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The Role of Mixing in the Mechanisms of Water Bloom by Microcystis spp.

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Abstract

Studies on the role of water mixing in the mechanisms of water bloom b *Microcystis* spp., which were composed from the three species, *M. aeruginosa, M. virid* and *M. wesenbergii,* were conducted during the summer of 1983. *Microcystis* spp. are species of blue-green algae that appears regularly in the summer in Lake Suwa, Japa

Centrifugation was used in order to separate *Microcystis* spp. from othe phytoplankton. Thus, the floating part of the centrifugal tube was occupied b *Microcystis* spp. only, and the settling part was by other phytoplankton. It was clea from the experimental results of the present study that the *Microcystis* cells in th floating part were active in photosynthesis, while the cells in the settling part were less so.

The results of the water mixing experiments showed that water mixing had a positive effect on the photosynthesis and on the growth of this algal population compared to non-mixing. At a mixing speed of 2.2 cm/sec, it was known to be an optimum condition for photosynthesis. The light saturation points changed by the mixing speeds, and were in the range of 10 to 20 Klux. It was concluded that the photosynthesis of the algal population was not inhibited under strong light in the summer season by forming the unique productive structure concentrated the algal colonies in the surface layer. However, a single colony was inhibited by a strong light of more than 20 Klux.

Introduction

Microcystis spp. are a species of blue-green algae which has been reported recently as a species causing water-bloom in some fresh-waters of the world. In temperate regions these algae are overabundant during the summer season when limnological conditions favor their growth.

Numerous investigators have reported various factors at work in the formation of

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Microcystis bloom. The main factors promoting growth of blue-green algae are light condition, water temperature, and the chemical composition of waters. In a lake the bloom is directly affected by water movement and thermal stratification (REYNOLDS and WALSBY, 1975 ; STEWART, 1976). Similarly, HARIS (1980) stated that mixing of the algal suspension had a close relationship with the photosynthetic activity of phytoplankton populations.

The effect of water mixing either on the growth or photosynthetic activity of algal species has been reported (FOGG and THAN-TUN, 1960 ; STEWARD, 1960 ; OLOFSON, 1980 ; OKADA, 1983). In an aquatic environment such as a lake, and particularly in a shallow eutrophic lake, mixing of algal suspension has a far-reaching influence on phytoplankton distribution (GANF, 1974) as well as physico-chemical and biological parameters (ARCIFA et al., 1981).

It is well known that *Microcystis* generally blooms in shallow eutrophic lakes. Several correlative components of physical factors with water-bloom have been well documented by many workers. However, little attention has been paid to the role of water mixing in the mechanisms of *Microcystis* bloom.

In the present studies the focus of attention was to clarify the growth of *Microcystis* in relation to water mixing by means of combining data obtained from field and laboratory experiments.

Materials and methods

Lake Suwa was chosen as a study area due to its limnological characteristics. It has attracted much attention among numerous Japanese limnological workers over the past few decades. Lake Suwa is located in the central part of Japan, and geographically its position lies between 36°03′N and 136°05′E with the altitude of 759 m above sea level. It is categorized as a eutrophic lake ; it is relatively small and shallow, with a surface area of 13.30 km^2 and a maximum depth of 6.5 m, and a mean depth of 4.1 m. The retention time of the lake water is calculated to be about 50 days. Tectonic in origin, Lake Suwa is surrounded by hills and mountains. Twenty-five streams and rivers flow into the lake, bringing in much more nutrients, and speeding up the eutrophication processes of the lake. The outlet from the lake is only one, Tenryu River.

For more detailed information on the limnological features of this lake, refer to SAKAMOTO et al. (1975), and to KURASAWA and OKINO (1975).

Water samples were taken at various depths from the center of Lake Suwa using a 5-liter Van Dorn water sampler, except for water samples from a depth of 0.2 m and 0.5 m ; a small rubber tube with a diameter of 1 cm equipped with a mini pump was used.

Sampling was usually done at around 9 : 00 A.M. and finished within about 30

minutes. The observations were mainly done during the blooming of *Microcystis* spp. in the summer of 1983.

The measurements of water temperature, transparency, light attenuation and dissolved oxygen content were made *in situ* by the following methods. Water temperature was measured by thermistor thermometer (Model ET-3, Toho Dentan Co., Ltd.) with its sensor lowered from the surface water layer to the bottom at a depth interval of 0.5 m. The measurement of under-water light intensity was made by selenium photocell (Type BMW-12K, Murayama Denki Co., Ltd.) with a neutral filter. Transparency was measured using Secchi's disk which was painted white (diameter, 23 cm). Dissolved oxygen content was determined by Winkler's method.

After sampling all water samples were brought back to the laboratory at Suwa Hydrobiological Station for further analysis of chlorophyll amounts, identification and enumeration of phytoplankton cells, and photosynthesis experiments. For chlorophyll determination, 100 ml of sample water was filtered through a Millipore glass fiber filter with a diameter of 47 mm and a mean pore size of about 1.2 μm. Algal pigment cells retained on the filter were manually ground in a glass grinder and extracted by the addition of a few drops of 90 % acetone. Extracts were then put into cylindrical plastic centrifuging tube with a cap for centrifuging at 3,000 rpm for 15 minutes. The supernatat was poured into a spectrophotometer cell for chlorophyll-a analysis spectrophotometrically following the formula of SCOR/UNESCO in STRICKLAND and PARSONS (1968). There were two kinds of chlorophyll-a determination. One was used for the floating part and the other for lake water without filtering. The difference between the latter and the former was called the settling part.

The separation of the phytoplankton population cells into the floating and settling parts could be done by centrifugation. The 100 ml water samples were put into the cylindrical centrifuging tube and centrifuged at 3,000 rpm for 15 minutes. The identification and the numeration of phytoplankton cells for floating and settling parts were also accomplished microscopically.

The experimental conditions for the photosynthetic activities of the algal populations taken from the lake were as follows. Water temperature in the thermostatic water bath was fixed at 20°C, and the light source came from an incandescent lamp which was set at 20 klux. The exposure time ranged from 5 to 60 minutes depending on the density of phytoplankton populations. During the measurement of algal photosynthesis, the algal suspension was continuously mixed by a magnetic stirrer at a mixing speed of 5 rpm. Oxygen evolution was monitored by a polarographic oxygen analyzer (YSI Model 57 oxygen meter) with an automatic recorder.

The experiments on *Microcystis* growth were also conducted in the following conditions. The concentrated samples of *Microcystis* were collected from Lake Suwa

during the water bloom in August of 1983. To obtain sample free from other algal contamination, fractionation and washing were undertaken. Fractionation was done by centrifuging at 3,000 rpm for 15 minutes. Filtrate of the water of Lake Suwa was used as a washing constituent and as an algal culture medium. The experimental conditions were as follows. The water temperature in the incubator was fixed at 10, 15 and 20°C. In each incubator chamber two bottles of algal culture were maintained; one was treated with continuous mixing at 5 rpm, and the other served as the control. The light source was cool white fluorescent lamps set at 20 klux. Nutrient enrichment was not performed in the algal medium, which was the filtrate of the lake water.

As an indicator of algal growth, the chlorophyll concentration of algal culture was analyzed once a day by subsampling a 10 ml aliquot. To refresh the algal medium, the same quantity (10 ml) of filtrate was put into the culture medium each time subsampling was undertaken.

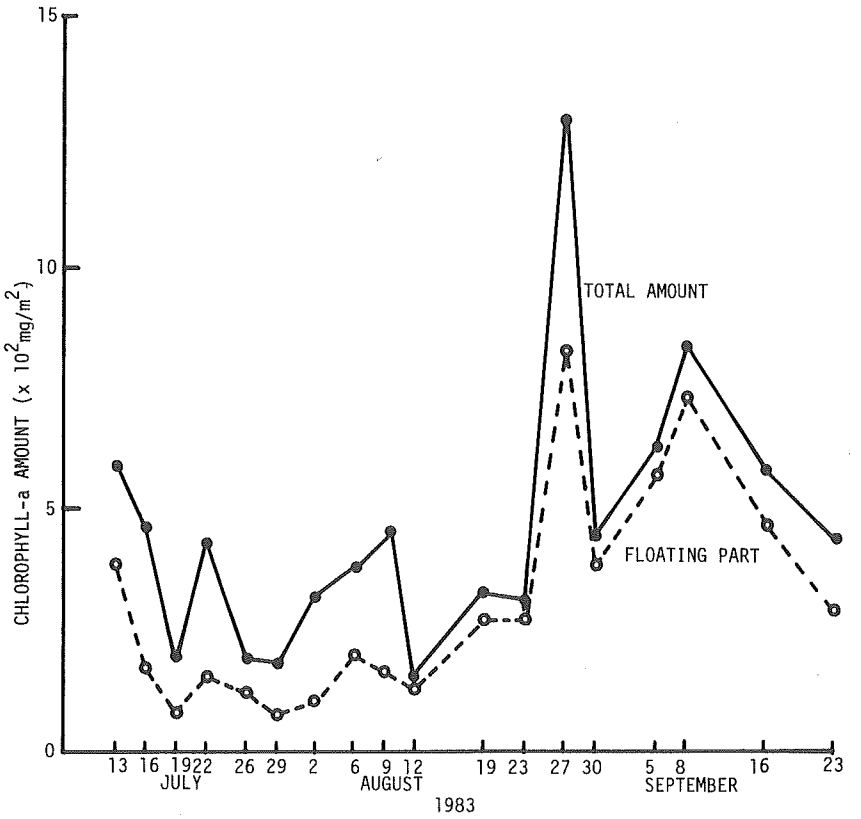

Figure 1. Changes in the chlorophyll amounts of the floating part and the total phytoplankton during the *Microcystis* bloom of Lake Suwa in 1983.

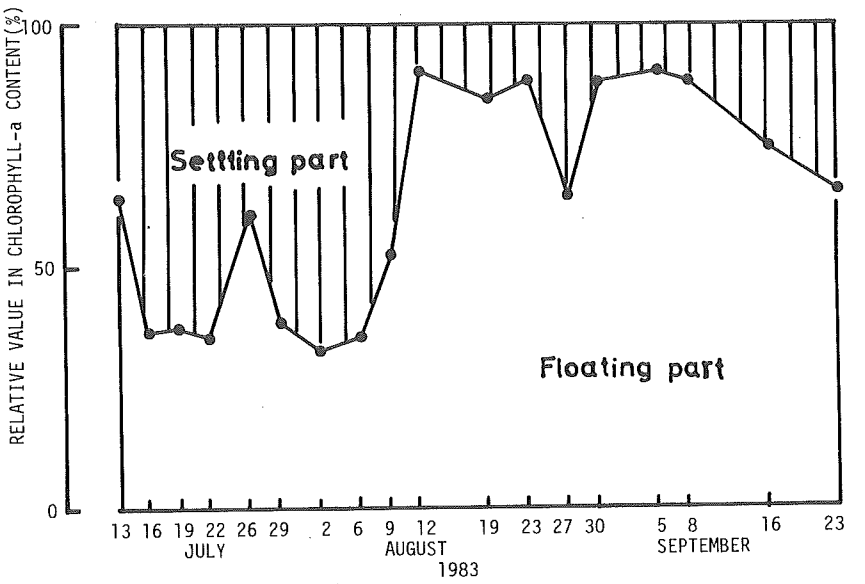

Figure 2. Relative values in chlorophyll-a between the floating part and the settling part of phytoplankton populations fractionated by centrifugating teqhnique during *Microcystis* bloom in 1983.

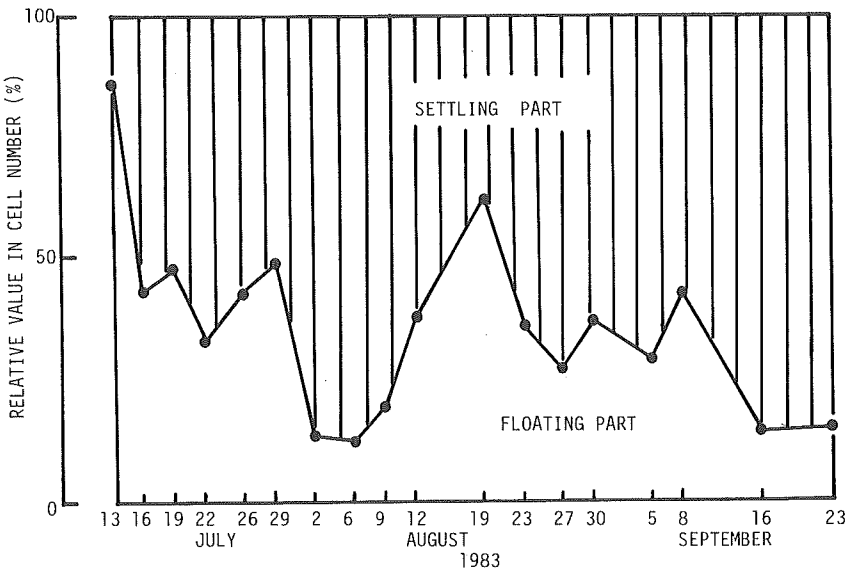

Figure 3. Changes in the relative amounts in cell numbers of the floating and settling parts of phytoplankton populations fractionated by centrifugating technique during *Microcystis* bloom in 1983.

Results

The physical data during the field observations were as follows. Water temperature at the surface ranged from 19.4 to 27.5°C, against 17.6 to 19.8°C at the bottom. Transparency varied from 30 to 90 cm. The depth at which 10 % of incident light penetration showed a great fluctuation was from 0.5 to 3.5 m. The value of 0.5 m was reached at the middle stage of water bloom, while the value of 3.5 m was obtained in the early stage.

Dissolved oxygen concentration as the mean value in the water column during the *Microcystis* bloom was in the range of 6.7 to 15.0 mg O_2/L. The difference between the dissolved oxygen concentration at the surface water and at the bottom was high at approximately 10 mg/L.

Changes in chlorophyll-a amount during the bloom are illustrated in Fig. 1, which shows that minimum and maximum total chlorophyll-a amounts occurring on August 12 and August 27 amounted to 140 mg/m^2 and 1,300 mg/m^2, respectively. The same figure indicates that the minimum and maximum chlorophyll-a amount for the floating part, occurring on July 29 and August 27, accounted for 68 mg/m^2 and 830 mg/m^2, respectively.

From the identification and enumeration of phytoplankton under microscope, besides *Microcystis* spp. there were other phytoplankton species like *Navicula cryptocephala, Anabaena spiroides, Phormidium mucicola, Cyclotella meneghiniana, Melosira granulata, Pediastrum duplex, Micractinium pussilum* and *Scenedesmus dimorphus*. Cell numbers of *Microcystis* spp. made up 50 to 70 % of the total phytoplankton cells.

In the early stage of *Microcystis* bloom, the percentage of chlorophyll-a amount for the floating part was lower than for the settling part. It is clear that the relative chlorophyll-a content of the floating part in the early stage of the bloom was lower, ranging from 32 to 65 %. Thereafter, it tended to increase up to 90 % in August, while

Table 1. Results of the experiments on the effect of mixing speed of the water in culture bottle on the optimum light intensity in the photosynthesis of *Microcystis* spp.

Mixing speed (rpm)	Optimum light intensity (k lux)	Maximum photosynthetic rate (O_2mg/chl. -a mg/hr)
0	5	2.37
5	10	4.50
6	10	4.80
7	20	4.27

at the end in September it decreased (Fig. 2).

Owing to the comparison between the relative cell numbers of *Microcystis* spp. for both of the floating and the settling parts, it is known that the cells of the latter outnumbered the former (Fig. 3). It is interesting to note that the chlorophyll-a content per cell of *Microcystis* for floating and settling parts showed a different order of magnitude.

The results of the experiments on the relation between photosynthesis and mixing speeds of water are given in Table 1, which indicates that the mixing speed of 6 rpm corresponding to a water transfer of 2.2 cm/sec is the optimal mixing speed for the photosynthesis of *Microcystis*.

Discussion

The measurement of chlorophyll-a content from algal materials in natural waters can be used as a general index of algal growth. From the observational results of chlorophyll-a amount, it was evident that *Microcystis* growth in Lake Suwa showed an exponential phase in August. This finding is also consistent with OKINO (1973), who found that the algal standing crop in Lake Suwa peaked in August during the *Microcystis* bloom. However, the chlorophyll-a concentration varied considerably, showing a patchy distribution during the blooming season. It was assumed that the characteristics of *Microcystis,* which contained gas vacuoles in its cell, might check to obtain the true standing crop. GANF (1974) observed that surface accumulation of *Microcystis* occur only during periods of extreme calm, and are quickly dispersed by wave action. IMBERGER et al. (1983) suggested that the migration of *Microcystis* colonies, accumulation to surface or dispersion to lower layer, was influenced by the physiological state of the algal cells combined with the motion and mixing of lake water.

During *Microcystis* growth in natural waters there is an alteration in its physiological condition. According to STRÖMGREN (1983), who studied temperature-length growth strategies in the littoral alga, *Ascophyllum nodosum* (L), this alga is floating during high tide and the top shoots of them get the best light condition. With falling tide this alga sinks to the bottom, and the position of the different algal tips in the algal bed varies more or less at random from one tidal cycle to the next, depending on water movements. This determines their exposure to light, temperature, and dessication. Also, HARRIS (1980) stated that mixing will not only maintain the phytoplankton populations in the surface mixed layer, but also it will have a profound effect on photosynthetic rates of phytoplankton populations in the photic zone.

Taking into consideration the physical conditions during field observations, the light intensity at the beginning of water bloom was relatively higher than that of the

end. In addition, water temperature at the bottom layer gradually increased. It is assumed that these two factors enhance the floating of *Microcystis* to the surface layer in calm weather.

Several studies were conducted on the buoyancy of *Microcystis* cells by REYNOLDS and WALSBY (1975) and WALSBY (1969). WALSBY (1970) proposed two mechanisms which could account for an inverse correlation between gas vacuolation and light intensity. The first mechanism depends on the differences in kinetics of cell growth and gas vacuole formation. Under light intensities which are suboptimal for growth, the doubling time for gas vacuole is shorter than that of other cell materials. Under optimal illumination, however, gas vacuoles are diluted out by the higher growth rate sustained. The second mechanism is one in which a proportion of gas vacuole in an algal cell may collapse by the rise in turgor pressure which takes place within the cell on exposure to increased light intensities.

As shown in Fig. 1, the present study demonstrates that in the early stage of water bloom there are great differences between the overall chlorophyll-a amount and that of the floating portion. In other words, the chlorophyll-a amount for the settling part is larger than for the floating part. This may well prove that there is weak gas vacuolation in *Microcystis* cells. It is also supported by the findings of LEHMANN and JOST (1971), who reported that gas vacuolation is least in exponentially growing cultures and greatest in the stationary phase of growth (SMITH and PEAT, 1967).

OKINO pointed out that *Microcystis* showed different patterns in photosynthesis-light curve and chlorophyll contents during the early and middle stages of blooming (OKINO, 1973). In addition to, the maximum rate of photosynthesis of the algal population in the blooming stage was high at about 30 mg O_2/mg Chl. -a/hr. The appearance of *Microcystis* bloom seems to correlate with the environmental conditions as follows: (1) water temperature at the bottom of above 20°C; (2) formation of weak thermocline; (3) high pH value of 9 to 11 in surface layer; and (4) strong solar radiation for a certain period time. There are physiological differences between the alga in the supernatant and precipitating parts in which the nitrogen content differs from one to the other, and the physiological differences between the parts might also bring about different phosphate accumulation rates. The supernatant part showed higher rate than the precipitating part.

Recently, the relation between *Microcystis* growth and water mixing during summer season was discussed from simulation studies (OKINO et al., 1977; OKADA, 1983). According to these reports, it was expected that the water exchange developing between the lower and the upper layers may influence the growth of some algal species. Several models on the *Microcystis* growth have been illustrated in which moderate water exchange between the layers can cause an increase in the algal amounts in lakes compared with that of strong water exchange. The increase of the floating cell, which

is high chlorophyll content and high photosynthetic activty, promote the *Microcystis* growth during the summer bloom.

REYNOLDS and WALSBY (1975) have discussed the factors which may bring an end to the growth of this alga lodged at the surface thin layer and which may lead to their rapid deterioration at the end of the bloom. They pointed out that the buoyancy of this alga was lost at the end of the bloom. PAERL and USTACH (1982) explained that it is the form of senescence preceding the massive death of cyanobacteria which have lost the ability to regulate buoyancy. In fact, according to REYNOLDS and LOGERS (1976), the *Microcystis* life cycle is closely related to the cycle of thermal stratification. Hence, the colonies of this alga present on the bottom mud overwinter, and then migrate to the epilimnion in summer, returning again to the bottom in late autumn and winter. These assumptions were also supported by the findings of FALLON and BROCK (1981). However, there were still unclear on the physiological state of the *Microcystis* cells in the lake bottom during the winter. TAKAMURA (1984) studied the photosynthetic activity of *Microcystis* in the bottom mud of Lake Kasumigaura, but no clear explanation as to the life form of this alga has been given to date.

The circulation of lake water is caused by wind, and the extent of water circulation may be influenced by the wind velocity. According to POND and PICKARD (1978), the mixing layer by wind can be estimated by the equation as written below.

$$D_e = \frac{4.3\,W}{\sqrt{\mathrm{Sin}\ \theta}}$$

where D_e is Ekman depth, W is wind velocity and θ is the latitude.

Therefore, Ekman depth, or the calculated mixed layer for Lake Suwa based on the records of Suwa Weather Station, is 11.2 m, indicating that the whole water from the surface to the bottom of Lake Suwa can be mixed thoroughly with a wind speed of 2 m/sec. If the calculation above is combined with the records on the vertical variations in dissolved oxygen content during the summer water bloom of *Microcystis* in Lake Suwa, the water column would have mixed thoroughly 3 times on the sampling dates of August 19, September 16 and September 23, 1983. It can be noted that the difference between dissolved oxygen content in the surface water and in the bottom layers was quite high except on the sampling dates mentioned previously. This situation has also been observed by YAMAGISHI and OKINO (1967), who explained that wind and waves usually were not favorable to increase oxygen into the surface water.

There have been many reports on the regulation of gas vacuoles in *Microcystis* blooming. GANF (1974) measured positive and negative sinking rates of *Microcystis* colonies in the laboratory. The positive sinking rate was 1 mm/sec and the negative rate was nearly the same. OKADA (1983) has reviewed mathematically the mechanisms in the occurrence of *Microcystis* blooming.

A series of artificial mixing experiments which have been conducted in the

present studies showed a positive effect either on photosynthesis or growth of *Microcystis*. From three selected mixing speeds it is evident that the mixing speed at 6 rpm corresponding to 2.2 cm/sec gives an optimal result on the photosynthetic activity of *Microcystis* (Table 1). KING (1970) elucidated that the rapid mixing of water should reduce the gradient of carbon dioxide immediately adjacent to the algal cells and allow photosynthesis to continue to lower equilibrium carbon dioxide levels. HARRIS and LOTT (1973) explained that variation in the asymmetry and in photoinhibition appears to be correlated with the light regimes experienced by the population. And other workers stated that photoinhibition and photorespiration of algae were also both time dependent (HARRIS, 1980 ; FARMER and TAKAHASHI, 1982).

From the present study, it can be concluded that the optimum light intensity for photosynthesis of *Microcystis* lies in a range of 10-20 klux and beyond 20 klux it appears to be photoinhibition to the photosynthesis under the experimental mixing condition. However, many reports showed that the photosynthetic activity of *Microcystis* was not affected by strong light beyond 20 klux. Their results were obtained in the stationary condition, whereas the present results were obtained in the mixing condition, and the colonies were constantly exposed to strong light. Judging from the difference in both conditions, it may be elucidated that the *Microcystis* cells are inhibited in their photosynthetic activity by strong light, but the *Microcystis* colonies in nature can exclude the photoinhibition owing to the colony formation and the unique productive structure concentrated in the surface layer. Thus the high productivity of *Microcystis* in natural waters is maintained by a unique productive structure which exclude the weak point of the alga, in spite of the fact that the cell itself has its photosynthetic character inhibited by strong light.

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