Effect of phosphorus fluctuation caused by river water dilution in eutrophic lake on competition between blue-green alga Microcystis aeruginosa and diatom Cyclotella sp.

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Received 29 December 2009; revised 21 May 2010; accepted 25 May 2010

Abstract

Tega-numa (Lake Tega) is one of the eutrophic lakes in Japan. For the improvement of water quality in Lake Tega, the North-chiba Water Conveyance Channel was constructed in 2000, which transfer water from Tone River into the lake. After 2000, the dominant species of diatoms, mainly Cyclotella sp., have been replacing blue-green algae, mainly Microcystis aeruginosa in Lake Tega. This transition of dominant species would be due to the dilution, but the detail mechanism has not been understood yet. This study examined the relationship between phosphorus fluctuation caused by river water dilution to Lake Tega and dominance of algal species, M. aeruginosa or Cyclotella sp. based on the single-species and the mixed-species culture experiments. The single-species culture experiment showed that the half-saturation constant and uptake rate of phosphorus were one order lower and seven times higher for M. aeruginosa than those for Cyclotella sp. These findings implied that M. aeruginosa would possess a potential for the growth and survival over Cyclotella sp. in the phosphorus limited condition. The superiority of M. aeruginosa was reflected in the outcome of the mixed-species culture experiment, i.e., dominance of M. aeruginosa, even phosphorus concentration was lowered to 0.01 mg-P/L. Therefore, it could be concluded that the decrease in phosphorus concentration due to the river water dilution to Lake Tega would be interpreted as a minor factor for the transition of dominant species from M. aeruginosa to Cyclotella sp.

Key words: eutrophic lake; water quality improvement; water blooms; Microcystis aeruginosa; Cyclotella sp.

DOI: 10.1016/S1001-0742(09)60304-1

Introduction

Lake eutrophication is one of the worldwide water environmental problems (Schindler, 2006). In such an environment, dense and nuisance water blooms, especially blooms formed by blue-green algae, have occurred and caused negative impacts such as the production of toxins as microcystin and anatoxin (Meißner et al., 1996; Van Apeldoorn et al., 2007), musty-odor compounds like geosmin and 2-methylisoborneol (Newcombe et al., 2002), and mass mortality of various organisms resulted from oxygen depletion by bacterial degradation (Clark et al., 1971; Pearl, 1998). For water quality improvement as well as control of water blooms, a number of on-site purifications have been practiced (Gelin and Ripl, 1978; Brandrud, 2002; Amano et al., 2008). Dilution is a conventional way to reduce eutrophication level of lake. Oglesby (1969) reported the influence of dilution in Green Lake, USA, and represented that the nutrients such as nitrogen and phosphorus concentrations decreased accompanying to the dilution, but there was little effect on the control of water blooms. Welch et al. (1992) investigated the effect of dilution on the occurrence of water blooms in Moses Lake, USA, and found that dominance of blue-green algae has still been persisted despite the fact that water quality was well improved. Recently, dilution of river water to Tega-numa (Lake Tega), Japan, lead to a decrease in nutrient concentration as well as change in dominant algal species from blue-green algae, mainly Microcystis aeruginosa (M. aeruginosa), to diatoms, mainly Cyclotella sp. (Yamasaki et al., 2003; Tatsumoto et al., 2007). Thereby, it is obvious that dilution would enable to enhance the water quality lowering nutrient concentration, but water blooms would not be necessarily controlled.

In the previous study, the influence of dilution rate, defined as the influent volume per unit time divided by the total volume, was examined in the mixed-species culture experiment to find the mechanisms of dominant species transition as observed in Lake Tega (Tatsumoto et al., 2007).
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The results showed that the change in dilution rate did not cause the transition from *M. aeruginosa* to *Cyclotella* sp. Many publications have reported the critical parameters such as nutrient concentration, light intensity and temperature for the growth of blue-green algae and diatoms. However, the factors affecting the transition from *M. aeruginosa* to *Cyclotella* sp. observed in Lake Tega have not been revealed. One of the possible factors on the transition is considered as phosphorus concentration, which has been widely understood as the critical factor for the growth of blue-green algae, especially *Microcystis* species.

The present study aimed to examine the relationship between phosphorus fluctuation caused by river water dilution to Lake Tega and dominance of algal species, *M. aeruginosa* or *Cyclotella* sp. For this purpose, (1) the single-species culture experiment for their growth characteristics; (2) the single-species culture experiment for their phosphorus uptake capacity; and (3) the mixed-species culture experiment for understanding their competitive superiority were conducted. The results from the experiments were then discussed to assess the role of phosphorus on the transition of dominant species from blue-green algae to diatoms as observed in Lake Tega.

1 Study site

1.1 Outline of Lake Tega

For Lake Tega, the water surface area, volume and mean depth are respectively 6.5 km², 5.6 × 10⁶ m³ and 0.9 m. As shown in Fig. 1, Lake Tega receives river inflows from Ohori, Otsu and Somei-riiotoshi, and the lake drains water out into Tone River through Tega River.

In 1970’s, the population growth in the lake’s basin caused the rise of trophic level in Lake Tega. From 1974 to 2000, the water quality in this lake had marked the worst, based on the chemical oxygen demand (COD) values that fluctuated approximately around 20 mg/L, in all Japanese lakes. For the improvement of water quality, the Ministry of Land, Infrastructure and Transport, the Japanese government, began to construct the North-chiba Water Conveyance Channel in 2000. The channel can transport water from Tone River to Edo River for the purpose of water control at the time of the flood in watershed in Tone River, and of the water shortage in watershed in Edo River. In the process of water transfer, a part of the river water is discharged into Lake Tega with the maximum amount of 10 m³/sec. After 2000, the COD value in Lake Tega has been lowered.

1.2 Water quality

The data of nutrient concentration and water volume on the river inflows as well as North-chiba Water Conveyance Channel are summarized in Table 1. Before 2000, dissolved total nitrogen (D-TN) and phosphate phosphorus (PO₄-P) concentrations were in the range of 5.0–6.0 mg-N/L and ca. 0.1–0.6 mg-P/L for three river inflows. While, D-TN and PO₄-P concentrations were in the range of ca. 3.0–6.0 mg-N/L and ca. 0.1–0.2 mg-P/L after 2000. D-TN and PO₄-P concentrations for the channel showed the lower values than other river inflows. The water volume for the channel represented one order greater than that for each river inflows. Accordingly, the nutrient concentrations discharged into Lake Tega after 2000 would considerably depend on the channel.

2 Materials and methods

2.1 Cultures

*M. aeruginosa* (UTEX 2061, The Culture Collection of Algae at the University of Texas at Austin, USA) and *Cyclotella* sp. (CCAP 1070/4, The Culture Collection...
of Algae and Protozoa, UK) were individually grown in Wright’s cryptophytes (WC) medium with pH value of 7.0 (Guillard and Lorenzen, 1974). The incubation temperature was set at 20°C, and the light illumination was controlled as 2000 lx, which gives photosynthetic photon flux of 27 μmol photons/(m²·sec) (Thimijan and Heins, 1983), with a light and dark cycle of 14 hr:10 hr.

2.2 Growth characteristics of *M. aeruginosa* and *Cyclotella* sp.

Growth characteristics of *M. aeruginosa* and *Cyclotella* sp. were examined through the single-species culture experiment. Prior to the experiment, both species were individually grown in 200 mL of phosphorus-free WC medium in 500 mL Erlenmeyer flask for 10 days. WC media with phosphorus concentration ranging from 0.005 to 0.5 mg-P/L were individually inoculated into each phosphorus depleted medium to give an initial cell density of 5000 cells/mL. Temperature and light intensity in the incubator were set as 20°C and 27 µmol photons/(m²·sec), respectively. All flasks were randomly displaced and manually stirred several times a day. The experiment was conducted in duplicates, and the results were represented with the mean value.

To evaluate the growth characteristics of *M. aeruginosa* and *Cyclotella* sp., the growth rate, \( \mu \) (day⁻¹), was estimated by Eq. (1):

\[
\mu = \frac{1}{C} \cdot \frac{dC}{dt}
\]  

(1)

where, \( C \) (N) and \( t \) (day) are the cell number and cultivation time, respectively.

The maximum growth rate, \( \mu_m \) (day⁻¹), and half-saturation constant, \( K_p \) (mg-P/L), can be calculated from Monod equation (Eq. (2)), applying the \( \mu \) values obtained in each phosphorus concentration level.

\[
\frac{\mu}{\mu_m} = \frac{P}{K_p + P}
\]  

(2)

where, \( P \) (mg-P/L) is the initial phosphorus concentration in medium.

2.3 Phosphorus uptake of *M. aeruginosa* and *Cyclotella* sp.

The single-species culture experiment was conducted to examine the characteristics of phosphorus uptake for both *M. aeruginosa* and *Cyclotella* sp. Fresh 800 mL of WC medium with phosphorus concentration of 0.1 mg-P/L in 1000 mL Erlenmeyer flask was inoculated into the phosphorus depleted medium of both *M. aeruginosa* and *Cyclotella* sp. to give an initial cell density of 5000 cells/mL. Details of the experimental condition were the same as mentioned above. The experiment for phosphorus uptake was performed in duplicates, and the results were represented with the mean value.

The reduction of phosphorus concentration by assimilating was faster in few days after the experiment was started, then gradually decreased, and finally label-off. Therefore, the uptake rate of phosphorus for *M. aeruginosa* and *Cyclotella* sp. was calculated with the steeper slope of phosphorus concentration based on Eq. (3):

\[
V_p = \frac{(P_1 - P_2)}{(C_2 - C_1) \cdot (t_2 - t_1)}
\]  

(3)

where, \( P_1 \) (mg-P/L), \( P_2 \) (mg-P/L) and \( C_1 \) (cells/mL), \( C_2 \) (cells/mL) are the phosphorus concentration and cell density at the cultivation time of \( t_1 \) (day) and \( t_2 \) (day), respectively.

2.4 Competition of *M. aeruginosa* and *Cyclotella* sp.

Competitive abilities of *M. aeruginosa* and *Cyclotella* sp. were examined in the mixed-species culture experiment. Fresh 200 mL of WC medium in 500 mL Erlenmeyer flask was inoculated into the mixed phosphorus depleted culture to give an initial cell density of 5000 cell/mL for each species. The experimental conditions were the same as above. The mixed-species culture experiment was conducted in triplicates, and the result was represented as the mean value with standard deviation.

2.5 Analyses

Cell density was evaluated by a direct count using the plankton counting chamber (MPC-200, Matsunami Glass Ind., Japan) and binocular microscope under magnification of 300. Phosphorus concentration was measured according to the molybdenum blue spectrophotometric method (Japanese Industrial Standards, 1998).
3 Results

3.1 Growth characteristics of *M. aeruginosa* and *Cyclotella* sp.

The relationship between the growth rate and phosphorus concentration for *M. aeruginosa* and *Cyclotella* sp. is displayed in Fig. 2. The growth rate calculated from Eq. (1) was lower for *M. aeruginosa* with the value of 0.17 day$^{-1}$ in the phosphorus concentration of 0.005 mg-P/L, while in the other phosphorus levels the growth rate showed almost similar values ranged from 0.21 day$^{-1}$ at 0.05 mg-P/L to 0.24 day$^{-1}$ at 0.5 mg-P/L. Using the Monod expression shown in Eq. (2), the maximum growth rate, $\mu_m$, of 0.24 day$^{-1}$ and half-saturation constant, $K_p$, of 0.002 mg-P/L were estimated by linear regression of the hyperbolic relationship. Both values of the $\mu_m$ and $K_p$ for phosphorus were in agreement with the value of 0.25 day$^{-1}$ and 0.006 mg-P/L, at 20°C under the phosphorus limited condition, reported by Holm and Armstrong (1981). On the other hand, the growth rate for *Cyclotella* sp. was calculated to be 0.08 and 0.09 day$^{-1}$ at phosphorus concentration as low as 0.005 and 0.01 mg-P/L, respectively, which represents much lower values than the value of 0.23 day$^{-1}$ at 0.05 mg-P/L and of 0.27 day$^{-1}$ at both 0.1 and 0.5 mg-P/L. The $\mu_m$ and $K_p$ of phosphorus for *Cyclotella* sp. were 0.29 day$^{-1}$ and 0.014 mg-P/L, respectively, representing that the $K_p$ was one order greater than that for *M. aeruginosa* although little difference of the $\mu_m$ was found. Both $\mu_m$ and $K_p$ values were quite different compared with the $\mu_m$ of 0.80 day$^{-1}$ and the $K_p$ of 0.003 mg-P/L for *Cyclotella meneghiniana* (Titman, 1976).

3.2 Phosphorus uptake rate of *M. aeruginosa* and *Cyclotella* sp.

The change in phosphorus concentration in culture medium accompanying the growth of *M. aeruginosa* and *Cyclotella* sp. is shown in Fig. 3. The phosphorus concentration showed the similar trend for *M. aeruginosa* and *Cyclotella* sp., representing rapid decrease at the early phase of their growth (day 2–4), and also reaching
stable phosphorus level at elapsed time of day 10–15. For the phosphorus uptake rate, for both *M. aeruginosa* and *Cyclotella* sp. the phosphorus concentration and cell density up to elapsed time of day 4 are substituted to the Eq. (3). Then the phosphorus uptake rate for *M. aeruginosa* was calculated to be $7.55 \times 10^{-9}$ (mg-P/(cell-day)), while for *Cyclotella* sp. the uptake rate was obtained as $1.08 \times 10^{-9}$ (mg-P/(cell-day)). The value of uptake rate for *Cyclotella* sp. is approximately seven times lower than that for *M. aeruginosa*.

3.3 Competition between *M. aeruginosa* and *Cyclotella* sp. under the phosphorus limited condition

The results of the mixed-species culture experiment between *M. aeruginosa* and *Cyclotella* sp. are illustrated in Fig. 4. At the phosphorus concentration of 0.01 mg-P/L, the cell density of *M. aeruginosa* was significantly higher ($t$-test, $P < 0.001$) than that of *Cyclotella* sp., increasing up to $6.7 \times 10^6$ cells/mL, while little increase in *Cyclotella* sp. cells was observed with the cells density of 7600 cells/mL at day 20. In the phosphorus concentrations of 0.1 and 0.5 mg-P/L, *M. aeruginosa* cells were significantly greater ($t$-test, $P < 0.001$ for 0.1 mg-P/L and $P < 0.001$ for 0.5 mg-P/L) than that of *Cyclotella* sp. *M. aeruginosa* cells reached ca. $1.8 \times 10^6$ cells/mL at 0.1 mg-P/L and $2.9 \times 10^7$ cells/mL at 0.5 mg-P/L, and dominated over *Cyclotella* sp. with the proportions of more than 99% in total cells at the end of the experiment.

4 Discussion

Although it has been widely recognized that the growth of *M. aeruginosa* would be limited in the low phosphorus condition (Olsen, 1989; Søndergaard et al., 2000; Amano et al., 2002; Xie et al., 2003), the results obtained in the present study do not support the generally accepted trend. This study clearly showed that *M. aeruginosa* dominated over *Cyclotella* sp. in all phosphorus concentration levels ranged from 0.01 to 0.5 mg-P/L.

The single-species culture experiment revealed that the values of maximum growth rate for *M. aeruginosa* and *Cyclotella* sp. were 0.24 day$^{-1}$ and 0.29 day$^{-1}$, respectively, which represent little difference between both species. While, the half-saturation constant and uptake rate of phosphorus were one order lower and seven times higher for *M. aeruginosa* than those for *Cyclotella* sp., implying that *M. aeruginosa* would possess a potential for growth and survival over *Cyclotella* sp. in the phosphorus limited condition.

Egge (1998) demonstrated the mesocosm experiment to examine the effect of phosphorus on the competition between diatoms and flagellates, and found that diatoms were significantly affected by phosphorus even with high nitrogen and silicate condition. Also, a morphological discussion regarding to the competition between both algae was made in this article, representing that diatoms possessing a larger cell surface were inferior to smaller flagellates for phosphorus uptake, and were therefore outcompeted by flagellates in the phosphorus limited condition. In the present study, *M. aeruginosa* showed faster uptake rate than *Cyclotella* sp., and the cell surface of *M. aeruginosa* was visually three or four times smaller than that of *Cyclotella* sp. In addition, high demand of phosphorus, i.e., the higher half-saturation constant of phosphorus ($K_p$), by *Cyclotella* sp. seems to be in agreement with other diatom species as presented by Egge (1998). Therefore, our results would be consistent with the general trend of algal nutrient kinetics.

The superiority of *M. aeruginosa* in the phosphorus limited condition could be represented as the difference in growth rate between the single-species and the mixed-species culture experiments. As shown in Fig. 3, the growth rate for both species showed a similar trend, but the $P$ uptake rate of *M. aeruginosa* ($7.55 \times 10^{-9}$ mg-P/(cell-day)) was faster than that of *Cyclotella* sp. ($1.08 \times 10^{-9}$ mg-P/(cell-day)) as previously described in Section 3.2. In the mixed-species culture experiment with the condition of $P = 0.1$ mg-P/L, the growth rate of *Cyclotella* sp. was restrained compared to that in the single-species culture experiment. This would be caused by faster phosphorus absorption of *M. aeruginosa*, and thereby *Cyclotella* sp. could not sufficiently use phosphorus. Another possibility was that *Cyclotella* sp. was sensitive in the phosphorus limited condition because of much higher $K_p$ value (0.014 mg-P/L) than *M. aeruginosa* (0.002 mg-P/L). In the mixed-culture experiment,
leading the disappearance of the algae. In a study of phosphorus concentration of 0.01 mg-P/L and 0.9% at both 0.1 and 0.5 mg-P/L in total cell. Thus, *M. aeruginosa* could be dominant species in the phosphorus limited condition even the concentration is 0.01 mg-P/L, of which concentration indicates the boundary between higher oligotrophic and lower mesotrophic state of eutrophication level suggested by Xu et al. (2001). In a study of phosphorus concentration leading the disappearance of *M. aeruginosa*, Gelin and Ripl (1978) reported that *M. aeruginosa* was disappeared in Lake Tummen by mean of dredging for the sediment with reduction of the phosphorus from approximately 0.2 mg-P/L to less than 0.01 mg-P/L. In the present study, the single-species culture experiment for *M. aeruginosa* indicated that the growth rate of *M. aeruginosa* was much smaller with the value of 0.17 day⁻¹ at 0.005 mg-P/L compared with those of 0.21–0.24 day⁻¹ at the phosphorus levels of higher than 0.01 mg-P/L. Thus, the phosphorus concentration of around 0.005 mg-P/L or less than 0.01 mg-P/L would be a critical level controlling the growth of *M. aeruginosa* based on the evidence obtained in this study and the observation in Lake Tummen reported by Gelin and Ripl (1978). Therefore, it could be concluded that the decrease in phosphorus concentration due to the river water dilution would be interpreted as a minor factor for the transition of dominant species from *M. aeruginosa* to *Cyclotella* sp. in Lake Tega.

Similar to phosphorus, silicon has been focused as a decisive factor on the competition between blue-green algae and diatoms. Holm and Armstrong (1981) demonstrated the mixed-species culture experiment between blue-green alga *M. aeruginosa* and diatom *Asterionella formosa* (A. formosa) in semi-continuous culture, and found the superiority of A. *formosa* when the Si:P molar ratio was more than 105, which gives the Si:P mass ratio of ca. 95, in the phosphorus limited condition. Figure 5 shows the relationship between silicon and phosphorus concentration on the competition of *M. aeruginosa* and A. *formosa* based on their results, in which the results from the present study were added. Also in Fig. 5, the Si:P mass ratio of 95 was represented as the dashed line. The SiO₂-Si concentration for the mixed-species culture experiment in the present study was adjusted as 11 mg-Si/L, of which concentration was much higher than the threshold value for diatom growth (approximately 0.06 mg-Si/L (2 μmol/L), found by Egge and Aksnes (1992). The SiO₂-Si reflects the concentration of water discharged from the North-chiba Water Conveyance Channel, which gives the Si:P mass ratio of 1100, 110 and 22 at the phosphorus concentration of 0.01, 0.1 and 0.5 mg-P/L, respectively. Notwithstanding the experimental conditions of the Si:P mass ratio, our results showed the dominance of *M. aeruginosa* suppressing *Cyclotella* sp. These results would be due to the difference in sensitivity toward silicon as well as phosphorus between *Cyclotella* sp. and *A. formosa*. Also, it might be considered as the influence of difference between the semi-continuous system and batch system, namely, the influence of continuous silicon supply on the growth characteristics and/or response of *Cyclotella* sp. Thus, the shift of dominant species between *M. aeruginosa* and *Cyclotella* sp. would not be related to the Si:P mass ratio.

There would also be a possibility that *M. aeruginosa* underwent CO₂ limitation although *M. aeruginosa* became a superior competitor in all of mixed-species culture experiment. Cliescher et al. (1989) represented an importance of the surface-to-volume ratio of medium in flask because of CO₂ limitation, which has been understood as an influential factor for the growth of blue-green algae (Scheller et al., 1997). Most of experiments employed 200 mL of culture medium in 500 mL flask, while 800 mL medium in 1 L flask was used in the experiment for P uptake rate based on the method of Marinho and Azevedo (2007). This would cause CO₂ limitation to *M. aeruginosa* under phosphorus concentration of 0.1 mg-P/L and the limitation would be reflected as a lag phase in Fig. 3 although no significant lag phase was observed in the mixed-species culture experiment as seen in Fig. 4b.

The decisive parameters on the transition of dominant species in Lake Tega could be considered as the nutrients other than phosphorus and silicon, e.g., nitrogen, magnesium and calcium, trace metals, organic ligands as well as light intensity and temperature. The dilution for the improvement of water quality in Lake Tega would cause the significant effects of either decreasing concentrations of various substances due to dilution or increasing the
concentrations due to the supply from the channel, or their interaction. Further experiments and field investigations are needed for the understanding of the rule of dilution and of the critical factors determining dominant species in the phytoplankton community in Lake Tega. Findings of the factors and mechanisms for the transition of algal species would be important for lake’s environment to suppress water blooms, to preserve the ecosystems, and thereafter to manage water quality.

5 Conclusions

This study aimed to examine the relationship between phosphorus fluctuation caused by river water dilution to Lake Tega and dominance of algal species, *M. aeruginosa* or *Cyclotella* sp. based on the single-species and the mixed-species culture experiment. The single-species culture experiment revealed that the half-saturation constant and uptake rate of phosphorus were one order lower and seven times higher for *M. aeruginosa* than those for *Cyclotella* sp. The superiority of *M. aeruginosa* in the phosphorus limited condition was reflected in the outcome of the mixed-species culture experiment, i.e., dominance of *M. aeruginosa* in all of phosphorus levels ranged from 0.01 to 0.5 mg-P/L. Therefore, the phosphorus fluctuation caused by river water dilution to Lake Tega would not influence the transition of dominant species from *M. aeruginosa* to *Cyclotella* sp. even phosphorus concentration was decreased as low as 0.01 mg-P/L. Although the influence of the Si:P mass ratio was also discussed, *Cyclotella* sp. never became a dominant species despite the fact that the Si:P mass ratio was more than 95, which was a condition promoting the growth of diatoms. The transition of dominant species in Lake Tega was considered as a consequence of either event, i.e., decreasing concentrations of various substances by dilution or increasing the concentrations by the supply from the North-chiba Water Conveyance Channel, or their interaction. Further experiments as well as field investigations would be required to understand the rule of dilution for the improvement of water quality and of the critical factor determining dominant species in the phytoplankton community in Lake Tega.

Acknowledgments

The authors wish to express gratitude to Prof. Dr. Masami Aikawa of Kisarazu National College of Technology for his fruitful discussion. Gratitude is also extended to Prof. Dr. Keiichi Nagao, Head of Safety and Health Organization, Chiba University, for his encouragement and support on this work.

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