Note

Effects of Temperature and Illumination on the Growth of Blue-green Alga *Microcystis viridis*

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Abstract

A field study was carried out from summer to fall in 1988 to clarify the change in the dominant species of *Microcystis* in Lake Kasumigaura. *Microcystis aeruginosa* f. *aeruginosa* and *M. aeruginosa* f. *flos-aquae* were dominant in the summer, and *M. viridis* became dominant in the fall. A high correlation was found between the standing crop of *M. aeruginosa* and the water temperature, but the standing crop of *M. viridis* and *M. wesenbergii* showed no correlation with the water temperature.

Effects of temperature and illumination on the specific growth rates (μ) and the algal yield of *M. viridis* N-1 and *M. viridis* NIES 102 were studied with a batch culture. Maximum specific growth rates ($\mu_{\max}$) of *M. viridis* N-1 and *M. viridis* NIES 102 were observed at 25 and 30°C, respectively. *M. viridis* could grow at lower temperature than the other species of *Microcystis*. *M. viridis* N-1 could not grow under continuous illumination of 2,000 lx but did so under periodic illumination. The specific growth rate of *M. viridis* was lower than for other *Microcystis* species.

These results suggested that *M. viridis* was more difficult to dominate than other *Microcystis* in summer.

Key words: blue-green alga, *Microcystis*, specific growth rate, water bloom

1. Introduction

Many workers have studied the ecological and physiological characteristics of *Microcystis* both in the field and in the laboratory. Some environmental factors, such as high water temperatures above 20°C, the formation of thermocline, the high pH value from 9 to 11 in the water surface layer, and the high irradiance were important for water bloom formation of *Microcystis* (OKINO, 1973).

Physiological studies on *Microcystis* developed by the use of the pure culture (GERLOFF *et al.*, 1950). McLACHLAN and GORHAM (1962) reported that the pH from 6.5 to 10 and nitrogen sources such as nitrate, ammonium and urea were important for the growth of *M. aeruginosa*. We have studied the growth charac-

teristics of two species of *Microcystis* (*M. aeruginosa* K-3A and *M. flos-aquae* K-5 strains) isolated from Lake Kasumigaura and reported the following results (YAGI *et al.*, 1984). The maximum specific growth rate ($\mu_{\max}$) of these *Microcystis* strains was observed at 35°C, and the nitrogen and phosphorus concentration for the $\mu_{\max}$ of these strains were 1.0 and 0.05 mg·l⁻¹, respectively. Moreover, one of the heterotrophic bacteria, *Moraxella nonliquefaciens*, increased the specific growth rate and the maximum growth yield of *M. aeruginosa* K-3A (KUMAGAI *et al.*, 1986). AMEMIYA *et al.* (1990) investigated the factors affecting the dominance of *Microcystis* species in Lake Suwa. They reported that *M. aeruginosa* became dominant at a relatively high nitrogen concentration and both *M. aeruginosa* and *M. wesenbergii*

could grow better than *M. viridis* at a higher light intensity.

In recent years, KUSUMI *et al.* (1987) reported that *M. viridis* produced a toxic substance which was a pentapeptide. It is very important to control the growth of *M. viridis*. However, only a few researchers have studied its growth characteristics. The present study was conducted to clarify the factors influencing the growth of *M. viridis*. We studied the change in the dominant species of *Microcystis* from summer to fall in Lake Kasumigaura, and the temperature and illumination effects on the growth of *M. viridis* in the laboratory.

2. Materials and methods

2-1. Determination of algal standing crop

Algal cell numbers were counted with a photomicroscope. The algal flocks were compressed previously to count the cell numbers. The classification of the four species of *Microcystis* was based on the system as reported by KOMÁREK (1958). Water samples were collected from the yacht harbor in Lake Kasumigaura using a 100 cm water column sampler. The water temperature was measured by thermometer.

2-2. Test algae

M. viridis N-1 and *M. viridis* NIES 102 were used in this experiment. A single cell of *M. viridis* N-1 was isolated from an algal flock sample in Lake Kasumigaura utilizing the capillary-pipette method in 1986. An axenic strain of *M. viridis* NIES 102 was provided from the culture collection of The National Institute for Environmental Studies. *M. viridis* N-1 has the characteristic of colony forming, but *M. viridis* NIES 102 loses that characteristic.

2-3. Medium

Table 1 shows the composition of M-11 medium which is a modified Gorham medium (HUGHES *et al.*, 1958). To avoid the formation of precipitation, the solutions of K_2HPO_4 and ferric citrate were autoclaved separately at 121°C for 15 min. These solutions were added axenically before using the M-11 medium.

2-4. Cultivation

Test algae were cultured in 100 ml of M-11

Table 1. Composition of M-11 medium.

NaNO ₃	100 mg
K ₂ HPO ₄	10 mg
MgSO ₄ ·7H ₂ O	75 mg
CaCl ₂ ·2H ₂ O	40 mg
Na ₂ CO ₃	20 mg
Fe-citrate	6 mg
Na ₂ EDTA·2H ₂ O	1 mg
Deionized Water	1 l
pH	8.0

medium in a 200 ml Erlenmeyer flask under illumination provided by a fluorescent lamp. The illumination cycle consisted of alternating 12 hr periods of illumination and darkness at 25°C. One milliliter of 100 mg·l⁻¹ of pre-cultured algae was inoculated in 100 ml of M-11 medium and cultured under the same conditions as the pre-culture.

2-5. Measurement of cell growth and specific growth rate

The biomass was estimated from algal cell numbers and cell volume using a coulter counter (Coulter Counter Electronics Inc., Model ZM-II). The algal cell numbers and cell volume were converted to algal dry weight (mg·l⁻¹) by a calibration curve. The specific growth rate, μ (d⁻¹) was calculated in the logarithmic phase using the following equation (1).

$$\mu = \frac{1}{t - t_0} \cdot \ln \frac{N}{N_0} \quad (1)$$

N : Algal concentration at t time (mg·l⁻¹)

N_0 : Algal concentration at t_0 time (mg·l⁻¹)

t : Cultivation time (day)

t_0 : Starting time of logarithmic phase (day)

3. Results and discussion

3-1. Changes in components of *Microcystis* species in Lake Kasumigaura

Figure 1 shows the seasonal changes in abundance of four species of *Microcystis* in Lake Kasumigaura in 1988. The abundance of cell numbers of *M. aeruginosa* f. *aeruginosa* and *M. aeruginosa* f. *flos-aquae* were ca. 6×10^2 and 1×10^3 cells·ml⁻¹ on 12 July, and increased to the maximum amounts of ca. 1×10^5 and 7×10^4

Fig. 1. Seasonal changes in cell numbers of four species of *Microcystis* in Lake Kasumigaura.

cells·ml⁻¹ on 24 August. The abundance of cell numbers of *M. aeruginosa* f. *aeruginosa* and *M. aeruginosa* f. *flos-aquae* reduced to ca. 4×10^3 and 2×10^3 cells·ml⁻¹ on 8 September, respectively, and decreased to less than 10^2 cells·ml⁻¹ on 4 October. *M. viridis* maximized ca. 1×10^4 cells·ml⁻¹ on 24 August and did not decrease from September to October. The maximum cell number of *M. wesenbergii* was ca. 4×10^2 cells·ml⁻¹ on 8 September. *M. aeruginosa* tended to be dominant in summer, while *M. viridis* did so in fall.

TAKAMURA and WATANABE (1987) also reported that *M. aeruginosa* f. *aeruginosa* and *M. aeruginosa* f. *flos-aquae* tended to predominate in the early stage of the bloom or sometimes throughout the bloom, while *M. viridis* tended to predominate in the middle and late stage of

the bloom from 1982 to 1985.

Table 2 shows the correlation coefficients between water temperature and the cell number of each species from June to November. There are high correlations between the water temperature and the cell number of *M. aeruginosa* or total cell number of four *Microcystis*. But no correlation was observed between water temperature and the cell number of *M. viridis* or *M. wesenbergii*. TAKAHASHI *et al.* (1981) also reported a positive correlation between total cell number of *Microcystis* and water temperature by the method of multi-regression analysis. These results suggested that the effect of water temperature on the standing crop of *Microcystis* was different for each species.

Table 2. Correlation coefficients between cell numbers of *Microcystis* and water temperature in Lake Kasumigaura.

Species	Regression equation	Correlation coefficient	Sample number
<i>M. aeruginosa</i> f. <i>aeruginosa</i>	$\log Y = 0.27x - 3.09$	0.867	22
<i>M. aeruginosa</i> f. <i>flos-aquae</i>	$\log Y = 0.27x - 4.11$	0.716	15
<i>M. viridis</i>	$\log Y = 0.23x - 4.06$	0.393	19
<i>M. wesenbergii</i>	$\log Y = 0.11x - 2.30$	0.314	22
Total of four <i>Microcystis</i>	$\log Y = 0.25x - 3.14$	0.838	22

3-2. Effect of temperature on the algal growth

Effects of temperature on μ values of the N-1 and NIES 102 strains under the periodic or continuous illumination of 2,000 lx are shown in Figure 2. Since the N-1 strain did not grow under the continuous illumination of 2,000 lx, it was cultivated only under the periodic illumination, when μ values of the N-1 and NIES 102

Fig. 2. Effect of temperature on the specific growth rate of *Microcystis* at 2,000 lx.

- : *M. viridis* N-1, periodic illumination*
- ◇: *M. viridis* NIES 102, periodic illumination*
- : *M. viridis* NIES 102, continuous illumination*
- : *M. aeruginosa* K-5, continuous illumination**
- △: *M. wesenbergii* K-3A, continuous illumination**

*Present experiment **YAGI (1984)

strains were 0.11 and $0.2 \cdot \text{d}^{-1}$ at 20°C, 0.17 and $0.32 \cdot \text{d}^{-1}$ at 25°C, and 0.09 and $0.32 \cdot \text{d}^{-1}$ at 30°C, respectively. The μ_{max} values of the N-1 and NIES 102 strains were observed at 25°C and 25–30°C, respectively. The μ value of the N-1 strain was lower than that of the NIES 102 strain above 20°C. The N-1 strain could not grow at 35°C, but could do so at 15°C. This result indicates that the N-1 strain can grow at lower temperature than the NIES 102 strain. The μ value of the NIES 102 strain under continuous illumination was 1.6-fold of that under periodic illumination at 30°C. We earlier observed μ_{max} values of *M. aeruginosa* K-5 and *M. wesenbergii* K-3A at 35°C (YAGI *et al.*, 1984). These facts show that the N-1 and NIES 102 strains grow more slowly above 30°C than the K-5 and K-3A strains.

Figure 3 shows the effect of temperature on the algal yields of the N-1 and NIES 102 strains under continuous or periodic illumination at 2,000 lx. The algal yield indicated normal growth for long periods. Maximum algal yields of the N-1 and NIES 102 strains were $580 \text{ mg} \cdot \text{l}^{-1}$ at 25°C, and *ca.* $400 \text{ mg} \cdot \text{l}^{-1}$ between 20 and 30°C, respectively, under periodic illumination. The algal yield of the N-1 strain was $180 \text{ mg} \cdot \text{l}^{-1}$ at 15°C, whereas that of NIES 102 strain was not detected at 15°C. Therefore, the N-1 strain can grow at a lower temperature than the NIES 102 strain.

3-3. Effect of light intensity on algal growth

Figure 4 shows the effect of light intensity on the μ values of four strains of *Microcystis*. The μ values of the N-1 strain ranged from 0.09 to $0.18 \cdot \text{d}^{-1}$ between 500 and 10,000 lx, while those

Fig. 3. Effect of temperature on the algal yield of *M. viridis* N-1 and *M. viridis* NIES 102 at 2,000 lx.

- : *M. viridis* N-1, periodic illumination
- ▲: *M. viridis* NIES 102, periodic illumination
- △: *M. viridis* NIES 102, continuous illumination

of the NIES 102 strain ranged from 0.10 to 0.34 d^{-1} between 500 and 5,000 lx under periodic illumination. The μ value of the N-1 strain was lower than that of the NIES 102 strain. The μ_{max} value of the N-1 strain was 0.18 d^{-1} between 2,000 and 3,000 lx, and that of NIES 102 strain was 0.34 d^{-1} at 4,000 lx under periodic illumination.

The effect of periodic or continuous illumination on the μ value of the NIES 102 strain is also shown in Figure 4. The continuous illumination increased the μ value of the NIES 102 strain at the range of 1.3- to 2.0-fold compared to the periodic illumination at 25°C through all light intensity. However, the continuous illumination reduced the μ value of the NIES 102 strain above 4,000 lx at 30°C. The μ_{max} value of *M. aeruginosa* K-5 was ca. 1.4 d^{-1} at 5,000 lx under continuous illumination, and the μ_{max} value of *M. wesenbergii* K-3A was ca. 0.8 d^{-1} between 3,000 and 4,000 lx under continuous illumination at 30°C. On the other hand, the μ_{max} value of *M. viridis* NIES 102 was ca. 0.5 d^{-1} between 2,000 and 3,000 lx.

Figure 5 shows the effect of light intensity on the algal yields of *M. viridis* N-1 and NIES 102

Fig. 4. Effect of light intensity on the specific growth rate of *M. viridis* N-1 and *M. viridis* NIES 102 at 25 and 30°C.

- : *M. viridis* N-1, at 25°C, periodic illumination*
- : *M. viridis* NIES 102, at 25°C, periodic illumination*
- △: *M. viridis* NIES 102, at 25°C, continuous illumination*
- ▲: *M. viridis* NIES 102, at 30°C, continuous illumination*
- : *M. aeruginosa* K-5, at 30°C, continuous illumination**
- : *M. wesenbergii* K-3A, at 30°C, continuous illumination**

*Present experiment **YAGI (1984)

strains under continuous or periodic illumination. The N-1 strain showed a high maximum algal yield of 570 $\text{mg}\cdot\text{l}^{-1}$ at 2,000 lx under periodic illumination. The maximum algal yield of the NIES 102 strain at 25°C was ca. 400 $\text{mg}\cdot\text{l}^{-1}$ between 2,000 and 4,000 lx under periodic illumination, and 300 $\text{mg}\cdot\text{l}^{-1}$ under continuous illumination. The maximum algal yield of the NIES 102 strain at 30°C was 480 $\text{mg}\cdot\text{l}^{-1}$ at 1,000 lx under continuous illumination.

The μ value and the algal yield are very important factors in algal bloom formation. The μ values of *M. viridis* N-1 and NIES 102 were lower than those of *M. aeruginosa* K-5 and

Fig. 5. Effect of light intensity on the algal yields of *M. viridis* N-1 and *M. viridis* NIES 102 at 25 and 30°C.

- : *M. viridis* N-1, at 25°C, periodic illumination
- : *M. viridis* NIES 102, at 25°C, periodic illumination
- △: *M. viridis* NIES 102, at 25°C, continuous illumination
- ▲: *M. viridis* NIES 102, at 30°C, continuous illumination

M. wesenbergii K-3A. A low μ is disadvantageous for the water bloom formation of *M. viridis*. It is not certain why the μ value of *M. viridis* is low. Since the N-1 strain could not grow under continuous illumination, we investigated the effects of continuous or periodic illumination on the specific growth rate and the algal yield. The μ values under continuous illumination were generally higher than with periodic illumination. However, continuous illumination inhibits algal growth under specific conditions.

BRAND and GUILLARD (1981) reported that many phytoplankton species cultured under the continuous illumination showed higher cell multiplication than under periodic illumination. However, a few species of phytoplankton had a high sensitivity to continuous illumination, and could not grow even if light intensity was very low. LOOGMAN *et al.* (1980) investigated the effect of the length of light period on the growth of *Oscillatoria agardhii* under saturated light

irradiance and reported that the efficiency of light-energy incorporation decreased as the length of the light period increased. There are two possible explanations for this phenomenon: (1) The faster growing cells under the longer photoperiod may have a higher respiratory rate, and (2) there may be a time-dependent inhibition of photosynthesis which progressively increases during the light period. In the present study, *M. viridis* (N-1 strain) exhibited no tolerance to continuous illumination above 25°C. The effects of continuous and periodic illumination on the growth of *M. viridis* are clarified.

4. Conclusion

From the above observations and experiments, we concluded as follows.

- 1) *M. aeruginosa* f. *aeruginosa* and *M. aeruginosa* f. *flos-aquae* were dominant in summer, but *M. viridis* became dominant in the fall of 1988 in Lake Kasumigaura.
- 2) *M. viridis* could grow at lower temperature than other *Microcystis* species.
- 3) The specific growth rate of *M. viridis* was lower than for other species of *Microcystis*.
- 4) In summer, *M. aeruginosa* was dominant but *M. viridis* was not.

摘 要

藍藻類 *Microcystis viridis* の増殖に及ぼす温度と照度の影響

霞ヶ浦に出現した *Microcystis* 属 4 種の現存量の変化ならびに、霞ヶ浦の水の華より単藻化した *M. viridis* N-1 株および国立環境研究所保存菌株である *M. viridis* NIES 102 株を用いて増殖に及ぼす温度と照度の影響を調べ、霞ヶ浦における *M. viridis* の発生要因について検討を加えた。

1988 年の霞ヶ浦土浦港では、*M. aeruginosa* f. *aeruginosa* と *M. aeruginosa* f. *flos-aquae* が夏に優占種となり、*M. viridis* は秋に優占種となる傾向が認められた。*M. aeruginosa* f. *aeruginosa* および *M. aeruginosa* f. *flos-aquae* の現存量は水温と高い相関を示したが、*M. viridis* および *M. wesenbergii* の現存量と水温との間に相関は認められなかった。

M. viridis N-1 は 25°C で最大の比増殖速度 (μ) を示し、15°C でも増殖が可能であった。また

M. viridis NIES 102 は 25~30°C で最大の μ を示した。*M. viridis* N-1 株は他の *Microcystis* 属よりも低温で増殖が可能な株と考えられ、夏期には優占種になりにくいものと考えられた。

References

- AMEMIYA, Y., T. OKINO and O. NAKAYAMA (1990): Factors possibly affecting dominance of *Microcystis* species. *Jpn. J. Limnol.*, **51**: 9-13.
- BRAND, L. E. and R. R. L. GUILLARD (1981): The effects of continuous light and light intensity on the reproduction rates of twenty-two species of marine phytoplankton. *F. exp. mar. Biol. Ecol.*, **50**: 119-132.
- GERLOFF, G. C., G. P. FITZGERALD and F. SKOOG (1950): The isolation, purification and culture of blue-green algae. *Am. J. Bot.*, **37**: 216-218.
- HUGHES, E. O., P. R. GORHAM and A. ZEHNDER (1958): Toxicity of a unialgal culture of *Microcystis aeruginosa*. *Can. J. Microbiol.*, **4**: 225-236.
- KOMÁREK, J. (1958): Die taxonomische Revision der planktischen Blaualgen der Tschechoslowakei, p. 10-206. In J. KOMÁREK and H. ETTL (eds.), *Algologische Studien*. Tschechoslowakischen Akademie der Wissenschaften.
- KUMAGAI, M., Y. TAKAMURA, O. YAGI and R. SUDO (1986): Interactions between *Microcystis aeruginosa* and *Moraxella nonliquefaciens*. *Jpn. J. Limnol.*, **47**: 219-228 (in Japanese).
- KUSUMI, T., T. OOI, M. M. WATANABE, H. TAKAHASHI and H. KAKISAWA (1987): Cyanoviridin RR, a toxin from the cyanobacterium (blue-green alga) *Microcystis viridis*. *Tetrahedron Letters*, **28**: 4695-4698.
- LOGGMAN, J. G., A. F. POST and L. R. MUR (1980): The influence of periodicity in light conditions as determined by the trophic state of the water, on the growth of the green alga *Scenedesmus protuberans* and the cyanobacterium *Oscillatoria agardhii*, p. 79-82. In J. BARICA and L. R. MUR (eds.), *Hypertrophic Ecosystem*, Junk, Den Haag.
- McLACHLAN, J. and P. R. GORHAM (1962): Effect of pH and nitrogen sources on growth of *Microcystis aeruginosa* Kütz. *Can. J. Microbiol.*, **8**: 1-11.
- OKINO, T. (1973): Studies on the blooming of *Microcystis aeruginosa*. *Jpn. J. Bot.*, **20**: 381-402.
- TAKAHASHI, M., I. NOMURA, M. KOMATSU and S. ICHIMURA (1981): Multi-regression analysis of *Microcystis* bloom with various environmental parameters in eutrophic Lake Kasumigaura, Japan. *Verh. Internat. Verein. Limnol.*, **21**: 659-663.
- TAKAMURA, N. and M. M. WATANABE (1987): Seasonal changes in the biomass of four species of *Microcystis* in Lake Kasumigaura. *Jpn. J. Limnol.*, **48**: S139-S144.
- YAGI, O., T. HAGIWARA, Y. TAKAMURA and R. SUDO (1984): Growth characteristics of axenic and unialgal *Microcystis* isolated from Lake Kasumigaura. *Jpn. J. Wat. Pollut. Res.*, **7**: 496-503 (in Japanese).
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Received: 25 June 1990

Accepted: 25 June 1991