Water Supply

IWA PUBLISHING

Water Supply Vol 22 No 5, 4864 doi: 10.2166/ws.2022.159

Effective dilution rate to suppress the risk of *Microcystis* blooms in Lake Tega, Japan, based on a competitive growth simulation model

Jingnan Li [a,*], Masato Chujo[a], Yoshimasa Amano[b,c] and Motoi Machida[b,c]

[a] Graduate School of Science and Engineering, Department of Applied Chemistry and Biotechnology, Chiba University, Chiba City, Japan
[b] Graduate School of Engineering, Department of Applied Chemistry and Biotechnology, Chiba University, Chiba City, Japan
[c] Safety and Health Organization, Chiba University, Chiba City, Japan
*Corresponding author. E-mail: lijingnan94@gmail.com

JL, 0000-0003-4503-3984

ABSTRACT

Although water transfer as an efficient method to improve water quality and control *Microcystis* blooms in lakes has been executed for several decades, few studies have examined effective dilution rates depending on various water qualities. Therefore, to clarify the effective dilution rate to suppress *Microcystis* blooms, a competitive growth simulation model developed for eutrophic conditions was utilized. A competition experiment between *Microcystis* sp. and *Cyclotella meneghiniana* under limited phosphorus and sufficient nitrogen concentration was conducted to investigate the mechanism of dilution effect and verify the broad applicability of the simulation model. Experimental results revealed that there was no remarkable discrepancy in *Microcystis* sp. cell density among different dilution groups ($p>0.05$), while *C. meneghiniana* cell density was significantly different between groups ($p<0.05$). The accuracy of the simulation model under limited phosphorus as well as sufficient nitrogen concentration was verified by comparing the simulated value with experimental results. Based on the simulated results, it was suggested that a dilution rate of over 13.3% can suppress *Microcystis* blooms effectively in Lake Tega, Japan, as a case study. The predicted data was also compared with the field data collected over years in Lake Tega, and its effectiveness has been confirmed.

Key words: bloom suppression, competitive growth model, *Cyclotella meneghiniana*, dilution rate, *Microcystis* sp., phosphorus limitation

HIGHLIGHTS

- The cell density of *Cyclotella meneghiniana* in competition experiments was more affected by dilution than that of *Microcystis* sp. under limited nutrient concentration.
- The simulated model used in this study had broad applicability that can be applied in both eutrophic condition and limited nutrient concentration.
- The precise dilution rate to effectively suppress *Microcystis* blooms in Lake Tega, has been simulated.

NOMENCLATURE

N	Nitrate-nitrogen concentration (mg-N L^{-1})
N_0	Initial nitrate-nitrogen concentration (mg-N L^{-1})
P	Phosphate-phosphorus concentration (mg-P L^{-1})
P_0	Initial phosphate-phosphorus concentration (mg-P L^{-1})
C	Cell density (cells mL^{-1})
Q	Cell quota (pg $cell^{-1}$)
ρ_{max}	Highest uptake rate(pg $cell^{-1}$ day^{-1})
ρ_{max}^{hi}	Highest uptake rate at the beginning of cultivation (pg $cell^{-1}$ day^{-1})
ρ_{max}^{lo}	Highest uptake rate at the maximum growth rate (pg $cell^{-1}$ day^{-1})
K_μ	Half-saturation constant for growth rate (mg L^{-1})
K_ρ	Half-saturation constant for uptake rate (mg L^{-1})
$K_\rho{}^{hi}$	Half-saturation constant for uptake rate at the beginning of cultivation (mg L^{-1})
$K_\rho{}^{lo}$	Half-saturation constant for uptake rate at the maximum growth rate (mg L^{-1})
μ_{max}	Maximum growth rate at a certain nutrient concentration (day^{-1})
μ'_{max}	Highest specific growth rate (day^{-1})

Q_{min} Minimum cell quota (pg cell^{-1})
Q_{max} Maximum cell quota (pg cell^{-1})
D Dilution rate (day $^{-1}$)
d Daily renewal rate (day^{-1})
α Inter-specific competition rate

Subscripts A and B, respectively, refer to two cocultured algal species (*Microcystis* sp. and *Cyclotella meneghiniana*)

Subscripts n and p, respectively, refer to nitrate-nitrogen and phosphate-phosphorus

1. INTRODUCTION

The severe impact of cyanobacterial blooms in eutrophic lakes on water quality has caused a lot of environmental problems worldwide in recent decades. Many treatment methods to remove cyanobacterial blooms, such as pump suction, bubble floatation, chemical coagulation and so on, have been carried out and examined in several studies (Bui *et al.* 2015; Cottingham *et al.* 2015; Türkmen & Kütük 2017; Farnham *et al.* 2019). Water transfer is one of the effective treatment methods among them, not only for suppressing the formation of cyanobacterial blooms but also for ameliorating the water quality. The transferred water can wash the algae away and dilute or enrich the nutrient concentrations of water to inhibit or promote algal growth (Amano *et al.* 2012; Sugimoto *et al.* 2016). Although there have been many studies focusing on the inhibitory effect of water transfer on cyanobacterial blooms, the limited experience in some certain lakes could not be applied to other lakes worldwide universally.

From 1974 to 2000, the lake ranked with the worst water quality in Japan was Lake Tega (35° 51′ N, 140° 02′ E) (Chiba Prefectural Government 2019), which suffered from cyanobacterial blooms (mainly the genus *Microcystis*) frequently in summer. To improve the deteriorated water quality, a water channel was constructed in 2000 to convey a large amount of water (maximum volume: 8.6×10^5 m^3 day^{-1}) from the Tone River to Lake Tega. After water conveyance, the highest total nitrogen (TN) concentration in Lake Tega has decreased to 2.2 mg-N L^{-1} in the past five years from 5.3 mg-N L^{-1} in the 1990s. Simultaneously, the highest concentration of total phosphorus (TP) in the past five years has dropped to nearly one-half of the highest value in the 1990s (Chiba Prefectural Government 2019). The dominant algal species changed into diatoms (mainly the genus *Cyclotella*) in summer instead of *Microcystis*, which reduced the frequency of *Microcystis* blooms' occurrence (Sugimoto *et al.* 2016).

To explore the effect of water transfer on the growth and dominant shift of *Microcystis* and *Cyclotella*, Sugimoto *et al.* (2016) have concluded that the dilution rate of up to 36% per day or more was effective to control *M. aeruginosa* growth according to the result of competition experiments with *Cyclotella* sp. However, the inhibitory effect of water transfer on the growth of *M. aeruginosa* was still not considered when the dilution rate was lower than 36%. Afterward, domination characteristics for each species under various nitrate-nitrogen (NO_3-N): phosphate-phosphorus (PO_4-P) mass ratios and dilution rates were predicted by Mikawa *et al.* (2017) based on their algal growth simulation model. The effective dilution rate to suppress the growth of *M. aeruginosa* was proposed at 20% per day by a simulated result in the study. However, the predicted cell densities were always overestimated compared with experimental values due to the defect of the simulation model. In order to modify the overestimation problem in their models, Chujo *et al.* (2021) introduced an additional growth limitation term from Lotka-Volterra model into the previous model to develop an improved simulation model. The developed simulation model could present a more accurate prediction of the growth patterns of *Microcystis* sp. and *C. meneghiniana*, which were isolated from Lake Tega, in competition experiments under high nitrogen and phosphorus concentrations.

Although Chujo's model has been confirmed to work under eutrophic nutrient concentrations (Chujo *et al.* 2020), the accuracy of the model simulation has been still unclear under the condition with the combination of one nutrient being limited and another one being enough. Maberly *et al.* (2020) have estimated the nutrient concentrations of 17 freshwater meres in Shropshire and Cheshire, England. They found that some meres contained high nitrogen and limited phosphorus, while the nutrient concentration conditions in some other meres were opposite. Takamura *et al.* (1992) reported that the concentration of nitrogen exceeded that of phosphorus by 20 times after 1986 in Lake Kasumigaura, Japan. The dominant algae had shifted from *Microcystis* spp. to *Planktothrix agardhii* at that time, which was related to the phosphorus limitation and the disparity between nitrogen and phosphorus concentrations. For revealing a more accurate dilution rate that can effectively suppress *Microcystis* blooms in such actual lakes, it should be examined whether Chujo's model is practical under various combinations of nutrient concentration. Otherwise, the predicted values by the model would not be applicable to *Microcystis* bloom suppression.

In this study, we aimed to explore the mechanism of suppressive effect of dilution on the growth of two different species and examine the broad applicability of Chujo's model, then indicate an effective dilution rate to suppress *Microcystis* blooms in Lake Tega. For exploring the mechanism of dilution's suppressive effect, a competition experiment of two algal species, *Microcystis* sp. and *C. meneghiniana*, was conducted in phosphorus-limited and nitrogen-enough conditions under various dilution rates. Based on the model, the growth curves were simulated, and the accuracy of simulated data was tested by comparing with the experimental data. After the accuracy was verified, the growth of the two algal species under simultaneous changes of various nitrogen, phosphorus concentrations, and daily renewal rates (used instead of dilution rate in semi-continuous culture system) was simulated based on the model. The simulated values could evaluate an effective dilution rate which suppresses *Microcystis* blooms effectively under various nitrogen and phosphorus concentrations. The outcome was compared with the field observation of *Microcystis* growth in Lake Tega under different dilution rates to discuss the optimum way to suppress *Microcystis* blooms.

2. MATERIALS AND METHODS

2.1. Test algae and culture conditions

Microcystis sp. and *C. meneghiniana* in Lake Tega water were isolated (Chujo *et al.* 2019) and used as test algae in this study. The average single cell volume of the two species was measured to be 9.73 μm^3 for *Microcystis* sp. and 243.35 μm^3 for *C. meneghiniana*. Wright's cryptophytes (WC) medium (Guillard & Lorenzen 1972) at pH 8.0 that can cultivate both cyanobacteria and diatoms (Anderson *et al.* 2005) was selected as culture medium. Sodium nitrate ($NaNO_3$) and dipotassium hydrogen phosphate (K_2HPO_4) were used to adjust the initial nitrate-nitrogen (N) and phosphate-phosphorus (P) concentrations of the culture medium to be 14 mg-N L^{-1} and 1.55 mg-P L^{-1}, respectively. Silicate-silicon (Si) concentration in the medium supplied by sodium metasilicate (Na_2SiO_3) was raised from 2.8 mg-Si L^{-1} to 11 mg-Si L^{-1}, which is the same as the concentration in the Tone River water. Carbon source was provided by sodium hydrogen carbonate ($NaHCO_3$). The medium was autoclaved at 121 °C for 20 minutes in all experiments.

For subculture, *Microcystis* sp. and *C. meneghiniana* were separately cultivated in WC medium in a 300 mL Erlenmeyer flask. Each flask was incubated in an incubator (MTI-202, EYELA, Japan) at a temperature of 25 °C, and the light intensity was adjusted to 10,000 lux using a light meter (LX-1102, Lutron, America). The light-dark cycle was set as 14 hours-light and 10 hours-dark. The two subcultured species were inoculated to fresh medium every two or three weeks. All the operations of inoculation and sampling were conducted in a clean bench to minimize bacterial contamination.

2.2. Semi-continuous competition experiments for *Microcystis* sp. and *C. meneghiniana*

Semi-continuous competition experiments were conducted with various dilution rates under the limited phosphorus and enough nitrate concentrations, to investigate the combined effect of these conditions on *Microcystis* sp. and *C. meneghiniana*.

Prior to the competition experiment, both species were precultured in nitrogen- and phosphorus-free medium for 7 days to deplete intracellular N and P, thereby eliminating the effect of intracellular nutrients. After the preculture, both species were inoculated together in 200 mL sterilized medium in a 300 mL Erlenmeyer flask for semi-continuous competition experiments. The initial cell density of *Microcystis* sp. was adjusted to 1.0×10^4 cells mL^{-1} and that of *C. meneghiniana* was 3.98×10^2 cells mL^{-1} to make the two species' cell volume equivalent to the same volume of 10^5 μm^3.

In actual lakes, there was continuous water inflow from river, which could be modeled as a continuous culture system. The dilution rate (D, %) was used to represent the continuous inflow of the water per day. In this study, the culture medium was diluted with fresh medium once a day instead of the continuous water inflow, which should be modeled as a semi-continuous culture system. The daily renewal rate (d, %) was used to describe the medium replacement in this culture system. The daily renewal rate in the semi-continuous culture system can be converted to the dilution rate by the following equation (Tilman & Kilham 1976):

$$D = ln\frac{100}{100 - d} \times 100. \quad (1)$$

The daily renewal rates of the competition experiment were set as 0, 5, and 15%. An appropriate volume of culture medium was removed and as soon an equal volume of fresh medium was added to each flask once a day. In addition to the different daily renewal rates, the P concentration of each group was limited to 0.1 mg-P L^{-1}, which was the same as the minimum P

concentration in Lake Tega in the last five years (Chiba Prefectural Government 2019). The initial N concentration of 14 mg-N L^{-1} was adequate for the growth of both species (Anderson *et al.* 2005). The cell density was measured every 2–5 days and continued until it tended to be constant. The nutrient concentrations were measured once a week. The experiments with different dilution rates were conducted in triplicate. All the experimental results were presented as [the mean value] ± [standard deviation].

2.3. Measurements and statistical analysis

The cell density of samples was measured by directly counting cell in a plankton counting plate (MPC-200, Matsunami Glass Industry, Japan) using an optical microscope (ECLIPSE E100, Nikon, Japan) after appropriately diluted. The entire cell volume of each species in the flask was calculated by multiplying single cell volume and cell density for each, and it was defined as the biovolume. The following Equation (2) was employed to calculate the average growth rate (μ, day^{-1}) at a certain growth period:

$$\mu = \frac{\ln C_2 - \ln C_1}{t_2 - t_1}, \tag{2}$$

where C_1 and C_2 are the cell densities at time points t_1 (day) and t_2 (day), respectively.

Concentrations of N were measured by ion chromatography (ICS-1100, Nippon Dionex, Japan), and molybdenum blue method was used to measure P concentration. The solution pH was monitored by a pH meter (D-51, Horiba, Japan).

Differences in experimental parameters of *Microcystis* sp. and *C. meneghiniana* in each condition were analyzed by a one-way analysis of variance (ANOVA) with a post hoc comparison being performed with Turkey's test, via SPSS Statistics (Ver. 23, IBM Corporation, USA). The results were judged to be a significant difference at $p < 0.05$.

2.4. Mathematical model and simulation

To predict the cell densities of *Microcystis* sp. and *C. meneghiniana* when they grow together and the trends of the appearance of *Microcystis* blooms under various nutrient concentrations and daily renewal rates, the model constructed by Chujo *et al.* (2020) was used. The equations of the model are tabulated in Table 1.

The accuracy of a previous model (Mikawa *et al.* 2016) tended to decrease under high nutrient concentrations. In the previous model, the limiting nutrient was determined as the relationship between the mass ratio of minimum cell quota of assimilated N:P (the optimum N:P ratio) and the external dissolved N:P mass ratio. While in the Chujo's model, the relationship between the mass ratio of cell quota of assimilated N:P (Q_n:Q_p) and the optimum N:P ratio was taken to determine the limiting nutrient (as shown in the tag of Table 1).

The model equations were calculated via a fourth-order form of the Runge-Kutta method with the time step of $\Delta t = 0.01$ day, using Microsoft Excel. The values of each constant parameter in the equations taken to calculate were shown in Table 2, which were obtained from monoculture experiments in the study of Chujo *et al.* (2021). Furthermore, to investigate the accuracy of the model simulation under combined nutrient concentrations, the growth curves of both species were simulated under the same experimental condition as the semi-continuous competition experiments mentioned above.

In the investigation of effective dilution rate, the growth of both species was simulated under various nutrient concentrations and dilution rates based on the Chujo's model. The initial N and P concentrations were ranged from 0 to 5.0 mg-N L^{-1} and 0 to 0.5 mg-P L^{-1}, respectively, reflecting the nutrient concentration in Lake Tega. The daily renewal rate (d) was increased from 0 to 20% with a 2.5% interval for each step. Since the two species always reached saturation within around 20 days (Chujo *et al.* 2021), the cell density in day 30 was used as the final result for prediction.

3. RESULTS

3.1. Growth characteristics of *Microcystis* sp. and *C. meneghiniana* at different daily renewal rates under phosphorus limited condition

Growth characteristics for *Microcystis* sp. and *C. meneghiniana* in P limited culture experiment are shown in Figure 1. The simulated growth curves of both species are also depicted in the same figure.

In all daily renewal rates (d) experimental groups, the biovolume of both species has reached saturation within 25 days. At $d = 0\%$, the biovolume of *Microcystis* sp. was $8.13 \times 10^6 \pm 1.76 \times 10^6$ μm^3 L^{-1} at day 24, and after that, the biovolume

Table 1 | Model equations used in this study

State variable	Modeling of each state variable
N concentration	$\frac{dN}{dt} = -C_A \frac{\rho_{nA,max} N}{K_{nA} + N} - C_B \frac{\rho_{nB,max} N}{K_{nB} + N} + D(N_0 - N)$
P concentration	$\frac{dP}{dt} = -C_A \frac{\rho_{pA,max} P}{K_{pA} + P}\left(1 - \frac{Q_{pA}}{Q_{pA,max}}\right) - C_B \frac{\rho_{pB,max} P}{K_{pB} + P} + D(P_0 - P)$
Cell quota of assimilated N of *Microcystis* sp.	$\frac{dQ_{nA}}{dt} = \frac{\rho_{nA,max} N}{K_{nA} + N} - \left[\mu'_{nA,max}\left(1 - \frac{Q_{nA,min}}{Q_{nA}}\right), \mu'_{pA,max}\left(1 - \frac{Q_{pA,min}}{Q_{pA}}\right)\right]^{*}\left(1 - \frac{C_A - \alpha_{AB} C_B}{K_A}\right) Q_{nA}$
Cell quota of assimilated N of *C. meneghiniana*	$\frac{dQ_{nB}}{dt} = \frac{\rho_{nB,max} N}{K_{nB} + N} - \left[\mu'_{nB,max}\left(1 - \frac{Q_{nB,min}}{Q_{nB}}\right), \mu'_{pB,max}\left(1 - \frac{Q_{pB,min}}{Q_{pB}}\right)\right]^{**}\left(1 - \frac{C_B - \alpha_{AB} C_B}{K_B}\right) Q_{nB}$
Cell quota of assimilated P of *Microcystis* sp.	$\frac{dQ_{pA}}{dt} = \frac{\rho_{pA,max} P}{K_{pA} + P}\left(1 - \frac{Q_{pA}}{Q_{pA,max}}\right) - \left[\mu'_{nA,max}\left(1 - \frac{Q_{nA,min}}{Q_{nA}}\right), \mu'_{pA,max}\left(1 - \frac{Q_{pA,min}}{Q_{pA}}\right)\right]^{*}\left(1 - \frac{C_A - \alpha_{AB} C_B}{K_A}\right) Q_{pA}$
Cell quota of assimilated P of *C. meneghiniana*	$\frac{dQ_{pB}}{dt} = \frac{\rho_{pB,max} P}{K_{pB} + P} - \left[\mu'_{nB,max}\left(1 - \frac{Q_{nB,min}}{Q_{nB}}\right), \mu'_{pB,max}\left(1 - \frac{Q_{pB,min}}{Q_{pB}}\right)\right]^{**}\left(1 - \frac{C_B - \alpha_{BA} C_A}{K_B}\right) Q_{pB}$
Cell density of *Microcystis* sp.	$\frac{dC_A}{dt} = \left\{\left[\mu'_{nA,max}\left(1 - \frac{Q_{nA,min}}{Q_{nA}}\right), \mu'_{pA,max}\left(1 - \frac{Q_{pA,min}}{Q_{pA}}\right)\right]^{*}\left(1 - \frac{C_A - \alpha_{AB} C_B}{K_A}\right) - D\right\} C_A$
Cell density of *C. meneghiniana*	$\frac{dC_B}{dt} = \left\{\left[\mu'_{nB,max}\left(1 - \frac{Q_{nB,min}}{Q_{nB}}\right), \mu'_{pB,max}\left(1 - \frac{Q_{pB,min}}{Q_{pB}}\right)\right]^{**}\left(1 - \frac{C_B - \alpha_{BA} C_A}{K_B}\right) - D\right\} C_B$

*In case of *Microcystis* sp.,
If the Q_{nA}:Q_{pA} ratio is higher than the optimum N:P ratio (=14.7): $\mu'_{pA,max}\left(1 - \frac{Q_{pA,min}}{Q_{pA}}\right)$.
if not: $\mu'_{nA,max}\left(1 - \frac{Q_{nA,min}}{Q_{nA}}\right)$.
**In case of C. meneghiniana,
If the Q_{nB}:Q_{li} ratio is higher than the optimum N:P ratio (=7.13): $\mu'_{pB,max}\left(1 - \frac{Q_{pB,min}}{Q_{pB}}\right)$.
if not: $\mu'_{pB,max}\left(1 - \frac{Q_{pB,min}}{Q_{pB}}\right)$.

Table 2 | Parameters for the competition growth model which referred to Chujo *et al*. (2021)

	Microcystis sp.	***C. meneghiniana***	***Microcystis sp.***	***C. meneghiniana***
Parameter	**Nitrogen**		**Phosphorus**	
Growth parameters				
μ_{max} (day^{-1})	0.587	1.13	0.625	1.21
K_{μ} (mg L^{-1})	8.75×10^{-3}	4.48×10^{-4}	0.021	1.21×10^{-4}
Cell quota parameters				
μ'_{max} (day^{-1})	0.688	1.15	0.761	1.22
Q_{max} (pg cell^{-1})	5.00	104	0.28	5.08
Q_{min} (pg cell^{-1})	0.730	1.81	0.050	0.254
Uptake parameters				
ρ_{max}^{hi} (pg cell^{-1} day^{-1})	11.5	27.2	3.12	54.6
K_{ρ}^{hi} (mg L^{-1})	1.21	6.13	0.02	1.21
ρ_{max}^{lo} (pg cell^{-1} day^{-1})	2.31	9.35	0.3	8.67
K_{ρ}^{lo} (mg L^{-1})	0.435	3.76	0.15	0.5

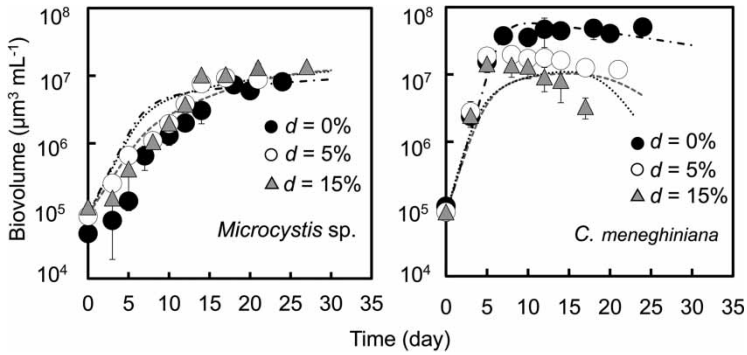

Figure 1 | Biovolume of *Microcystis* sp. and *C. meneghiniana* under limited phosphorus and sufficient nitrogen concentration with various daily renewal rates of $d = 0\%$ (black filled circle), $d = 5\%$ (open circle), and $d = 15\%$ (grey filled triangle), and the simulated competitive growth curves of two species. Dotted-dashed line indicates the simulated cell volume of *Microcystis* sp. under $d = 0\%$, dashed line indicates that under $d = 5\%$, dotted line indicates that under $d = 15\%$.

remained constant within 7 days. For the other two experimental groups ($d = 5$ and 15%), there was no significant discrepancy in the biovolume ($p > 0.05$). In the $d = 5\%$ group, the biovolume in saturation was obtained at day 17 as $9.31 \times 10^6 \pm 1.29 \times 10^6\ \mu m^3\ L^{-1}$ and that for the 15% group was $1.31 \times 10^7 \pm 1.17 \times 10^6\ \mu m^3\ L^{-1}$ at day 21. The average growth rate of *Microcystis* sp. in the logarithmic phase of the three groups was also similar ($p > 0.05$). The value was $0.313 \pm 0.007\ day^{-1}$ in the $d = 0\%$ group, and $0.318 \pm 0.005\ day^{-1}$ in the $d = 5\%$ group. When the daily renewal rate increased to 15%, the average growth rate in the logarithmic phase was obtained to be $0.291 \pm 0.002\ day^{-1}$.

On the other hand, the growth of *C. meneghiniana* was affected obviously by the daily renewal rate under phosphorus limitation. *C. meneghiniana* reached saturation at day 7 with the biovolume of $3.76 \times 10^7 \pm 1.01 \times 10^6\ \mu m^3\ L^{-1}$ in the $d = 10\%$ group and remained stationary until the end of the experiment. In comparison, the growth of *C. meneghiniana* at $d = 15\%$ has entered the death phase directly after it reached saturation at day 5. Moreover, the biovolume in this group at the end of the experiment was $2.18 \times 10^6 \pm 1.01 \times 10^6\ \mu m^3\ L^{-1}$, which decreased by nearly one order of magnitude compared with that in the $d = 5\%$ group. As a result of the competition experiments, the dominant species, defined as the species which take the most biovolume in the flask, was always *Microcystis* sp. in each group. The obvious effect of the daily renewal rate was also reflected in the growth rate of *C. meneghiniana*. At the beginning of the experiment (from day 0 to day 5), although the differences in growth rate among each group were not obvious ($p > 0.05$), the average growth rates of the three groups displayed different characteristics after the logarithmic phase. From day 12 to day 18, the average growth rate in the $d = 0\%$ group was $0.005 \pm 0.001\ day^{-1}$, and then the growth rate of *C. meneghiniana* gradually changed to 0 until the end of the experiment. On the contrary, in the groups of $d = 5\%$ and $d = 15\%$, the average growth rate has become negative after the logarithmic phase.

From the result of nutrient analysis as shown in Figure 2, it was observed that the dilution had scant effect on the change in nutrient concentrations in the competition experiments. The differences in the N and P concentrations among each group were not significant ($p > 0.05$), and the decline of both nutrient concentrations was mainly attributed to the growth of two species as the competition experiment progressed. The concentration of P dropped rapidly during the logarithmic phase of the two species and remained at $0.012 \pm 0.002\ mg\text{-}P\ L^{-1}$ and $0.016 \pm 0.004\ mg\text{-}P\ L^{-1}$ at $d = 0\%$ and $d = 15\%$, while the N concentration remained at $9.25 \pm 0.39\ mg\text{-}N\ L^{-1}$ and $8.62 \pm 0.22\ mg\text{-}N\ L^{-1}$ at $d = 0\%$ and $d = 15\%$, respectively. These values portrayed that the nutrient concentrations were similar at the end of the competition experiments, despite the differences in daily renewal rates.

3.2. Accuracy of competitive growth simulation model under limited P concentration

The simulated growth curves in Figure 1 displayed that the simulated data could well correspond with the experimental growth patterns for *Microcystis* sp. In the $d = 0\%$ group, the trend of experimental cell growth data and simulated curve changing trend have coherence. The two simulated growth curves in the $d = 5\%$ and $d = 15\%$ group almost coincided with each other, which is consistent with the fact that there was not a significant difference in experimental data between the two groups ($p > 0.05$). The coefficient of determination (R^2) for the simulated curve at $d = 0\%$ was 0.827 and the values at $d = 5\%$ and $d = 15\%$ were 0.894 and 0.915, respectively. Thus, the feasibility of Chujo's model under limited phosphorus and sufficient

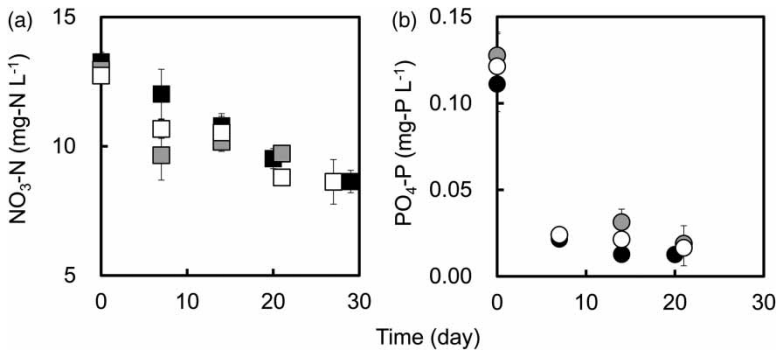

Figure 2 | Nutrient concentration in competition experiments. (a) Nitrogen concentrations change in experiments under various dilution rates of $d = 0\%$ (black filled square), $d = 5\%$ (grey filled square) and $d = 15\%$ (open square). (b) Phosphorus concentrations change in experiments under various dilution rates of $d = 0\%$ (black filled circle), $d = 5\%$ (grey filled circle) and $d = 15\%$ (open circle).

nitrogen conditions could be verified. It would be used to predict the growth of *Microcystis* sp. under various nutrient conditions including phosphorus deficiency. Similarly, the simulated values also matched with the experimental patterns in the growth of *C. meneghiniana* at $d = 0\%$ under low P concentration ($R^2 = 0.973$). Although a little discrepancy between simulated and experimental biovolume was observed at $d = 5$ and 15%, the model can still be valid to simulate the growth trend of *C. meneghiniana*.

3.3. Prediction of *Microcystis* blooms under various dilution rates

Since the accuracy of the model was verified, the cell density of *Microcystis* sp. was predicted under the same N and P concentrations and daily renewal rate as those in Lake Tega before ($d = 5\%$) and after ($d = 15\%$) water transfer. The estimated *Microcystis* sp. cell densities in saturation were 2.72×10^6 cells mL^{-1} (before water transfer) and 1.36×10^6 cells mL^{-1} (after water transfer), respectively. Based on these values, it could be assumed that, when the estimated cell density was more than 2.72×10^6 cells mL^{-1}, there is a high possibility of *Microcystis* blooms occurrence, while the cell density of less than 1.36×10^6 cells mL^{-1} implies that blooms would occur hardly. The plane view of contour figures in Figure 3 shows the predicted values at various daily renewal rates and different nutrient concentrations. The black area was taken as the bloom occurrence area where the predicted cell densities were equal to or more than 2.72×10^6 cells mL^{-1}. The dark-grey area was taken as the non-bloom occurrence area where the predicted cell densities were equal to or less than 1.36×10^6 cells mL^{-1}. The light-grey area between the black and dark-grey area implies the state that suffers the risk of bloom appearance although the blooms have not still occurred. Solid lines and dashed lines were used to indicate the boundary of the bloom occurrence area and non-bloom occurrence area, respectively.

When the daily renewal rate changed from 0 to 5%, the bloom area was obviously enlarged. However, as the daily renewal rate gradually increased from 5%, the bloom area gradually decreased. At the same time, the non-bloom occurrence area increased. It could be anticipated that the *Microcystis* bloom area would disappear at a certain dilution rate. In particular, it can be found in the figures that when the renewal rate was equal to or higher than 12.5%, the bloom area no longer existed. Based on Equation (1), the dilution rate D was 13.3% when the renewal rate was 12.5%.

The data from Chiba Prefectural Government (Chiba Prefectural Government 2019) was used to calculate the annual average of dilution rate (D_{avg}) and the cell densities of *Microcystis* sp. in every month of each year in Lake Tega. The results are displayed in Figure 4. The field observation data indicated that when the dilution rate was above 13.3%, *Microcystis* sp. was hard to proliferate, and the cell density was less than 3×10^3 cells mL^{-1}. This trend apparently corresponds to the predicted data as shown in Figure 3. Thus, the dilution rate $D = 13.3\%$ could be considered to suppress *Microcystis* blooms effectively.

4. DISCUSSION

Semi-continuous competition experiments showed that the growth of *C. meneghiniana* isolated from Lake Tega was more affected by the replacement of medium (dilution) than that of *Microcystis* sp. under phosphorus limitation. Meanwhile, the dilution still had a suppressive effect on the growth of *Microcystis* sp. The simulation results for the competitive

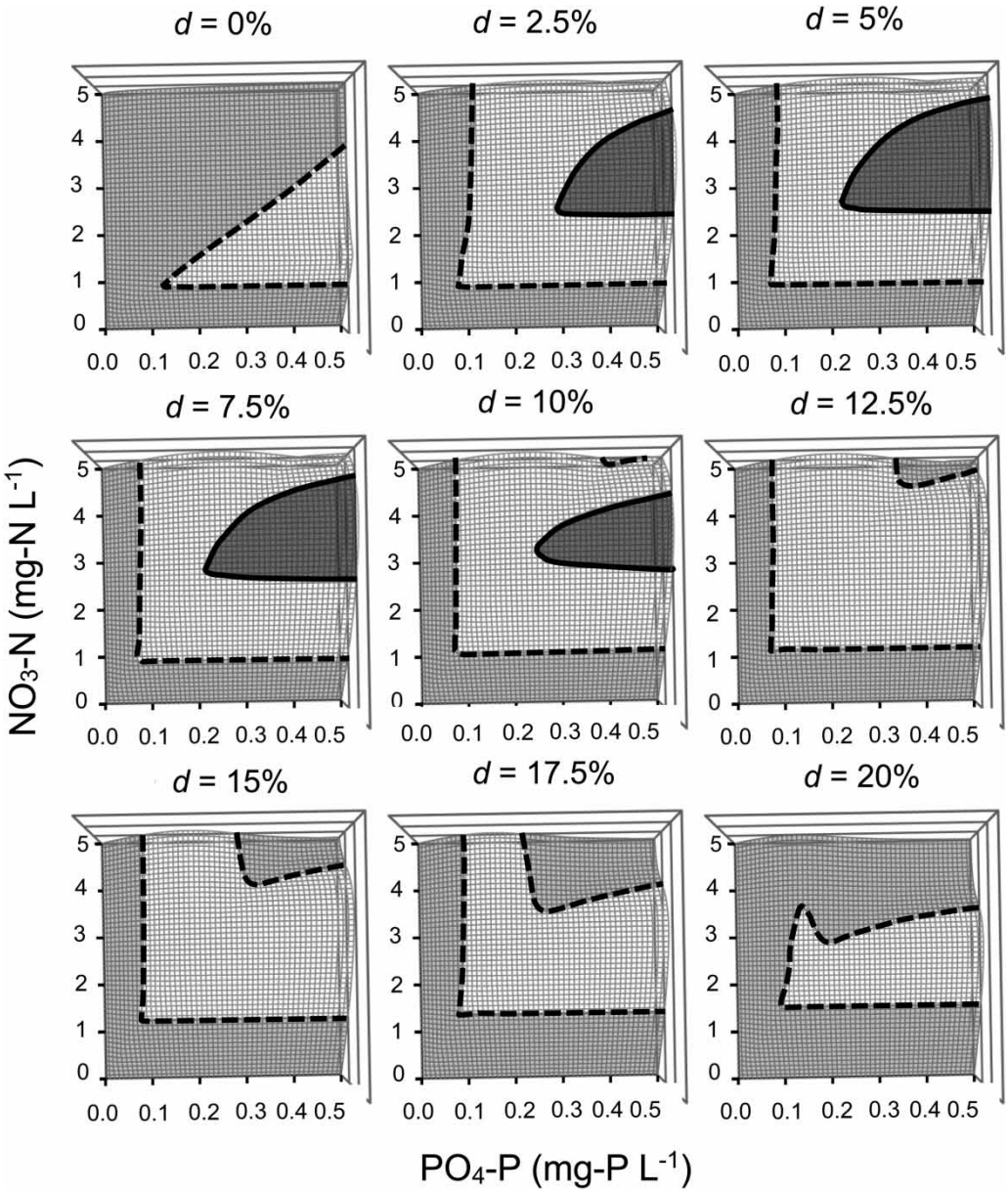

Figure 3 | Contour figures of simulated *Microcystis* sp. cell density at day 30 under various daily renewal rates at plane view. Black area and dark-grey area indicate bloom occurrence and non-bloom occurrence area, respectively. Light-grey area suffers a high risk of bloom appearance although no bloom has occurred yet.

growth elucidated that the lowest dilution rate to suppress *Microcystis* blooms was $D = 13.3\%$, which is consistent with the field observation results in Lake Tega.

In semi-continuous competition experiments, *Microcystis* sp. showed better adaptability than *C. meneghiniana* under the phosphorus limited condition. The growth parameters obtained from monoculture experiments of the two species in the previous study (Chujo *et al.* 2021) as shown in Table 2 indicate that the maximum cell quota (Q_{max}) values of *C. meneghiniana* for N and P ($Q_{max,n} = 104$ pg cell^{-1}, $Q_{max,p} = 5.08$ pg cell^{-1}) were nearly 20 times higher than those of *Microcystis* sp. ($Q_{max,n} = 5$ pg cell^{-1}, $Q_{max,p} = 0.28$ pg cell^{-1}), whereas the uptake rate ρ_{max}^{hi} for N was only twice that of *Microcystis* sp. This indicates that the deficiency of phosphorus would influence the growth of *C. meneghiniana* more than *Microcystis* sp., and that *Microcystis* sp. would grow more advantageously with adequate nutrients, excluding the effect of other factors such as

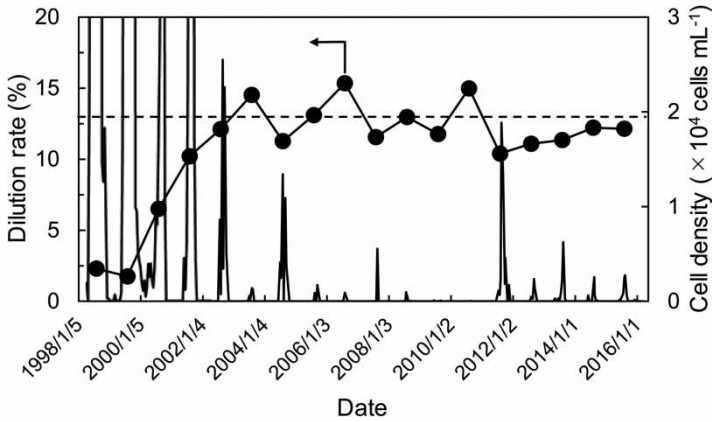

Figure 4 | Monthly cell density of *M. aeruginosa* and annual average dilution rates in Lake Tega. A dashed line indicates 13.3% of the dilution rate.

temperature and dilution. Based on the changes of nutrient concentration as shown in Figure 2, there should be no remarkable differences in cell densities of both species in each group. However, *C. meneghiniana* cell densities decreased sharply with the increase in daily renewal rate. Thus, it could be considered that the number of *C. meneghiniana* cells washed away everyday was greater than that grown under the phosphorus limitation. Furthermore, the fluctuation of phosphorus concentration caused by water transfer has been proven that it can not lead to the shift of dominant species and suppress the formation of *Microcystis* blooms (Amano *et al.* 2010). This was consistent with our results in the semi-continuous competition experiment.

Tatsumoto *et al.* (2008) indicated that the ignition loss of the Lake Tega sediment was measured to be ca. 16% and the upper 20 cm of the sediment was supposed to contribute to nutrient elution, which may provide nutrient constantly after water transfer. Water pH is also an important influential factor of the nutrient release from sediment, although the release of ammonium-nitrogen increases with pH and the release of nitrate-nitrogen has no obvious relation with pH (Hu *et al.* 2003). The release of phosphorus will decrease with increasing pH until 7.0, and increase with rising pH after higher than 8.0 (Wu *et al.* 2014). Furthermore, the contribution of different ratios of various nitrogen sources to the growth of *Microcystis* sp. has also been observed by several studies (Junfeng *et al.* 2017; Krausfeldt *et al.* 2020). In the north part of Lake Taihu, China, when the molar ratio of ammonium to nitrate was below 1, *Microcystis* blooms tended to be dominant during summer (Liu *et al.* 2011). Therefore, methods of controlling the internal pollution, adjusting the nutrient concentrations, and controlling the pH of the water body were essential for the prevention and management of cyanobacterial (*Microcystis*) blooms and should be developed in the future.

As for the simulated results shown in Figure 3, the prediction of *Microcystis* sp. growth displayed a decrease at daily renewal rates of lower than 5%, and it was very nearly impossible for the cyanobacteria blooms to occur when the daily renewal rate was 0%. However, in reality, the smaller the daily renewal rate is, the more possibility the cyanobacterial blooms occurrence should be (Romo *et al.* 2013). The discrepancy would be due to a shortcoming with the nutrient uptake term of the Chujo's model. According to the equations of the model (Table 1), the value of the uptake rate term ρ_{max} was always obtained when the nutrient concentration was adequate. However, it could not always reach the maximum value and would decrease with algal growth as well as nutrient concentration decline in the competition process. As a result of the shortcoming, the simulated nutrient concentrations always decreased exponentially when the cell densities of *Microcystis* sp. increase exponentially. In comparison, in reality, when the cell density increased exponentially with initial sufficient nutrients, the decrease in nutrient concentration was only proportionally reduced (Wang *et al.* 2010; Chujo *et al.* 2021). For this reason, the predicted nutrients uptake of *Microcystis* sp. after the logarithmic phase would be overestimated.

However, when the two species were grown together under eutrophic conditions, *C. meneghiniana* showed a very advantage in nutrient uptake over *Microcystis* sp. as shown in our previous study (Chujo *et al.* 2021). At the daily renewal rate of 0%, both the experimental and the simulation results indicated that the nutrient was almost exclusively occupied by *C. meneghiniana*, which led to the suppression of the proliferation of *Microcystis* sp. This mechanism provided a reasonable explanation

and support for the predicted cell density of *Microcystis* sp. at $d = 0\%$. Nevertheless, there was still a gap between the experimental and predicted values of the *C. meneghiniana* cell densities when it was under relatively scarce nutrient concentration. With the objective of improving the accuracy of the simulation model, the amelioration of the uptake rate term and other environmental factors such as temperature and light intensity should be focused on in the future study.

5. CONCLUSION

The replacement water (medium) restricted both the growth of *C. meneghiniana* and *Microcystis* sp. in competition experiments. Especially, the restricted effect was more significant on the growth of *C. meneghiniana* compared with that of *Microcystis* sp. under a nutrient condition with the combination of limited P and sufficient N. The difference was caused by the more excellent ability of nutrient uptake of *Microcystis* sp., and *C. meneghiniana* was easier to flow away. The broad accuracy of Chujo's competition growth model in various nutrient concentrations was also verified based on the prediction curves and competition experiments results. The minimum effective dilution rate (D) to suppress *Microcystis* sp. bloom in Lake Tega was estimated to be 13.3% by this model, which was consistent with the field observation in Lake Tega. Since the accuracy of the Chujo's model in broad nutrient concentration range has been verified, it can be a powerful tool to apply for the management of *Microcystis* bloom in many actual lakes with complex nutrient conditions.

ACKNOWLEDGEMENTS

This work was supported in part by the Japan Society for the Promotion of Science under Grants-in-aid for Scientific Research (No. JP21K04311) and by the JST SPRING (No. JPMJSP2109). Authors are thankful to Prof. Dr. Fumio Imazeki, the head of Safety and Health Organization at Chiba University, for his encouragement and financial support.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Amano, Y., Sakai, Y., Sekiya, T., Takeya, K., Taki, K. & Machida, M. 2010 Effect of phosphorus fluctuation caused by river water dilution in eutrophic lake on competition between blue-green alga Microcystis aeruginosa and diatom Cyclotella sp. *J. Environ. Sci.* **22**, 1666–1673.

Amano, Y., Takahashi, K. & Machida, M. 2012 Competition between the cyanobacterium Microcystis aeruginosa and the diatom Cyclotella sp. under nitrogen-limited condition caused by dilution in eutrophic lake. *J. Appl. Phycol.* **24**, 965–971.

Anderson, R., Berges, R., Harrison, P. & Watanabe, M. 2005 Appendix A-Recipes for freshwater and seawater media; freshwater synthetic media. *Alga. Cult. Tech. Elsevier Academic Press*, Burlington, USA, 435–436.

Bui, T. T., Nam, S.-N. & Han, M. 2015 Micro-bubble flotation of freshwater algae: a comparative study of differing shapes and sizes. *Sep. Sci. Technol.* **50**, 1066–1072.

Chiba Prefectural Government 2019 *Water Quality for Lakes in Chiba Prefecture*. Chiba Prefectural Government, Chiba city. Available from: http://www.pref.chiba.lg.jp.

Chujo, M., Pang, L., Fujimura, Y., Amano, Y. & Machida, M. 2019 Composition of small *Thalassiosirales* under highly diluted conditions in a eutrophic lake. *J. Environ. Inf. Sci.* **2019**, 13–21.

Chujo, M., Li, J., Datta, T., Amano, Y. & Machida, M. 2021 A competitive growth model for the simulation of cyanobacterial blooms under eutrophic conditions. *Environ. Eng. Sci.* **38**, 15–23.

Cottingham, K., Ewing, H., Greer, M., Carey, C. & Weathers, K. 2015 Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling. *Ecosphere* **6**, 1–19.

Farnham, W., Murfin, C., Critchley, A. & Morrell, S. 1980 Distribution and control of the brown alga *Sargassum muticum*. *International Seaweed Symposium*. Edited by: Tore Levrig Göteborg, Sweden, 278–282.

Guillard, R. R. & Lorenzen, C. J. 1972 Yellow-green algae with chlorophyllide c. *J. Phycol.* **8**, 10–14.

Hu, Q., Zhu, Y., Song, J., Li, Z. & Wen, J. 2003 Effects of pH and Eh on release of nitrogen and phosphorus from sediments of West Lake. *J. Zhejiang Univ. Sci. A* **4**, 358–362.

Junfeng, S., Tingting, L., Tinglin, H., Donghui, L. & Min, M., & suo L. 2017 Microcystis aeruginosa flour as carbon and nitrogen source for aerobic denitrification and algicidal effect of Raoultella sp. *R11. Ecol. Eng.* **105**, 162–169.

Krausfeldt, L. E., Farmer, A. T., Castro, H. F., Boyer, G. L., Campagna, S. R. & Wilhelm, S. W. 2020 Nitrogen flux into metabolites and microcystins changes in response to different nitrogen sources in *Microcystis aeruginosa* NIES-843. *Environ. Microbiol.* **22**, 2419–2431.

Liu, X., Lu, X. & Chen, Y. 2011 The effects of temperature and nutrient ratios on *Microcystis* blooms in Lake Taihu, China: an 11-year investigation. *Harmful Algae* **10**, 337–343.

Maberly, S. C., Pitt, J.-A., Davies, P. S. & Carvalho, L. 2020 Nitrogen and phosphorus limitation and the management of small productive lakes. *Inland Waters* **10**, 159–172.

Mikawa, M., Sugimoto, K., Amano, Y., Machida, M. & Imazeki, F. 2016 Competitive growth characteristics between *Microcystis aeruginosa* and *Cyclotella* sp. accompanying changes in river water inflow and their simulation model. *Phycol. Res.* **64**, 123–132.

Mikawa, M., Datta, T., Amano, Y. & Machida, M. 2017 Dominant characteristics between *Microcystis aeruginosa* and *Cyclotella* sp. accompanying dilution process in eutrophic lake. *Water Air Soil Pollut.* **228**, 174.

Romo, S., Soria, J., Fernandez, F., Ouahid, Y. & Barón-Solá, Á. 2013 Water residence time and the dynamics of toxic cyanobacteria. *Freshwater Biol.* **58**, 513–522.

Sugimoto, K., Negishi, Y., Amano, Y., Machida, M. & Imazeki, F. 2016 Roles of dilution rate and nitrogen concentration in competition between the cyanobacterium *Microcystis aeruginosa* and the diatom *Cyclotella* sp. in eutrophic lakes. *J. Appl. Phycol.* **28**, 2255–2263.

Takamura, N., Otsuki, A., Aizaki, M. & Nojiri, Y. 1992 Phytoplankton species shift accompanied by transition from nitrogen dependence to phosphorus dependence of primary production in lake Kasumigaura, Japan. *Arch. Hydrobiol.* **124**, 129–148.

Tatsumoto, H., Amano, Y., Machida, M., Aikawa, M., Fujimura, Y., George, D., Berk, S. & Taki, K. 2008 Control of TN/TP ratio by sediment treatment for restraint of water blooms. *JECE.* **36**, 357–364.

Tilman, D. & Kilham, S. S. 1976 Phosphade and silicate growth and uptake kinetics of the diatoms *Asterionella formosa* and *Cyclotella mnenghiniana* in batch and semicontinuous culture. *J. Phycol.* **12**, 375–383.

Türkmen, A. & Kütük, Y. 2017 Effects of chemical fertilizer, algea compost and zeolite on green bean yield. *Turk. J. Agric. Food Sci. Technol.* **5**, 289–293.

Wang, X., Qin, B., Gao, G. & Paerl, H. W. 2010 Nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation. *J. Plankton Res.* **32**, 457–470.

Wu, Y., Wen, Y., Zhou, J. & Wu, Y. 2014 Phosphorus release from lake sediments: effects of pH, temperature and dissolved oxygen. *KSCE J. Civ. Eng.* **18**, 323–329.

First received 11 January 2022; accepted in revised form 27 March 2022. Available online 7 April 2022