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Cyanobacterium *Microcystis* Bloom in a Eutrophicated Regulating Reservoir

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Abstract

The effect of the hydraulic turnover rate on dominance of the cyanobacterium *Microcystis* was examined using field data from the summer of 2001 for the Minami-shio Reservoir, a shallow eutrophic regulating reservoir in Japan, where the water outflow and storage volumes are measured hourly. A *Microcystis* bloom occurred in late summer when the daily hydraulic turnover rate decreased to 15%. The hydraulic turnover rate provided an index of nutrient supply rate, because the nutrient load was input mostly in the inflowing water and because the water body was well mixed. Field measurement results indicate that the low hydraulic turnover rate led to the *Microcystis* bloom.

Discipline: Watershed and regional resources management
Additional key words: algal bloom, hydraulic turnover rate

Introduction

Massive growth of *Microcystis* in eutrophic freshwater systems is a serious problem. The growth of *Microcystis* produces bad-smelling and unsightly scum, preventing recreational use of water bodies, hampering the treatment of water for drinking, and clogging irrigation pipe. Some *Microcystis* species produce toxins that cause fatal poisoning of livestock and humans[3,15]. To predict and prevent nuisance *Microcystis* blooms in freshwater systems, it is necessary to understand the conditions that lead to *Microcystis* dominance.

Various explanations for *Microcystis* dominance have been proposed. A recent review[6] lists nine factors that promote *Microcystis* dominance: resource competition, light conditions, pH/CO_2 conditions, buoyancy, high-temperature tolerance, avoidance by herbivores, superior cellular nutrient storage, ammonium-N exploitation, and competition for trace elements. In addition to these explanations, water exchange can affect *Microcystis* dominance. *Microcystis* blooms often appear in still waters with low water exchange rates[4,11]. Furthermore, water exchange may be related to the nutrient supply, which can also be a critical environmental factor affecting *Microcystis* dominance.

Mass development of *Microcystis* frequently occurs under conditions of high water stability, particularly under stratified conditions when the nutrient supply rate is low[6,13,22]. On the other hand, under continuously flowing conditions and a continuous supply of nutrients, a shift in dominance from *Microcystis* to other species such as green algae and diatoms has been observed[2,19,20]. In laboratory competition experiments, a continuous nutrient supply does not necessarily favour *Microcystis*[10,16,21].

One of the specific characteristics of regulating reservoirs for irrigation in Japan is artificial exchange of water (hydraulic turnover rate) due to paddy irrigation demand. Hydraulic turnover rate during the paddy irrigation period from the end of Apr to the end of Aug is much higher than that of the other period. Hydraulic turnover rate affects hydrodynamic conditions, which will change the environmental conditions for phytoplankton growth.

In regulating reservoirs for irrigation, the nutrient supply rate tends to be related to the hydraulic turnover rate of storage water. Thermal stratification often disappears in summer, when increased mixing is accompanied by high hydraulic turnover rates due to irrigation

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demand. In chemostat experiments in which water was thoroughly mixed, the dominance of the cyanobacterium *Microcystis* varied with the turnover rate[18]. To our knowledge, few studies have examined the relationship between the hydraulic turnover rate and *Microcystis* dominance in regulating reservoirs.

We suggest that the hydraulic turnover rate is a critical factor for *Microcystis* dominance in regulating reservoirs for irrigation. In a small, shallow reservoir, the hydraulic turnover rate can be considered as an index of the nutrient supply rate to the whole water body. We examined the effect of the hydraulic turnover rate on *Microcystis* dominance using field data collected during the summer of 2001 from the Minami-shio Reservoir, a shallow, eutrophic regulating reservoir for irrigation in Japan, where hydraulic turnover rates have been calculated precisely. We discuss the effects of the turnover rate and nutrient supply on *Microcystis* dominance.

Materials and methods

Field studies were carried out in Minami-shio Reservoir (36°14′N and 140°4′E), which was constructed in 1991 as a facility of the Kasumigaura Irrigation Project. The reservoir has a maximum depth of 9.0 m, a surface area of 1.2×10^5 m^2, and a storage volume of 5.6×10^5 m^3 (Fig. 1). We used the storage volume of water in the reservoir and the volume of the outflow as measured by the Kasumigaura Canal Operating & Management Office (Japan Water Agency) to calculate the hydraulic turnover rates.

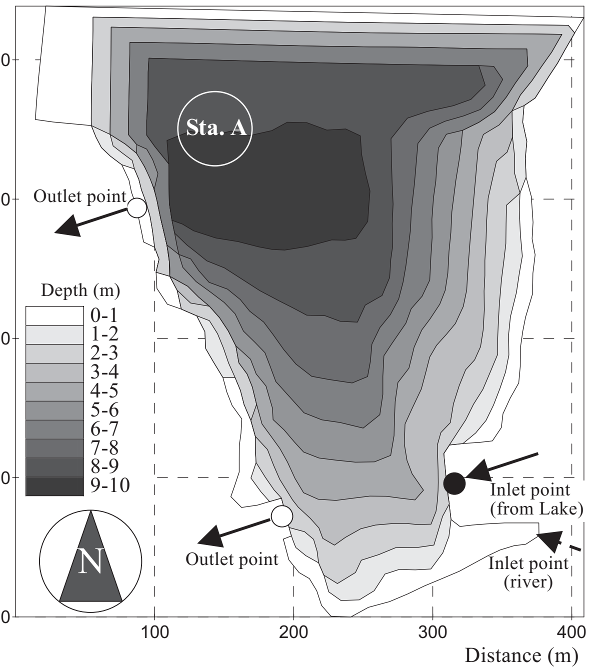


Fig. 1. Bathymetry of the studied reservoir

Five submerged 5-kW mixers have been installed in 0.5 m depths of the reservoir as a countermeasure for algal, especially cyanobacteria, bloom by artificial mixing but *Microcystis* is still present. The mixers have a 1-m-diameter propeller that generates a horizontal water flow and circulates the surface water. In 2001, the mixers were operated from 06:00 to 18:00 from 7 Jul to 10 Sep except when it was windy (about > 5 m/s). The mixers were operated by the caretaker who belonged to the farmer association and he tried to reduce the operating time in order to reduce its running cost. These mixers partly contributed to the mixing of water.

Sampling was conducted at station A (Sta. A) and at the inlet point (Fig. 1) weekly or once every 2 weeks from May to Oct 2001. Dissolved oxygen (DO), pH and water temperature were analyzed with a multi-monitoring system (U-20, Horiba, Japan). Other temperature sensors (StowAway TidbiT, Onset, USA) recorded water temperature at 10-min intervals at depths of 0.3, 2.0, 4.0, and 6.0 m on the north side. The profile of underwater photosynthetically active radiation (PAR) was measured on 2 Aug using LI-193SA spherical quantum sensor (LI-COR Inc., USA) at Sta. A. Water samples for determining the concentrations of nutrients and chlorophyll-*a* were collected with a van Dorn water sampler. After sampling, collected water samples were kept at 4°C in the dark and measured within 48 h. Qualitative phytoplankton samples were collected by passing a 72-µm-mesh plankton net through the open water at the center and the inlet point of the reservoir.

Nutrient concentrations were measured in unfiltered samples. Chlorophyll-*a* was estimated by spectrophotometry after filtering of the samples through glass fiber discs (GF/B, Whatman, UK) and extraction with cold 90% (v/v) acetone[14]. The concentration of the PO_4-P was measured by the methods of Murphy & Riley[9]. The concentrations of NH_4-N, NO_2-N and NO_3-N were measured by ion chromatography (DX-320, Dionex, USA). Phytoplankton species were observed at 100× magnification with an inverted microscope (DMIL, Leica, Germany) in order to determine *Microcystis* dominant periods by counting the ratio of *Microcystis* colonies to all other species.

Meteorological data, measured at intervals of an hour, were obtained from the Weather Data Acquisition System of the National Institute of Agro-Environmental Sciences, 20 km away from the reservoir.

Principal component analysis (PCA) was performed in order to determine external environmental factors for increasing chlorophyll-*a* concentration. Meteorological factor (wind speed and solar radiation), dissolved nutrient concentration (PO_4-P, NO_2+NO_3-N), and hydraulic turnover rate were supposed to be external environmental factors. Statistical analysis was carried out using three days of averaged values before the day of field measurement, since the phytoplankton growth rate is so low that the observed phytoplankton biomass was assumed to be dependent on the factors of three days before. In addition, the averaged hydraulic residential time during irrigation period was 2.7 days.

Results

The daily hydraulic turnover rate was calculated by dividing the daily out-flowing water volume by the maximum storage volume (Fig. 2). We used the maximum storage volume as the divisor because the observed water level was almost the same as the full reservoir level. From 20 Apr to 26 Aug 2001, the average daily hydraulic turnover rate was 37%. Other than this period, the average rate was a constant 15%.

The productive zone was assumed to be 2 m, since observed PAR at 2 m depth was 5 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and further measurement in summer 2004 showed that the average value of PAR at 2 m depth was 13 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (Max: 34, Min: 1).

Figure 3a shows the vertical profile of observed chlorophyll-*a* concentration. The average concentration of chlorophyll-*a* was 22.1 $\mu g \cdot L^{-1}$. The chlorophyll-*a* concentration at surface increased three times in the summer of 2001.

The first peak in May was caused by phytoplankton included in inlet water, because daily inflowing volume was more than 50% of the storage volume and because the phytoplankton mainly observed in inlet water and in the reservoir was also diatom (*Synedra*). From these results it could be concluded that diatom included in inlet water raised the chlorophyll-*a* concentrations in the reservoir.

The second peak of chlorophyll-*a* concentration by *Volvox* was from 19 Jul to 2 Aug. On 19 Jul the chlorophyll-*a* concentration reached to 46 $\mu g \cdot L^{-1}$ and green surface scum formed. The third peak of chlorophyll-*a* concentration by *Microcystis* was from 19 Sep to 11 Oct. The chlorophyll-*a* concentration began to increase on 6 Sep. During this peak, the concentration ranged between 18 and 50 $\mu g \cdot L^{-1}$ at 0 and 1 m depth. On 19 Sep and 3 Oct green granular surface scum formed. After 11 Oct, the concentration had decreased markedly.

In our investigation results, *Microcystis* was dominant from 6 Sep to 11 Oct. The dominant period of *Microcystis* was defined here as the period when the ratio of *Microcystis* colonies to other observed species exceeded 10%, since one *Microcystis* colony commonly contains more than one thousand cells. During the second peak of chlorophyll-*a* concentration in Jul *Microcystis* was not dominant, but *Volvox* was dominant.

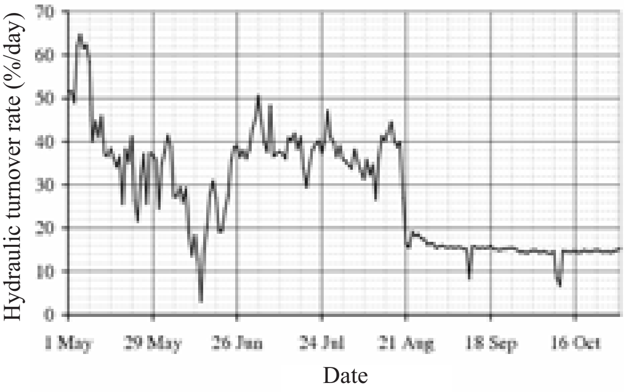
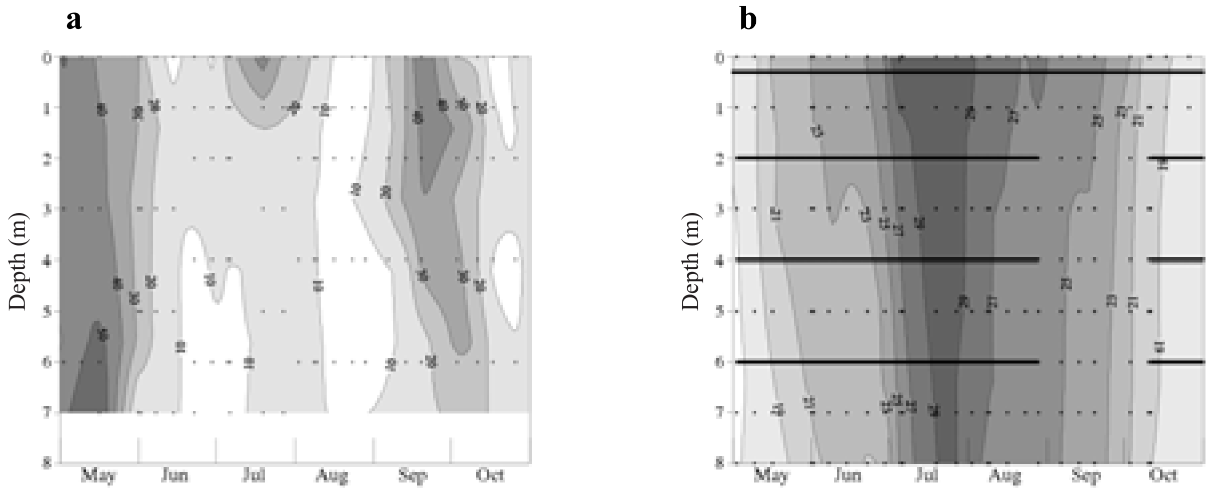


Fig. 2. Daily hydraulic turnover rate of the reservoir calculated from the daily out-flowing water volume and the storage water volume

Microcystis blooms, defined here as the averaged chlorophyll-*a* concentration value at the productive zone (0 to 2 m depth) greater than 20 $\mu g \cdot L^{-1}$ and the ratio of the colonies exceeding 10%, formed from 13 Sep to 11 Oct. It could be suggested that *Microcystis* biomass increased in Sep, since chlorophyll-*a* concentration, which is the index of phytoplankton biomass increased. A *Microcystis* bloom occurred 3 weeks after the hydraulic turnover rate decreased to 15%.

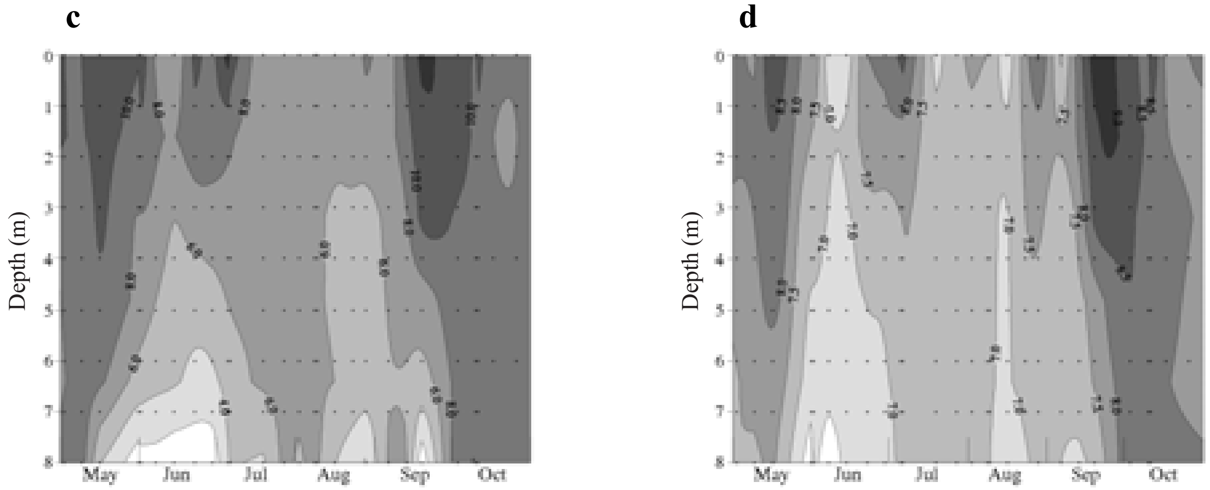
No clear thermal stratification developed (Fig. 3b). The difference in water temperature between 0 and 8 m depth was usually within 3°C. The maximum difference was 6.3°C on Jul 5. The DO concentration above 6 m depth was higher than 4 $mg \cdot L^{-1}$ (Fig. 3c). The pH differed by less than 1 pH unit between 0 and 8 m depth (Fig. 3d). The result of a homogeneous oxygen and pH distribution with depth supported that a strong thermocline did not form. High pH and over-saturated DO during the third peak indicated a high photosynthetic rate.

Figures 3e and 3f show the concentrations of PO_4-P and (NO_2+ NO_3)-N. During the second peak of chlorophyll-*a* concentration, the concentration of PO_4-P at both 0 and 1 m depth was around 0.03 $mg \cdot L^{-1}$, and the concentration of (NO_2+ NO_3)-N, 0.3 $mg \cdot L^{-1}$, was higher than during other periods. During the third peak of chlorophyll-*a* concentration by *Microcystis* bloom, the concentration of PO_4-P at both 0 and 1 m depth was less than 0.01 $mg \cdot L^{-1}$, and the concentration of (NO_2+ NO_3)-N, 0.1 $mg \cdot L^{-1}$, was lower than during other periods. The NH_4-N concentration was lower than the limit of detection (0.05



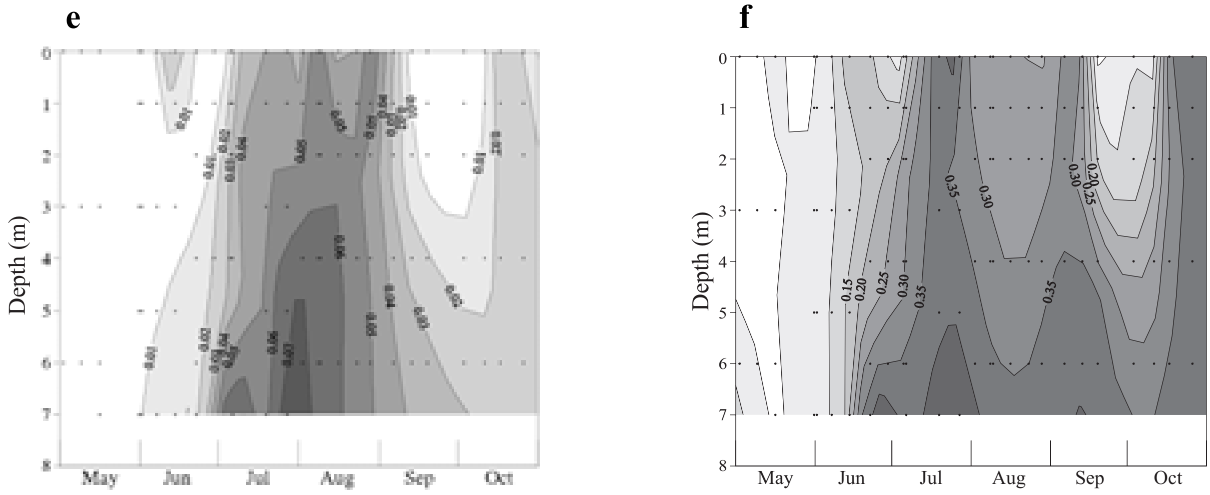
a : Chlorophyll-*a* concentration (unit: $\mu g \cdot L^{-1}$)

b : Water temperature (unit: °C)



c : Dissolved oxygen concentration (unit: $mg \cdot L^{-1}$)

d : pH



e : PO_4-P concentration (unit: $mg \cdot L^{-1}$)

f : $(NO_2 + NO_3)$-N concentration (unit: $mg \cdot L^{-1}$)

Fig. 3. Time series of the vertical profile of observed data
Black dots show the sampling times and depth.

mg·L^{-1}) (data not shown).

The nutrient concentration of the inlet water was constant. The inflowing water came from Lake Kasumigaura, which is hypertrophic. The average nutrient concentrations in the inlet water were NH_4-N, < 0.01 mg·L^{-1}, (NO_2+ NO_3)-N, 0.19 mg·L^{-1}, T-N, 1.1 mg·L^{-1}, PO_4-P, 0.02 mg·L^{-1}, and T-P, 0.1 mg·L^{-1}. More than 98% of the supplied nutrients came from the inflowing water, and less than 2% came from other sources such as the load in run-off from the reservoir's catchment (unpublished data).

Figure 4 shows the result of PCA analysis of determining factors for *Microcystis* dominance. As a first step, the data in May were excluded from the analysis, since diatom included in inlet water raised the chlorophyll-*a* concentrations in the reservoir. Secondly, the data of chlorophyll-*a* and dissolved nutrients were given by depth averaged (from 0 to 2 m) considering the productive zone for phytoplankton. The values for statistical analysis were given by linearly day interpolating from the depth-averaged values. Principal components analysis (PCA) was performed on the five indicator variables. The five variables explained 70.4% of the variation in the first two axes. Hydraulic turnover rate and solar radiation are the strongest axis-I variables. These variables make up the axis-I environmental gradient. Concentrations of dissolved nitrogen and phosphorus are stronger axis-II variables. The plots of *Microcystis* bloom are located on the impairment side of axis-I and over all of axis-II. These results pointed out that *Microcystis* favoured the opposite condition of axis-I variables and they were not as important as axis-II variables.

It could be interpreted that *Microcystis* bloomed during low hydraulic turnover rates and for a short duration of time, because the plots of the bloom are located on the impairment side of axis-I. Moreover, it could be suggested that dissolved nutrients in the surface of the reservoir were not a critical factor for *Microcystis* dominance since there was not a significant relationship in axis-II.

Discussion

Microcystis blooms often appear in eutrophic waters at high temperature. We hypothesized that the hydraulic turnover rate is a critical factor explaining *Microcystis* dominance in a small, shallow reservoir. To test this hypothesis, we examined the effect of the hydraulic turnover rate on *Microcystis* dominance using field data from the Minami-shio Reservoir, a shallow, eutrophic reservoir in Japan.

Our study showed that the period of *Microcystis* dominance coincided with that of low hydraulic turnover rates. When the daily hydraulic turnover rate was less than 15%, the chlorophyll-*a* concentration was more than 20 μg·L^{-1}, owing to a *Microcystis* bloom. A significant correlation was found between the chlorophyll-*a* concentration and a low hydraulic turnover rate (Fig. 4).

In the reservoir, the hydraulic turnover rate is an index of the nutrient supply rate, since the reservoir had three specific characteristics related to nutrient supply to whole water bodies. (1) Most nutrients were supplied from the inflowing water, and the dissolved nutrient concentrations in the inflowing water were constant. (2) Thermal stratification, which impedes vertical water movement, did not develop well (Figs. 3b & 3c). (3) The water body was nearly circular (shoreline development, the ratio of the length of the shore line to the circumference of a circle of area equal to that of the water body, = 1.3), so water could easily circulate horizontally. Thus, nutrients entering with the inlet water were distributed equally to the entire reservoir. Therefore, the hydraulic turnover rate well reflected the nutrient supply to the whole water body. Since the hydraulic turnover rate was low from the end of Aug to Oct (Fig. 2), the nutrient supply was also low. The observed dissolved nutrient depletion in the surface water column during the *Microcystis* bloom was consistent with a low nutrient supply rate (Figs. 3e & 3f).

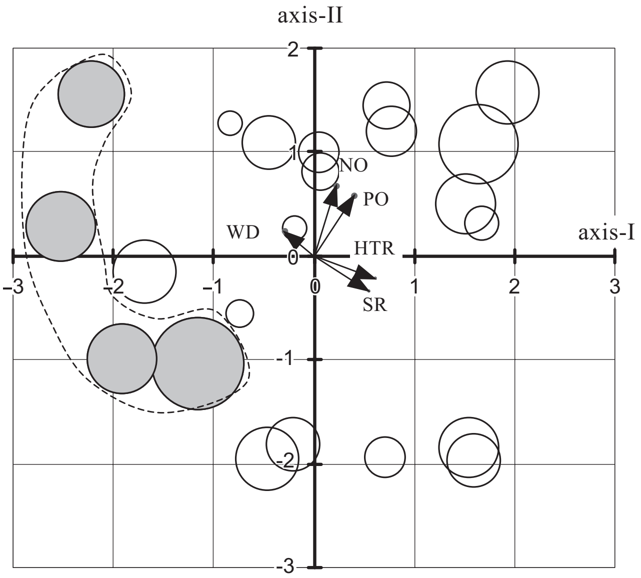


Fig. 4. PCA ordination of five indicator environmental variables for chlorophyll-*a* concentrations

WD: Wind speed, SR: Solar radiation, PO: PO_4-P, NO: (NO_2 + NO_3)-N, HTR: Hydraulic turnover rate. Area of circle represents the concentration of chlorophyll-*a*. Gray circles represent *Microcystis* dominance.

A low nutrient supply rate seems to cause *Microcystis* dominance. Mass development of *Microcystis* frequently occurs when the nutrient supply rate is low[4,11]. Olsen et al.[10] found that a low phosphorus supply rate favored *Microcystis* in competition experiments. Kuwata & Miyazaki[7] indicated that a low nitrogen supply rate could lead to *Microcystis* dominance in a simulation study, and Takeya et al.[18] confirmed this result by chemostat experiments. The dominance of *Microcystis* during summer is consistent with the results of these previous studies. In addition, the 3-weeks time lag between the bloom and environmental condition sufficient for water bloom formation was the same as that of a *Microcystis* bloom reported by Atkins et al.[1].

The phytoplankton composition of the inlet water was dissimilar to that of the reservoir water, and the number of phytoplankton in the inlet water was lower than that in the reservoir water. In addition, according to other studies carried out in the same water supply system, the physiological activity of phytoplankton in the inflow water is low because of high pressures during water pumping from Lake Kasumigaura into the reservoir, and the number of phytoplankton in the inflow water is low compared with that in the source water[8,17]. Thus, phytoplankton in the present reservoir grew independently of the phytoplankton in the water flowing into the reservoir. In addition, the effect of the inlet water on phytoplankton composition in the reservoir was less when the hydraulic turnover rates were low.

Submerged water pumps had less effect on *Microcystis* dominance, because the ratio of *Microcystis* in the whole observed phytoplankton sample and chlorophyll-*a* concentration increased during the operation term.

In conclusion, low hydraulic turnover rates favored *Microcystis* dominance in Minami-shio Reservoir. Our result is based on field data from the reservoir, in which ① the water balance was observed at 1-h intervals; ② the nutrient inflow rate depended on the inflowing water volume; and ③ the hydraulic turnover rate changed drastically. Although the field data demonstrated that a low hydraulic turnover rate resulted in *Microcystis* dominance, mechanisms other than low hydraulic turnover rate (avoidance by herbivores, buoyancy) can lead to *Microcystis* dominance[5,12]. To prevent *Microcystis* blooms, further investigations of the mechanisms of *Microcystis* bloom formation are needed.

Acknowledgments

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References

1. Atkins, R. et al. (2001) The Microcystis cyanobacteria bloom in the Swan River—February 2000. *Water Sci. & Technol.*, **43**(9), 107–114.
2. Berman, T. & Shteinman, B. (1998) Phytoplankton development and turbulent mixing in Lake Kinneret (1992–1996). *J. Plankton Res.*, **20**, 709–726.
3. Codd, G. A., Bell, S. G. & Brooks, W. P. (1989) Cyanobacterial toxins in water. *Water Sci. & Technol.*, **21**, 1–13.
4. Fogg, G. E. (1969) The physiology of an algal nuisance. *Proc. R. Soc. Lond.* B, **173**, 175–189.
5. Haney, J. F. (1987) Field studies on zooplankton–cyanobacteria interactions. *NZ. J. Mar. & Freshwater Res.*, **21**, 467–475.
6. Hyenstrand, P., Blomqvist, P. & Pettersson, A. (1998) Factors determining cyanobacterial success in aquatic systems—a literature review. *Arch. Hydrobiol., Spec. Issues: Adv. Limnol.*, **51**, 41–62.
7. Kuwata, A. & Miyazaki, T. (2000) Effects of ammonium supply rates on competition between *Microcystis novacekii* (Cyanobacteria) and *Scenedesmus quadricauda* (Chlorophyta): Simulation study. *Ecol. Modelling*, **135**, 81–87.
8. Miyazaki, T., Tajima, K. & Iriyama, Y. (1995) Carbon and nitrogen uptake activities in irrigation systems. Possible effects of pressures. *Arch. Hydrobiol.*, **135**, 101–111.
9. Murphy, J. & Riley, J. P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta*, **27**, 31–36.
10. Olsen, Y. et al. (1989) Competition between *Staurastrum luetkemuellerii* (Chlorophyceae) and *Microcystis aeruginosa* (Cyanophyceae) under varying modes of phosphate supply. *J. Phycol.*, **25**, 499–508.
11. Pearl, H. W. (1988) Growth and reproductive strategies of freshwater blue-green algae (Cyanobacteria). *In* Growth and reproductive strategies of freshwater phytoplankton, ed. Sandgren, C. D., Cambridge University Press, New York, 261–315.
12. Reynolds, C. S., Oliver, R. L. & Walsby, A. E. (1987) Cyanobacterial dominance: The role of buoyancy regulation in dynamic lake environments. *NZ. J. Mar. & Freshwater Res.*, **21**, 379–390.
13. Reynolds, C. S. (1993) Scales of disturbance and their role in plankton ecology. *Hydrobiologia*, **249**, 157–171.
14. SCOR-UNESCO (1966) Determination of photosynthetic pigments in seawater. *In* Monographs on oceanographic methodology vol. 1, UNESCO, Paris, 11–18.
15. Sivonen, K. (1996) Cyanobacterial toxins and toxin production. *Phycologia*, **35**, 12–24.
16. Sommer, U. (1985) Comparison between steady state and non-steady state competition: Experiments with natural phytoplankton. *Limnol. & Oceanogr.*, **30**, 335–346.
17. Tabuchi, T. et al. (1989) Change in water quality flowing

through a pipeline. Study of water quality in a water conveyance system (I). *Trans. Jpn. Soc. Irrig., Drain. & Reclam. Eng.*, **140**, 57–63 [In Japanese with English summary].

18. Takeya, K. et al. (2004) Effect of dilution rate on competitive interactions between the cyanobacterium *Microcystis novacekii* and the green alga *Scenedesmus quadricauda* in mixed chemostat cultures. *J. Plankton Res.*, **26**, 29–35.
19. Toetz, D. W. (1981) Effects of whole lake mixing on water quality and phytoplankton. *Water Res.*, **15**, 1205–1210.
20. Visser, P. M. et al. (1996) Artificial mixing prevents nuisance blooms of the cyanobacterium *Microcystis* in Lake Nieuwe Meer, the Netherlands. *Freshwater Biol.*, **36**, 435–450.
21. Watanabe, T. & Miyazaki, T. (1996) Maximum ammonium uptake rates of *Scenedesmus quadricauda* (Chlorophyta) and *Microcystis novacekii* (cyanobacteria) grown under nitrogen limitation and implications for competition. *J. Phycol.*, **32**, 243–249.
22. Zohary, T. & Breen, C. M. (1989) Environmental factors favouring the formation of *Microcystis aeruginosa* hyperscums in a hypertrophic lake. *Hydrobiologia*, **178**, 179–192.