Influence of Phosphorus and Silicon on Competitive Interaction between *Microcystis aeruginosa* and *Cyclotella* sp. by Monoculture and Competitive Biculture Experiments

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Abstract

In Lake Teganuma, the shift of dominant species from blue-green algae (*Microcystis aeruginosa* [*M. aeruginosa*]) to diatoms (*Cyclotella* sp.) has been observed since 2000 as a result of the discharge of Tone River water into Lake Teganuma through the North-chiba Water Conveyance Channel. In this study, the influence of phosphorus fluctuation under relatively high silicon concentration, reflecting the condition in Lake Teganuma after 2000, on the shift of dominant species was experimentally examined through individual monoculture and competitive biculture experiments. The results from monoculture experiment showed that the half-saturation constant and uptake rate of phosphorus for *M. aeruginosa* was respectively lower and faster than that for *Cyclotella* sp., representing that *M. aeruginosa* possesses much higher growth potential than *Cyclotella* sp. under the phosphorus limited condition. The outcome of competitive biculture experiment obviously reflected these results, i.e., dominance of *M. aeruginosa* with a proportion of 99 % in total cell. Therefore, phosphorus fluctuation under the high silicon concentration could be interpreted as a minor factor for the shift of dominant species, from *M. aeruginosa* to *Cyclotella* sp. as observed in Lake Teganuma.

Key words: *Microcystis*, *Cyclotella*, growth kinetics, competition, Lake Teganuma

INTRODUCTION

The occurrence of harmful blooms formed by blue-green algae in eutrophic lakes has often caused serious problems, such as fish mortality¹⁾, toxin production²⁾ and growth inhibition of aquatic macrophytes by shading³⁾. Taste and odor problems, especially in lakes used for drinking water, have also been raised from harmful blooms, posing serious threats to animals and humans⁴⁾.

Control of harmful blooms as well as reduction of eutrophication level has therefore been required for ecosystems and water environment in lakes.

Although blue-green algae form dense blooms and become dominant species in summer, change in dominant species is often observed in field and laboratory observations. In temperate lakes, algal species such as diatoms and/or green algae are observed as dominant species replacing blue-green algae

in spring and winter⁵⁾. Takamura et al.⁶⁾ showed that dominant species in Lake Kasumigaura (Ibaraki prefecture, Japan) shifted from blue-green algae, mainly *Microcystis* to *Phormidium*, according to increasing TN:TP mass ratio, in which transition was experimentally demonstrated by Fujimoto et al.⁷⁾ There has also been a report that drastic change in water environment as a consequence of water purification caused a shift of dominant species. For example, in Lake Teganuma (Chiba prefecture, Japan), the discharge of Tone River water through the North-chiba Water Conveyance Channel consequently caused the shift of dominant species from blue-green algae, mainly *Microcystis aeruginosa* (*M. aeruginosa*), to diatoms, *Cyclotella* sp.⁸⁾ The shift of dominant species could be considered as an influence of interaction among environmental parameters such as change in nutrient concentrations, light intensity and temperature. So far, this mechanism has not yet been understood in detail.

This study aimed to obtain fundamental information to understand how environmental factors changed by the discharge of Tone River water into Lake Teganuma affect the shift of dominant species from blue-green algae to diatoms. In general, phosphorus is acceptable as a major nutrient limiting growth of blue-green algae, on the other hand, silicon can promote growth of diatoms. In this study, influence of both nutrients on the growth of *M. aeruginosa* and *Cyclotella*

sp. was investigated through individual monoculture experiment to know their growth characteristics and through competitive biculture experiment to see their competitive ability, and then we discussed which species can become superior competitor under phosphorus and silicon limited conditions.

STUDY SITE

Outline of Lake Teganuma and the North-chiba Water Conveyance Channel Lake Teganuma, one of the eutrophic lakes in Japan, is located in the northwest part of Chiba prefecture. Water surface area, water volume and mean depth are about 6.5 km², 5.6 × 10⁶ m³ and 0.9 m, respectively. There are three river inflows to Lake Teganuma; Ohori, Otsu and Someiri River. In 2000, the North-chiba Water Conveyance Channel was constructed and large amount of water conveyed from the first pumping station near Sakae Bridge, Tone River, was discharged into Lake Teganuma for the purpose of purification.

The data of nutrient concentration and water volume on the river inflows as well as the channel were given in Table 1. Note that there are no available data for both phosphorus and silicon concentrations at the discharge point of the channel, we substituted both nutrient concentrations at Sakae Bridge near the first pumping station. Mean dissolved total nitrogen (D-TN), PO₄-P and SiO₂-Si concentrations for three river inflows before and after 2000 are practically same values except PO₄-P concentration for

Table 1 The data of nutrient concentration and water volume on the river inflows and the North-chiba Water Conveyance Channel

River inflow	1989 and 1998–1999 ^{9, 10)}				2000–2005 and 2007 ^{8, 10)}			
	D-TN (mg-N·l ⁻¹)	PO ₄ -P (mg-P·l ⁻¹)	SiO ₂ -Si (mg-Si·l ⁻¹)	WV*** (m ³ ·d ⁻¹)	D-TN (mg-N·l ⁻¹)	PO ₄ -P (mg-P·l ⁻¹)	SiO ₂ -Si* (mg-Si·l ⁻¹)	WV*** (m ³ ·d ⁻¹)
Ohori	5.0	0.55	16.9	80064	3.4	0.23	11.9	66240
Otsu	6.0	0.57	14.6	73440	5.6	0.23	14.1	15408
Someiri	5.0	0.09	13.4	10944	5.2	0.07	13.1	15840
Conveyance Channel	—	—	—	—	2.5**	0.06**	10.9	479120
Mean	5.3	0.40	15.0	54816	4.2	0.15	12.5	144152

* Authors (unpublished data)

** Data at Sakae Bridge near the first pumping station

*** WV: Water Volume

Someiri River. While, lower D-TN and, especially $\text{PO}_4\text{-P}$, concentrations than that for river inflows are discharged from the channel, although $\text{SiO}_2\text{-Si}$ concentration is nearly the same as river inflows.

The water volume discharged from the channel represented one order greater than that for each river inflow. When the inflow of nutrient concentration to Lake Teganuma after 2000 is assumed to be dependant with the water from the channel, it could be hypothesized that decrease in phosphorus concentration would limit growth of *M. aeruginosa* and that, in such the condition, *Cyclotella* sp. would become a superior competitor with a relatively high silicon concentration. This seems to be consistent with the experimental results that diatoms could become superior competitor under enough silicon and phosphorus limited conditions because of their much lower half-saturation constant of phosphorus than that for blue-green algae¹¹⁻¹³. Both individual monoculture and competitive biculture experiments were conducted to verify the influence of phosphorus and silicon conditions for the shift of dominant species from *M. aeruginosa* to *Cyclotella* sp. as observed in Lake Teganuma.

MATERIALS AND METHODS

Organisms *M. aeruginosa* and *Cyclotella* sp. cultures were obtained from the culture collection of algae at University of Texas (strain: UTEX 2061) and from the culture collection of algae and protozoa (strain: CCAP 1070/4), respectively. Both species were individually cultured in autoclaved WC medium¹⁴ (pH: 7.0) under temperature and light intensity of 20 °C and 2000 lx, respectively.

Characteristics of growth kinetics for *M. aeruginosa* and *Cyclotella* sp. in individual monoculture experiment Characteristics of growth kinetics for *M. aeruginosa* and *Cyclotella* sp. were examined in individual monoculture experiment in batch system.

WC medium with pH value of 7.0 was prepared with modifications; phosphorus concentration was reduced to 0.02, 0.1 and 0.5 $\text{mg-P}\cdot\text{l}^{-1}$ and silicon concentration was increased to 11 $\text{mg-Si}\cdot\text{l}^{-1}$. Phosphorus

concentration levels cover the concentrations of river inflows before and after 2000 and the level of silicon reflects the concentration of water discharged from the channel. Each medium of 200 ml in 500 ml Erlenmeyer flasks was sterilized by autoclaving.

As a preliminary experiment, *M. aeruginosa* and *Cyclotella* sp. were grown for 10 days in WC medium with phosphorus concentration of 0.1 $\text{mg-P}\cdot\text{l}^{-1}$ to allow them phosphorus depletion. For individual monoculture experiment, P-depleted cells of both species were then individually inoculated into sterile medium to give an initial density of 5000 $\text{cells}\cdot\text{ml}^{-1}$. The test flasks were placed in incubator in which the temperature was controlled at 20 °C and light intensity was provided at 2000 lx by 14 h light : 10 h dark cycle with cool-white fluorescent lighting. In experimental term, the flasks were stirred by hand several times per day. The monoculture experiment was conducted once and the counting for both species in each experimental day was carried out in duplicate.

To evaluate growth characteristics for *M. aeruginosa* and *Cyclotella* sp. under phosphorus limited condition, maximum growth rate, μ_m (d^{-1}), and half-saturation constant, K_p ($\text{mg-P}\cdot\text{l}^{-1}$), were examined. Growth rate, μ (d^{-1}), for both species in each phosphorus concentration level was determined, based on Eq. (1), from relationship between cell density and cultivation time during exponential growth phase, then the μ_m and K_p were estimated from Eq. (2) by linear regression analysis.

$$\mu = \frac{\ln C_2 - \ln C_1}{t_2 - t_1}, \quad (1)$$

$$\mu = \frac{\mu_m \cdot P}{K_p + P}, \quad (2)$$

where C_2 and C_1 are the cell density ($\text{cells}\cdot\text{ml}^{-1}$) at cultivation time of t_2 (d) and t_1 (d), respectively, and P is the initial phosphorus concentration ($\text{mg-P}\cdot\text{l}^{-1}$) for the experiment.

Phosphorus uptake rate (V_p , $\text{mg-P}\cdot\text{cell}^{-1}\cdot\text{d}^{-1}$) for *M. aeruginosa* and *Cyclotella* sp. was also examined. Sterile 500 ml of WC medium with phosphorus concentration of 0.1 mg-

$P \cdot t^{-1}$ and silicon concentration of $11 \text{ mg-Si} \cdot t^{-1}$ was prepared in 1000 ml Erlenmeyer flask, then the medium was individually inoculated into P-depleted cultures of *M. aeruginosa* and *Cyclotella* sp. to give an initial density of $5000 \text{ cells} \cdot \text{ml}^{-1}$. Details for incubation conditions were the same as above mentioned. Duration of experiment was 6 days. Similar to the monoculture experiment, the single experiment for phosphorus uptake was conducted with duplication of measurements for both cell counting and phosphorus concentration.

Based on the phosphorus concentrations in cultivation time of day 0, 4 and 6, phosphorus uptake rate was calculated by

$$V_p = \frac{P_1 - P_2}{t_2 - t_1} \cdot \frac{1}{C_2 - C_1}, \quad (3)$$

where P_1 and P_2 are the phosphorus concentration ($\text{mg-P} \cdot t^{-1}$) at cultivation time of t_1 (d) and t_2 (d), respectively. C_2 and C_1 are respectively the cell density ($\text{cells} \cdot \text{ml}^{-1}$) at cultivation time (d) of t_2 and t_1 .

Competitive ability of *M. aeruginosa* and *Cyclotella* sp. in competitive biculture experiment Competitive ability for *M. aeruginosa* and *Cyclotella* sp. were examined in competitive biculture experiment under the phosphorus limited and abundant silicon conditions.

Sterile WC medium with phosphorus concentration of $0.1 \text{ mg-P} \cdot t^{-1}$ and silicon concentration of $11 \text{ mg-Si} \cdot t^{-1}$ was inoculated in P-depleted culture mixture of both *M. aeruginosa* and *Cyclotella* sp. Initial cell densities were $5000 \text{ cells} \cdot \text{ml}^{-1}$ for each species. Culture conditions were the same as above. The competitive biculture experiment was conducted in triplicate and the counting in each experimental day was done in duplicate.

Measurements Cell number was counted using the plankton counting chamber (MPC-200, MATSUNAMI) and binocular microscope under magnification of $\times 300$. Phosphorus concentration was analyzed by molybdenum blue spectrophotometric method¹⁵⁾.

RESULTS

Characteristics of growth kinetics for *M. aeruginosa* and *Cyclotella* sp. Results of

individual monoculture experiment for both *M. aeruginosa* and *Cyclotella* sp. were shown in Fig. 1. Growth curves for *M. aeruginosa* were similar in both phosphorus conditions of 0.1 and $0.5 \text{ mg-P} \cdot t^{-1}$, reaching final cell density of approximately $2.0 \times 10^7 \text{ cells} \cdot \text{ml}^{-1}$. When phosphorus concentration was reduced to $0.02 \text{ mg-P} \cdot t^{-1}$, final cell density was much less ($1.3 \times 10^6 \text{ cells} \cdot \text{ml}^{-1}$) than that obtained in 0.1 and $0.5 \text{ mg-P} \cdot t^{-1}$. Having applied the cell density and cultivation time in each phosphorus condition to Eq. (1), maximum

Fig. 1 The growth curves for (a) *M. aeruginosa* and (b) *Cyclotella* sp. under the phosphorus limited and silicon concentration of $11 \text{ mg-Si} \cdot t^{-1}$ in individual monoculture experiment. The experiment was conducted once and the counting for both species in each experimental day was carried out in duplicate.

Symbols: ● $P=0.02 \text{ mg-P} \cdot t^{-1}$ (*M. aeruginosa*), ○ $P=0.02 \text{ mg-P} \cdot t^{-1}$ (*Cyclotella* sp.), ▲ $P=0.1 \text{ mg-P} \cdot t^{-1}$ (*M. aeruginosa*), △ $P=0.1 \text{ mg-P} \cdot t^{-1}$ (*Cyclotella* sp.), ■ $P=0.5 \text{ mg-P} \cdot t^{-1}$ (*M. aeruginosa*), □ $P=0.5 \text{ mg-P} \cdot t^{-1}$ (*Cyclotella* sp.)

growth rate and half-saturation constant based on Eq. (2) could be calculated to be $0.31 \text{ (d}^{-1}\text{)}$ and $0.003 \text{ (mg-P}\cdot\text{L}^{-1}\text{)}$, respectively, which agree to the experimental results ($\mu_m = 0.25 \text{ [d}^{-1}\text{]}$, $K_p = 0.006 \text{ [mg-P}\cdot\text{L}^{-1}\text{]}$) reported by Holm and Armstrong¹¹⁾. On the other hand, growth of *Cyclotella* sp. was similar in both phosphorus conditions of 0.1 and $0.5 \text{ mg-P}\cdot\text{L}^{-1}$, representing that final cell densities were $1.69 \times 10^6 \text{ cells}\cdot\text{mL}^{-1}$ and $1.08 \times 10^6 \text{ cells}\cdot\text{mL}^{-1}$, respectively. However, no increase in *Cyclotella* sp. cell was observed in $0.02 \text{ mg-P}\cdot\text{L}^{-1}$, which showed final cell density of $5180 \text{ cells}\cdot\text{mL}^{-1}$. Maximum growth rate and half-saturation constant of *Cyclotella* sp. were $0.44 \text{ (d}^{-1}\text{)}$ and $0.22 \text{ (mg-P}\cdot\text{L}^{-1}\text{)}$, respectively. Both values were apparently higher than that for *M. aeruginosa*, and differed from the values of $\mu_m = 0.80 \text{ (d}^{-1}\text{)}$ and $K_p = 0.003 \text{ (mg-P}\cdot\text{L}^{-1}\text{)}$ for *Cyclotella meneghiniana*¹²⁾.

Characteristics of phosphorus uptake for *M. aeruginosa* and *Cyclotella* sp. Fig. 2 represented phosphorus uptake rate for *M. aeruginosa* and *Cyclotella* sp. Cell density

Fig. 2 Change in phosphorus concentration and cell density with growth of *M. aeruginosa* and *Cyclotella* sp. under the phosphorus and silicon concentrations of $0.1 \text{ mg-P}\cdot\text{L}^{-1}$ and $11 \text{ mg-Si}\cdot\text{L}^{-1}$, respectively, in individual monoculture experiment. The single experiment was conducted with duplication of measurements for both cell counting and phosphorus concentration. Symbols: $\blacktriangle$ phosphorus concentration (*M. aeruginosa*), $\triangle$ phosphorus concentration (*Cyclotella* sp.), $\blacklozenge$ cell density (*M. aeruginosa*), $\diamond$ cell density (*Cyclotella* sp.)

and growth rate for both species showed nearly same values. However, there was a difference in phosphorus concentration in medium, representing that, in day 4, *M. aeruginosa* absorbed much amount of phosphorus than that for *Cyclotella* sp. When phosphorus concentrations were fitted to Eq. (3) for selected time interval from day 0 to day 4, then phosphorus uptake rate for *M. aeruginosa* was calculated to be $1.86 \times 10^{-9} \text{ (mg-P}\cdot\text{cell}^{-1}\cdot\text{d}^{-1}\text{)}$. While, *Cyclotella* sp. showed uptake rate of $1.13 \times 10^{-9} \text{ (mg-P}\cdot\text{cell}^{-1}\cdot\text{d}^{-1}\text{)}$, which displayed lower value than that for *M. aeruginosa*.

Competition of *M. aeruginosa* and *Cyclotella* sp. under phosphorus limited and abundant silicon conditions Results of competition between *M. aeruginosa* and *Cyclotella* sp. were illustrated in Fig. 3. In this figure, the results obtained from monoculture experiment at phosphorus concentration of $0.1 \text{ mg-P}\cdot\text{L}^{-1}$ were also described. In competition of both species, growth of *Cyclotella* sp. was significantly restrained, and cell density in

Fig. 3 The growth curves under the phosphorus and silicon concentrations of $0.1 \text{ mg-P}\cdot\text{L}^{-1}$ and $11 \text{ mg-Si}\cdot\text{L}^{-1}$, respectively, in competitive biculture experiment. The results of monoculture experiment for both species under the phosphorus concentration of $0.1 \text{ mg-P}\cdot\text{L}^{-1}$ were also described in this figure. The experiment was conducted in triplicate and the counting in each experimental day was done in duplicate. Symbols: $\blacktriangle$ *M. aeruginosa*, $\triangle$ *Cyclotella* sp., — *M. aeruginosa* (monoculture), - - - *Cyclotella* sp. (monoculture)

day 30 showed about 1.2 % compared with the case of monoculture. In contrast to *Cyclotella* sp., *M. aeruginosa* grew well until day 20, afterword leveled off, then cell density showed 19 % lower than that of the result from monoculture. Final cell density for both species was 1.8×10^6 cells·mL⁻¹ for *M. aeruginosa* and 2.0×10^4 cells·mL⁻¹ for *Cyclotella* sp, explaining that 99 % of total cell was dominated by *M. aeruginosa*.

DISCUSSION

It has been widely accepted that growth of blue-green algae tends to be limited in water with low phosphorus concentration compared with diatoms because of higher half-saturation constant for phosphorus¹¹⁻¹³. However, our results did not support the general trends reported. According to our experimental results, the value of half-saturation constant for *M. aeruginosa* was two order lower than that for *Cyclotella* sp., representing that *M. aeruginosa* possesses much higher growth potential than *Cyclotella* sp. under the phosphorus limited condition. In addition, phosphorus uptake rate of *M. aeruginosa* was faster than *Cyclotella* sp. From these results, it could be assumed that *M. aeruginosa* would possess superior ability than *Cyclotella* sp. to absorb phosphorus in early stage of growth. The outcome of competition experiment obviously reflected these results, i.e., dominance of *M. aeruginosa* with a proportion of 99 % in total cell. Therefore, decrease in phosphorus concentration due to the discharge of Tone River water could be interpreted as a minor factor for the shift of dominant species, from blue-green algae to diatoms, in Lake Teganuma.

Silicon concentration would be one of the important factors for competition between blue-green algae and diatoms. Holm and Armstrong¹¹ conducted competition experiment between *M. aeruginosa* and *Asterionella formosa* (*A. formosa*) in semicontinuous culture, and concluded that *A. formosa* was superior competitor when Si:P molar ratio was higher than 100. In our results, SiO₂-Si concentration for competitive biculture experiment reflected the concentration of discharged water from the channel, giving Si:P molar ratio of approximately 120.

Nevertheless, our results showed the dominance of not *Cyclotella* sp. but *M. aeruginosa*. These results would be due to difference in growth characteristics between *Cyclotella* sp. and *A. formosa*, and would be attributed to experimental manner, e.g., difference between batch and continuous culture. At least, silicon concentration would not affect the shift of dominant species between *M. aeruginosa* and *Cyclotella* sp.

As a possibility affecting the shift of dominant species in Lake Teganuma, change in water volume flowing to Lake Teganuma could be considered since blue-green algae prefer to stagnant condition¹⁶. Yamasaki et al.¹⁷ simulated the effect of increased water volume through the North-chiba Water Conveyance Channel on water quality, and revealed that improve of the quality was found with operation of the channel. However, Tatsumoto et al.⁸ pointed out from the results of competitive biculture experiment between *M. aeruginosa* and *Cyclotella* sp. based on semicontinuous culture that change in water volume did not promote growth of *Cyclotella* sp. in contrast to *M. aeruginosa*.

The shift of dominant species due to the discharge of Tone River water from North-chiba Water Conveyance Channel in Lake Teganuma would not be affected by phosphorus and silicon concentrations based on this study, and also by change in water volume based on other study. Accordingly, other factors such as nutrients (e.g., nitrogen, trace metals, organic ligands) as well as light intensity and temperature might influence determination of dominant species. When change in nutrients in Lake Teganuma is supposed to be one of the major factors for the shift of dominant species, it would be needed to consider either event; decreasing nutrients due to dilution or increasing nutrients due to supply from the channel, or their interaction. Further experiments as well as field investigations should be needed to understand critical factor determining dominant species in the phytoplankton community. Findings of the factor and/or mechanism for transition of algal species would be important for lakes environment, i.e., for suppression of water blooms, preservation of ecosystems, and thereafter

management of water quality.

CONCLUTIONS

This study aimed to obtain fundamental information to understand how environmental factors changed by the discharge of Tone River water into Lake Teganuma affect the shift of dominant species from blue-green algae (*M. aeruginosa*) to diatoms (*Cyclotella* sp.). The results showed that decrease in phosphorus concentration would not affect the shift of dominant species from *M. aeruginosa* to *Cyclotella* sp. Also, silicon supplied through the channel did not promote *Cyclotella* sp. growth contrary to *M. aeruginosa*, and did not lead *Cyclotella* sp. to dominant species based on competitive biculture experiment. It would be possible that the shift of dominant species in Lake Teganuma was occurred as a result of change in nutrients with either event; decreasing nutrients due to dilution or increasing nutrients due to supply from the channel, or their interaction. So far, further experiments as well as field investigations should be required to understand critical factor determining dominant species in the phytoplankton community in Lake Teganuma.

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