 |
| *M. varians* Sta | 14.22 | 2.19 | 1.1 | 1.99 | 0.14 |
| *M. varians* Tur | 17.73 | 1.24 | 1.1 | 1.13 | 0.06 |
| *S. minutulus* Sta | 13.18 | 5.32 | 3.68 | 1.45 | 0.11 |
| *S. minutulus* Tur | 12.87 | 4.29 | 3.68 | 1.17 | 0.09 |

*Sta* standing (0 m $s^{-1}$), *Tur* turbulence (0.38 m $s^{-1}$), $K_m$ the half-saturation constant, $\rho_{max}$ the maximum P uptake rate, $Q_0$ the minimum cell quota, $V_{max}$ $\rho_{max}/Q_0$, $V_{max}/K_m$ the competitive ability for P uptake

**Table 4** Significant differences of specific growth rate ($\mu$) and AP activity (APA) parameters between different P regimes and hydrodynamics regimes

| | Lotic diatoms | | | | Lentic diatoms | | |
|---|---|---|---|---|---|---|---|
| | *C. atomus* | *F. crotonensis* | *N. palea* | *S. hantzschii* | *C. meneghiniana* | *M. varians* | *S. minutulus* |
| Specific growth rate ($\mu$) | | | | | | | |
| P concentration effect | $F$=1.2 | $F$=0.51 | $F$=44.2 | $F$=3 | $F$=112.4 | $F$=10 | $F$=61.6 |
| | $P$=0.307 | $P$=0.054 | $P$=0.000*** | $P$=0.121 | $P$=0.000** | $P$=0.013* | $P$=0.000*** |
| Turbulence effect | $F$=205.7 | $F$=263 | $F$=112.4 | $F$=532.6 | $F$=306.1 | $F$=1,441 | $F$=344 |
| | $P$=0.000*** | $P$=0.000*** | $P$=0.000*** | $P$=0.000*** | $P$=0.000*** | $P$=0.000*** | $P$=0.000*** |
| Interaction effect | $F$=0.5 | $F$=0.4 | $F$=66 | $F$=1.3 | $F$=7.8 | $F$=4 | $F$=15.4 |
| | $P$=0.481 | $P$=0.561 | $P$=0.000** | $P$=0.283 | $P$=0.023* | $P$=0.096 | $P$=0.004** |
| APA | | | | | | | |
| P concentration effect | $F$=8.014 | $F$=28.16 | $F$=119.6 | $F$=191.8 | $F$=16.5 | $F$=78.94 | $F$=491.9 |
| | $P$=0.022* | $P$=0.001** | $P$=0.000*** | $P$=0.000*** | $P$=0.004** | $P$=0.000*** | $P$=0.000*** |
| Turbulence effect | $F$=2.544 | $F$=0.07 | $F$=0.7 | $F$=8.7 | $F$=141.4 | $F$=96 | $F$=46.4 |
| | $P$=0.149 | $P$=0.794 | $P$=0.43 | $P$=0.018* | $P$=0.000*** | $P$=0.000*** | $P$=0.000*** |
| Interaction effect | $F$=0.074 | $F$=2.2 | $F$=0.6 | $F$=7.9 | $F$=9.9 | $F$=10.94 | $F$=59.6 |
| | $P$=0.793 | $P$=0.176 | $P$=0.444 | $P$=0.023* | $P$=0.015* | $P$=0.011* | $P$=0.000*** |

$\alpha$=0.05, 0.01, 0.001 (factorial, ANOVA) were used to assess statistical significance

*$P$<0.05; **$P$<0.01; ***$P$<0.001

### *Alkaline Phosphatase Activity*

Extracellular APAs were affected differently by varying P concentration and turbulent conditions in the lotic and lentic diatoms (Fig. 3). Small-scale turbulence led to greater APA in lentic diatoms and *S. hantzschii* at low P concentrations compared to that under standing condition ($P$<0.05) (Table 4). The interaction analysis results (Table 4) indicated that APA in lentic diatoms and *S. hantzschii* were significantly affected by both P concentration and turbulent condition and their interaction while APA in three lotic diatoms (*C. atomus*, *F. crotonensis*, and *N. palea*) were only influenced by P concentration.

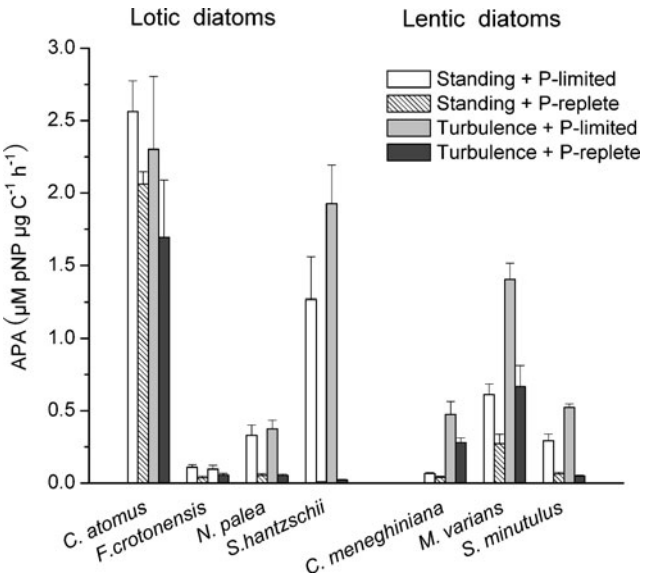


**Figure 3** Alkaline phosphatase activity of four lotic diatoms and three lentic diatoms grown for 18 days at varying P concentrations and hydrodynamic regimes

### *Photosynthetic Parameters and Cellular Chl a Concentration*

The maximum effective quantum yield of PSII ($F_v/F_m$) was measured under standing and turbulent conditions combined with two P concentrations at initial concentrations of 0.2 and 2 mg $L^{-1}$ (Table 5). The effects of small-scale turbulence on the PSII activities were different between the lotic and lentic diatoms. In the turbulent treatment, we found an increase of $F_v/F_m$ values in lotic diatoms but a decline in $F_v/F_m$ values for lentic diatoms. The $F_v/F_m$ values of *S. hantzschii* showed little change in four treatments. On the other hand, the effects of P on the PSII activities of diatoms were also related to their habitats. The $F_v/F_m$ values in the lotic diatoms did not significantly change with varying initial P concentrations except *N. palea* during the experiments, and those of lentic diatoms increased at initial P concentration of 2 mg $L^{-1}$ under standing condition. An identical phenomenon was also observed for the maximum electron transport rates ($ETR_{max}$). We found that the $ETR_{max}$ values varied accordingly with the cellular Chl *a* contents in all the diatoms during the experiments (Fig. 4).

**Table 5** Initial and 18-day maximum potential quantum yields $F_v/F_m$

| $F_v/F_m$ | Initial | 18 days: Sta and P-limited | 18 days: Sta and P-replete | 18 days: Tur and P-limited | 18 days: Tur and P-replete |
|---|---|---|---|---|---|
| Lotic diatoms | | | | | |
| *C. atomus* | 0.46 | 0.43±0.05 | 0.45±0.01 | 0.60±0.01 | 0.64±0.03 |
| *F. crotonensis* | 0.40 | 0.37±0.03 | 0.45±0.01 | 0.67±0.02 | 0.63±0.02 |
| *N. palea* | 0.35 | 0.39±0.01 | 0.66±0.01 | 0.60±0.02 | 0.71±0.01 |
| *S. hantzschii* | 0.66 | 0.73±0.00 | 0.72±0.01 | 0.72±0.02 | 0.72±0.01 |
| Lentic diatoms | | | | | |
| *C. meneghiniana* | 0.3 | 0.31±0.04 | 0.55±0.02 | 0.34±0.04 | 0.5±0.02 |
| *M. varians* | 0.43 | 0.54±0.01 | 0.59±0.02 | 0.35±0.02 | 0.37±0.04 |
| *S. minutulus* | 0.5 | 0.47±0.02 | 0.60±0.02 | 0.44±0.05 | 0.48±0.02 |

*Sta* standing (0 m $s^{-1}$), *Tur* turbulence (0.38 m $s^{-1}$)

## Discussion

### Comparison Between Diatom Blooms in Lake Taihu and Hanjiang River

In the field study, concentrations of TP and TN differed between Lake Taihu and the Hanjiang River during diatom blooms. This finding is consistent with results summarized from previous studies showing that diatom blooms usually occur in lakes with higher concentration of TP (0.15–0.2 mg $L^{-1}$) whereas in rivers at a wide range of TP concentrations (0.02–0.22 mg $L^{-1}$) (Table 6). This implies that elevated nutrient concentration, especially TP and TN, is a necessary and sufficient condition for diatom (especially for the centric diatoms) bloom formation in the lentic systems while elevated concentrations of TP and TN is a necessary but not sufficient condition for diatom blooms in lotic ecosystems. Hydrologic regime plays a prominent role in the distribution of algae in the freshwaters [31, 35]; this role is probably as important as nutrient concentration. Gosselain et al. [17] suggested that diatom communities seemed to develop in a characteristic way from the "relatively fast-flowing headwaters" to the downstream reaches. Therefore, the physiological mechanism of bloom formation of the same phytoplankton genus may differ in different habitats. In our study, centric diatom blooms occurred in the low-flow year of the Hanjiang River under lower nutrient concentrations than that in Lake Taihu, which suggests that diatoms develop different nutrient strategy to adapt to the change of hydrodynamics.

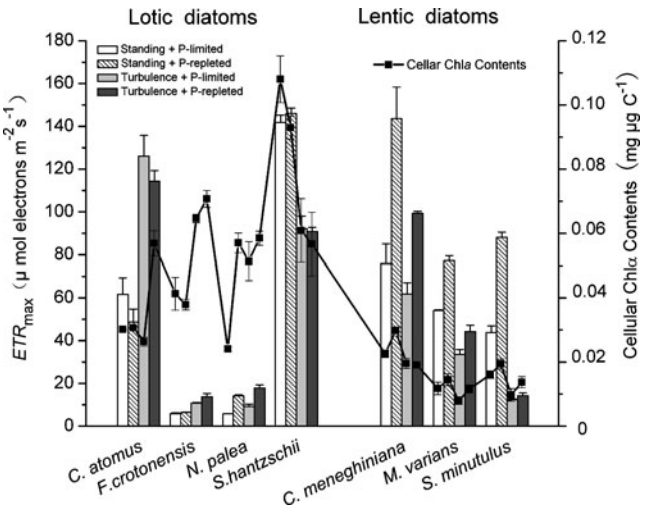


**Figure 4** $ETR_{max}$ and cellular Chl *a* contents of four lotic diatoms and three lentic diatoms grown for 18 days at varying P concentrations and hydrodynamic regimes

Turbulence exists in all kinds of water columns, to some extent, in terms of vertical exchange for resuspending sediments and certain flows. Reynolds et al. [59] demonstrated that artificial mixing in large enclosures could either prolong diatom dominance or induce new growth for diatoms. Such increases in phytoplankton biomass are related to new growth resulting from resuspended nutrients (such as TP and TN) in lentic waters, where typical mixing episodes in thermally stratified lake systems occur once or twice annually during destratification [2, 5, 64]. In some lakes, the inhibition of resuspension can decrease the nutrient release from sediments [64], and resuspension is associated with increases in the concentration of phytoplankton biomass and nutrients [27]. In addition, Lund [38] and Ferris and Lehman [13] both studied the contribution of artificial resuspension to the population dynamics of diatoms in the enclosed areas. Resuspending sediments can increase the nutrient content in the water column and thereby stimulate nutrient-limited algal growth in a few days. In the shallow lakes, small-scale turbulence can diminish the nutrient stratification and release amounts of nutrients from the sediments, which might favor the growth

**Table 6** Selected occurrences of algal blooms and site characteristics from previous studies

| Type of water body | Predominant algae | Biomass or cell density | Mean TP (mg $L^{-1}$) | Mean flow rate (m/s, mL $day^{-1}$ or $m^{-3}$ $s^{-1}$) | Name of water body | Reference |
|---|---|---|---|---|---|---|
| Lentic water | Centric diatoms | ≈10 mg $L^{-1}$ (biomass) | Data not shown | <0.01 m $s^{-1}$ | Spremberg impoundment (upstream of Spree River), Germany | [31] |
| | *Cyclotella* sp. | ≈3 mg $L^{-1}$ (biomass) | 0.15–0.2 | Data not shown | Donghu Lake, China | [78] |
| | *Cyclotella* sp. | 1–2 mg $L^{-1}$ (biomass) | 0.2 | Data not shown | Wuli Lake, China | [68] |
| | *Aulacoseira* sp., *Stephanodiscus neoastrae* | ≈15.7 mg $L^{-1}$ (biomass) | Data not shown | <0.01 m $s^{-1}$ | Müggelsee Lake, Belgium | [31] |
| | *Cyclotella meneghiniana* | >1×$10^6$ cell $L^{-1}$ | 0.03 (SRP) | Data not shown | Three Gorges Reservoir, China | [79] |
| Lotic water | *Stephanodiscus hantzschii* | >1.1×$10^8$ cell $L^{-1}$ | 0.049 | <298 $m^{-3}$ $s^{-1}$ | Nakdong River, South Korea | [20] |
| | *Thalassiosira* sp. | ≈20 μg $L^{-1}$ (Chl *a*) | 0.3 (SRP) | 30 $m^{-3}$ $s^{-1}$ | Middle of San Joaquin River, USA | [35] |
| | *Stephanodiscus hantzschii* | 50–100 μg $L^{-1}$ (Chl *a*) | >0.22 | >28 $m^{-3}$ $s^{-1}$ | Meuse River, Belgium | [17, 73] |
| | *Cyclotella meneghiniana* | ≈30 μg $L^{-1}$ (Chl *a*) | 0.1 (SRP) | 26 $m^{-3}$ $s^{-1}$ | Downstream of San Joaquin River, USA | [35] |
| | *Cyclotella* sp. | >4×$10^6$ cell $L^{-1}$ | 0.08 | 0.213 m $s^{-1}$ | Hanjiang River, China | [9] |
| | *Stephanodiscus hantzschii* | >1×$10^7$ cell $L^{-1}$ | 0.103 | 0.14 m $s^{-1}$ | Dongjin River, China | Yang et al. (unpublished data) |
| | *Cyclotella* sp., *Nitzschia* sp. | >1×$10^8$ cell $L^{-1}$ | <0.02 (SRP) | <300 mL $d^{-1}$ | Hunter River, Australia | [41] |
| | *Cyclotella meneghiniana* | ≈30 μg $L^{-1}$ (Chl *a*) | 0.1 (SRP) | 26 $m^{-3}$ $s^{-1}$ | Downstream of San Joaquin River, USA | [35] |
| | *Achnanthidium minutissimum*, *Cocconeis pediculus* | ≈2.88 μg $L^{-1}$ (Chl *a*) | 0.046 | mean 40 $m^{-3}$ $s^{-1}$ | Nicolet River, Canada | [33] |
| | *Achnanthidium minutissimum* | Data not shown | 0.07 | 0.7 m $s^{-1}$ | Yulong River, China | [74] |
| | *Nitzschia amphibia* | Data not shown | 0.1 | 0.4 m $s^{-1}$ | Bao River, China | [74] |
| | *Nitzschia* sp., *Diatoma* sp. | 0.63–8.95 mg $L^{-1}$ (biomass) | Data not shown | 0.5–0.6 m $s^{-1}$ | Spree River, Germany | [31] |
| | *Nitzschia acicularis*, *Aulacoseira ambigua*, *Cyclotella* sp. | ≈20 μg $L^{-1}$ (Chl *a*) | <0.02 (SRP) | 480 $m^{-3}$ $s^{-1}$ | Meuse River, Belgium | [17] |
| | *Asterionella japonica* | ≈7.3 μg $L^{-1}$ (Chl *a*) | 0.002–0.01 (SRP) | Data not shown | Bay of Bangal, Indian | [7] |

of some diatoms [3, 44]. This may explain high nutrients concentration in Lake Taihu when diatom dominance occurred, even in the period without turbulence. On the other hand, every part of the river flow simultaneously differs from every other part and, so, is quite counter-intuitive: "Flowing" does not necessarily mean either "mixed" or "uniform" [58]. Bonilla et al. [4] found that differences in phytoplankton biomass, main taxonomic groups, and strategies were more relevant to hydrological dynamics than seasonal and spatial differences. Many field studies have demonstrated that the biomass of diatoms depend on the change of hydrological regimes in the lotic waters [20, 35, 41]. In the present study, the spring diatom blooms always occurred in the years with low flow rates in Hanjiang River. Changes in hydrological conditions have been shown to play a vital part in retaining water for long enough for cell replication [17, 57], while the mechanisms of diatom selection in rivers have not been yet studied adequately. Based on our field observations, flow regimes and nutrients level together determine species selection among different water bodies. Dominant species might develop their own special survival strategies in order to adapt to the surrounding environments. In this study, we illustrate the difference in responses in hydrodynamics in species from two habitats, as identified by P uptake, APA, and photosynthesis activity.

## Growth and Nutrient Strategies of Diatoms Under Turbulent Conditions

In the laboratory experiment, the specific growth rates for all diatoms between P-limited and P-replete conditions indicated that the growth of lentic diatoms was restricted under low P concentration while growth for lotic diatoms

was not limited. Even among the same genus, such as *C. atomus* and *C. meneghiniana*, *S. hantzschii*, and *S. minutulus*, diatoms expressed different nutrient strategies to cope with $P_i$-limited conditions. Fu et al. [14] found that the diazotrophic cyanobacterium *Trichodesmium* isolated from the North Atlantic Ocean (IMS101) is better adapted to growing on low $P_i$ concentration than that from the $P_i$-enriched environment Great Barrier Reef, Australia (GBRTRLI101). We found that the different hydrological regimes can affect the growth. Under both P-limited or P-replete conditions, small-scale turbulence had a negative impact on the growth rates of the lentic diatoms *C. meneghiniana*, *M. varians*, and *S. minutulus* and the Hanjiang River spring bloom-forming diatom *S. hantzschii* but favored the growth of the lotic diatoms *C. atomus*, *F. crotonensis*, and *N. palea*, indicating that hydrodynamics also plays an important role in the occurrence of diatom blooms. Some previous laboratory studies reported that growth of some algae was not affected by turbulence when $P_i$ was sufficient [1, 51, 63], which is similar to our results for *N. palea*. Our results indicate that *N. palea* can be affected by turbulence and P concentration, suggesting that *N. palea* can grow quickly under either disturbed or nutrient rich conditions.

The lentic and lotic diatoms showed significantly habitat-dependent growth characteristic responses to $P_i$-limitation, which was dependent on P strategy. Although the connection between nutrients uptake and growth in algae is not strong on an instantaneous scale, it is on the scale of generation times where uptake is approximately similar to growth + excretion [10]. The half-saturation constant ($K_m$) is an index of the affinity to nutrient and reflects the relative ability of phytoplankton to use nutrients at low levels [11]. According to Table 3, the affinities for $P_i$ at low concentration in the lotic diatoms were much higher than those in the lentic diatoms, which demonstrated that the diatoms from Hanjiang River can survive in the lower P concentration waters compared to those lentic diatoms from nutrient rich water bodies. $K_m$ values for phosphate uptake tend to be higher with cell size [44]. Experimental and theoretical evidence demonstrates that smaller cells have higher rates of nutrient uptake per unit biomass and lower $K_m$ values [26]. In the present study, we found the same result in the lotic diatoms where the smaller cell sized diatom *C. atomus* had the lower $K_m$ value than *S. hantzschii*. Small cells have a lower minimum cellular metabolic requirement that allows them to grow at much lower resource concentrations than larger cells [19, 66]. But for lentic diatoms, there was not any relationship between cell size and P affinity. In addition, the growth rates of these three lotic diatoms (*C. atomus*, *F. crotonensis*, and *N. palea*) were improved by turbulence, which can be partially explained by the higher affinity for $P_i$ under turbulent condition than under the standing condition, probably because small-scale turbulence can enhance the diffusive transport of nutrients toward phytoplankton cells [29, 75], consequently favoring $P_i$ uptake by the algal cells. Enhancements of $P_i$ affinity by turbulence was also studied by Maar et al. [39] and Savidge [61]. $V_{max}/K_m$ is also considered as a better measure to evaluate the competitive ability for nutrient at low concentration [21]. Higher $V_{max}/K_m$ values were found for lotic diatoms *C. atomus* and *N. palea* under turbulence, suggesting that lotic diatoms have greater competition for $P_i$ in small-scale turbulent condition than in the standing condition. This nutrient competitive advantage facilitates competition for resources in rivers [50]. Diatom blooms occurred both in Lake Taihu and Hanjiang River, indicating that the different dominant diatoms developed respective nutrient strategies in the corresponding environments.

Increasing APA of diatoms is another physiological mechanism to adapt to environments with the P-limitation. The results for the APA values can be used to explain the low growth rate of the lentic diatoms *C. meneghiniana*, *M. varians*, and *S. minutulus* and the Hanjiang River spring diatom *S. hantzschii* under turbulence. The greater APAs were induced by the small-scale turbulence in lentic diatoms and *S. hantzschii* both at low and high $P_i$ concentration compared with under standing conditions, indicating that turbulence can lead to the change of $P_i$ demand in lentic diatoms and *S. hantzschii*. This implies that lentic diatoms and *S. hantzschii* need higher concentration of P for their growth under turbulence than standing condition. Previous research showed that APAs are affected by cell size [23] and that turbulence increased APA more significantly in *T. pseudonana* (6 μm in diameter) than in *Coscinodiscus* sp. (109 μm in diameter) [52]. In our study, however, this so-called cell size dependence was not observed. Moreover, the change of APA value between standing and turbulent conditions also showed habitat-dependent characteristics, although the mechanisms behind this need further investigation.

## Chlorophyll *a* Content and Photosynthetic Parameters

The change in cellular chlorophyll *a* concentrations under turbulence was species-specific: An increase occurred for *C. atomus*, *F. crotonensis*, and *N. palea* and a decrease in *S. hantzschii*, *C. meneghiniana*, and *S. minutulus* and no change for *M. varians*. A decrease in cellular chlorophyll *a* concentration under P-limitation has been observed for both marine and freshwater microalgae [15, 36, 37, 77]. We found that the chlorophyll *a* content in some lotic diatoms increased with small-scale turbulence even under P-limited condition while lentic diatoms showed the opposite response to turbulence. Thus, turbulence is also an important factor which can impact pigment content of algae. Mixing in the water column enhances the degree of

self-shading of algal cells and cellular chlorophyll *a* content due to self-shading [72]. The increase in cellular chlorophyll *a* concentration under self-shading appears to be similar to the increase under lower irradiance and has been observed in other species, such as the cyanobacterium *Oscillatoria agardhii* [55].

The photosynthetic efficiency ($F_v/F_m$) and maximum electron transport rates ($ETR_{max}$) indicate that PS II function are also considered as one of the most sensitive photosynthetic parameters to environmental stress [76]. Many studies [32, 49, 65] observed low values of $F_v/F_m$ in algae coincide with low surrounding P concentration. Under standing condition, both the lentic diatom *C. meneghiniana* and *S. minutulus* and the lotic diatom *N. palea* showed a reduction in the PS II quantum efficiency when $P_i$ was depleted, indicating that an important portion of the PS II reaction center might have been damaged. However, small-scale turbulence increased photosynthetic efficiency of the lotic diatom *C. atomus*, *F. crotonensis*, and *N. palea*, suggesting that turbulence imparts a positive effect on the structure of PS II in these species because they are characterized by a high $P_i$ affinity under turbulence. High photosynthetic efficiency in algae might reflect high metabolism. Lotic diatoms have higher metabolic rates under turbulence than under standing condition, which supported the photosynthetic parameters dependence of the growth rate and P affinity. Thus, they can maintain dominance for long periods under flowing conditions and fluctuating P concentrations. In contrast, the PSII function in lentic water algal cells was inhibited both by turbulence and $P_i$ concentration, which allows them to bloom and dominate the phytoplankton community only in standing eutrophic water bodies.

## Conclusions

In our field study, we found that both nutrient and hydrological regimes contributed to the freshwater diatom blooms. The dominant diatoms in lotic and lentic waters are not identical, and they bloom under different nutrient environments and hydrodynamics. By means of growth rate, P uptake, APA, and photosynthetic parameters in the laboratory experiment, isolated diatom cultures displayed different physiological responses to phosphorus concentration under different hydrological conditions. The lotic water diatom *C. atomus*, *F. crotonensis*, and *N. palea* were better growth competitors under small-scale turbulence both in $P_i$-limited and $P_i$-replete conditions while lotic water diatom *S. hantzschii* became dominant during calm periods in $P_i$-limited conditions. In contrast, the lentic water diatom *C. meneghiniana*, *M. varians*, and *S. minutulus* had a competitive advantage in the $P_i$-replete standing waters but were obviously suppressed by $P_i$-limitation and turbulence. These indicate that hydrodynamic conditions can substantially change phosphorus strategies in diatoms. Our study added additional support to the hypothesis that habitat-dependent physiological characteristics in diatoms are important for understanding the mechanism of diatom blooms formation in various hydrodynamic regimes. More detailed studies are needed on the physiological responses of diatom species to different hydrodynamics regimes and P status.

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