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Effect of Temperature on Blue-Green Algae (Cyanobacteria) in Lake Mendota

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The temperature optimum for photosynthesis of natural populations of blue-green algae (cyanobacteria) from Lake Mendota was determined during the period of June to November 1976. In the spring, when temperatures ranged from 0 to 20°C, there were insignificant amounts of blue-green algae in the lake (less than 1% of the biomass). During the summer and fall, when the dominant phytoplankton was blue-green algae, the optimum temperature for photosynthesis was usually between 20 and 30°C, whereas the environmental temperatures during this period ranged from 24°C in August to 12°C in November. In general, the optimum temperature for photosynthesis was higher than the environmental temperature. More importantly, significant photosynthesis also occurred at low temperature in these samples, which suggests that the low temperature alone is not responsible for the absence of blue-green algae in Lake Mendota during the spring. Temperature optima for growth and photosynthesis of laboratory cultures of the three dominant blue-green algae in Lake Mendota were determined. The responses of the two parameters to changes in temperature were similar; thus, photosynthesis appears to be a valid index of growth. However, there was little photosynthesis by laboratory cultures at low temperatures, in contrast to the natural samples. Evidence for an interaction between temperature and low light intensities in their effect on photosynthesis of natural samples is presented.

The effects of temperature on the rates of biological processes are well known, but the importance of temperature in determining the occurrence of particular phytoplankton species is uncertain. Goldman and Ryther (9) studied the effect of temperature on five species of marine phytoplankton. The cell yield was independent of temperature, but the outcome of competition between species was highly dependent on temperature. A literature survey of the effect of temperature on algal growth rate indicated that, as temperature increased, the algal group with the highest growth rate changed from diatoms to green algae to blue-green algae (cyanobacteria) (6). However, Fogg (7) has commented that because of the possibility of adaptation and interaction with other factors, it is unlikely that temperature alone is a major factor in determining the occurrence of a particular algal species.

A common observation is that blue-green algal dominance of aquatic communities is greater when water temperatures are warmer (3). In Lake Mendota, Wis., blue-green algae are an insignificant component of the phytoplankton in the spring, but become dominant as the water temperature warms. In the present investigation, the effect of temperature on photosynthesis of natural blue-green algal populations from Lake Mendota and on photosynthesis and growth of cultures isolated from this lake was studied. The objectives were to determine whether the absence of blue-green algae in the spring was due to the low temperatures present at that time and whether autumn populations were better adapted to lower temperatures than were the summer populations.

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MATERIALS AND METHODS

Study site and sampling procedure. Lake Mendota (Dane County, Wis.) has a surface area of 39.1 km^2, an average depth of 12.4 m, and a maximum depth of 24 m. Samples were collected at a station located above the deepest part of the lake (24 m) from a depth of 0.25 m.

Measurement of photosynthesis. The rate of photosynthesis was determined by adding 1 μCi of $NaH^{14}CO_3$ (10 μg/μCi; New England Nuclear) to 30 ml of lake water in a 32-ml serum vial. Three vials were incubated at a light intensity of 2,500 lux at each of seven temperatures: 4, 10, 15, 20, 25, 30, and 37°C. For 1 h before the addition of radioisotope, the vials were incubated at the appropriate temperature to equilibrate the contents to that temperature. A vial wrapped

in aluminum foil and one to which 2 ml of Formalin was added at the start of the experiment were also incubated. After 5 h, 2 ml of Formalin was added to each vial. The contents were filtered though a 0.45-μm membrane filter (Gelman GN-6), and the filter was washed, placed in fuming HCl for 12 to 18 h, and counted in a toluene-based scintillation cocktail containing 3.8 liters of toluene, 1.42 g of 2,5-diphenyloxazole, and 0.39 g of 1,4-bis-(2-[4-methyl-5-phenyloxazolyl])benzene by using a Packard Tri-Carb scintillation counter. Efficiency of counting was determined by the channel ratio method.

The incorporation of $^{14}CO_2$ into algal protein was determined as follows. After a 2-h incubation at 2,500 lux, the algae were filtered onto a glass fiber filter (Whatman GFC), and the filter was placed in 2 ml of 1 N NaOH and heated at 100°C for 10 min. After refiltration, sufficient trichloroacetic acid was added to the supernatant to give a 5% final concentration. One drop of carrier protein (lysozyme, 5 mg/ml) was added, and the liquid was incubated at 0°C for 30 min. The precipitated protein was filtered onto a 0.45-μm filter and counted.

Isolation and growth of cultures. Cultures of *Anabaena, Aphanizomenon,* and *Microcystis* were isolated from Lake Mendota by spreading water samples collected in July from a 4-m depth onto solid 1.5% agar medium with a nutrient composition described by Gerloff and Skoog (8). The plates were incubated at 20°C and a light intensity of 1,500 lux. Isolates were subsequently cultured in Gerloff-Skoog medium or ASM-1 (10).

The temperature range for growth was determined by inoculating 20 ml of growth medium into a screw-cap culture tube (25 by 150 mm) with the appropriate organism and incubating the tubes at 4, 10, 15, 20, 25, 30, or 37°C at 2,500 lux for 7 days. The cultures did not reach the stationary phase of growth during the 7-day incubation, so that relative growth rates at different temperatures could be deduced from the biomass concentration after 7 days. The contents of a tube were filtered onto a glass fiber filter, the filter was placed in 2 ml of 1 N NaOH and heated to 100°C for 10 min, and the supernatant was assayed for protein by the method of Lowry et al. (13). The temperature range for photosynthesis by cultures grown at 25°C was determined as described above for the natural samples.

Autoradiography. Light-dependent incorporation of $^{14}CO_2$ into individual algal species was followed by microautoradiography. A 4-μCi amount of $NaH^{14}CO_3$ was added to 30 ml of lake water, which was incubated for 5 h at 2,500 lux. Autoradiograms were prepared by the method of Brock and Brock (4).

RESULTS

Photosynthesis in natural populations. The temperature optimum of photosynthesis of Lake Mendota algae was determined nine times in the period of June to November 1976 (Table 1). During this period the in situ temperature ranged from 12 to 24°C, and blue-green algae were the dominant phytoplankton. In general, the temperature optima were very broad, although the optimal temperature for photosynthesis was lower in autumn than in summer. However, the difference in optima was only about 5°C, whereas the change in the environmental temperature was 12°C. On seven of the nine dates, the optimal temperature was higher than the environmental temperature. Significant photosynthesis also occurred at nonoptimal temperatures, especially at temperatures below the optimum (Fig. 1). For example, at 4°C the amount of carbon fixed was usually 50% of that fixed at the optimal temperature. Autoradiograms were prepared from samples incubated at all temperatures tested, and it was determined that it was the blue-green algae which were responsible for carbon fixation at low temperatures, although no quantitative estimates were made.

TABLE 1. *Optimum temperature for photosynthesis of Lake Mendota algae*[a]

Date	Optimum temperature (°C)	In situ temperature (°C)	Organisms present
8 June	30	22.5	*Aphanizomenon*
16 June	25	22	*Aphanizomenon*
24 June	25	21	*Aphanizomenon*
20 July	20	23	*Aphanizomenon*
23 August	25	24	*Anabaena* *Microcystis*
22 September	15	18	*Aphanizomenon* *Microcystis* *Anabaena*
7 October	20	15	*Aphanizomenon*
13 October	20	15	*Aphanizomenon*
8 November	20	12	*Aphanizomenon*

[a] A 1-μCi amount of $NaH^{14}CO_3$ was added to 30 ml of Lake Mendota water, and the sample was incubated at 2,500 lux at 4, 10, 15, 20, 25, 30, or 37°C for 5 h. Three samples were incubated in the light and one in the dark at each temperature.

Photosynthesis was routinely measured at a light intensity of 2,500 lux (approximately 3% of midsummer surface radiation). An experiment on 9 August 1977 indicated that there was an interaction between temperature and light intensity. The temperature optimum for photosynthesis was measured after incubation at 650 or 2,500 lux (Fig. 2). The optimal temperature was lower in samples incubated at 650 lux. In a parallel experiment, incorporation of $^{14}CO_2$ into protein was measured as a function of temperature; this optimum was the same as that for total incorporation (5% of the fixed CO_2 was present in the protein fraction after a 2-h incubation).

Photosynthesis and growth in cultures. The three predominant bloom-forming blue-green algae (*Aphanizomenon, Anabaena,* and

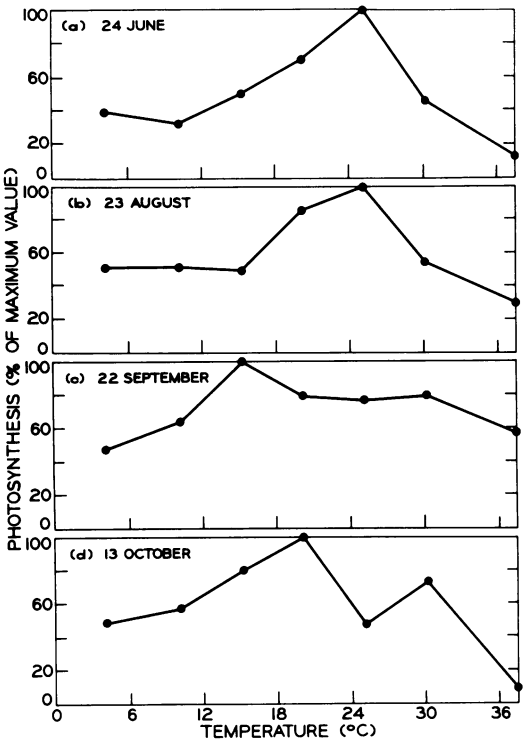

FIG. 1. *Photosynthetic rate of Lake Mendota algae at different temperatures. A 1-μCi amount of $NaH^{14}CO_3$ was added to 30 ml of lake water, the samples were incubated at 4, 10, 15, 20, 25, 30, or 37°C for 5 h at 2,500 lux, and the amount of $^{14}CO_2$ incorporated by the algae was determined. The amount of photosynthesis at each temperature is plotted as a percentage of the incorporation at the optimal temperature for that sample. The dates of the experiments and the amount of carbon fixed by photosynthesis at the optimum temperature were (a) 24 June, 340 μg/liter; (b) 23 August, 430 μg/liter; (c) 22 September, 690 μg/liter; and (d) 13 October, 160 μg/liter. Confidence intervals at the 95% level for each datum were less than or equal to ±12%.*

Microcystis) were isolated from Lake Mendota in unialgal culture, and the temperature optima for photosynthesis and growth at 2,500 lux were determined (Fig. 3). The growth optimum for all cultures was about 25°C. The apparent doubling times for the cultures at 25°C were 2 days for *Microcystis*, 2.5 days for *Anabaena*, and 5.5 days for *Aphanizomenon*. *Microcystis* only grew at temperatures above 15°C and below 37°C; in contrast, *Aphanizomenon* and *Anabaena* grew slowly at 10 and 15°C. *Anabaena* grew at 30°C, but growth of *Aphanizomenon* was minimal at this temperature. None of the cultures grew at 37°C. The same results for growth range, optimal growth temperature, and doubling time at the optimal temperature were obtained with cultures incubated at a greater light intensity, 10,000 lux.

The temperature range for photosynthesis was broader than that for growth in *Aphanizomenon* and *Anabaena*, and the ranges were similar for *Microcystis*. In all three cultures the optimal temperature for photosynthesis was 20°C. *Anabaena* photosynthesized at about the same rate in the temperature range of 20 to 30°C, whereas in the other two cultures photosynthesis at 25°C was about 70% of that at the optimum temperature. In general, though, growth and photosynthesis of the cultures as a function of temperature followed similar patterns, so that photosynthesis can be used as an index of growth.

DISCUSSION

One aim of this investigation was to determine whether temperature is a controlling factor in the occurrence of blue-green algae in Lake Mendota. Blue-green algal blooms were not found until early June, when the temperature of the lake was above 15°C. The relationship between lake temperature and blue-green algal growth is

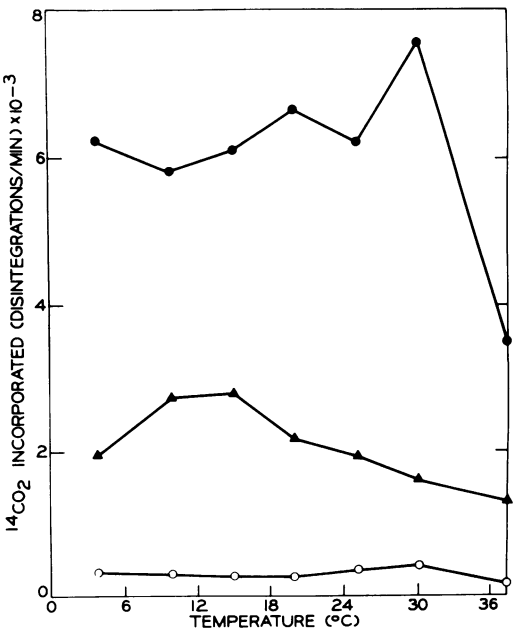

FIG. 2. *Effect of light intensity on photosynthetic rate at different temperatures. A 1-μCi amount of $NaH^{14}CO_3$ was added to 25 ml of Lake Mendota water collected on 9 August 1977; the samples were incubated for 2 h at 650 (▲) or 2,500 (●) lux at 4, 10, 15, 20, 25, 30, or 37°C. The amount of $^{14}CO_2$ incorporated by the algae was determined. The amount of $^{14}CO_2$ incorporated into protein after a 2-h incubation at 2,500 lux was also determined (○). Confidence intervals at the 95% level for each datum were less than or equal to ±16% of the plotted value.*

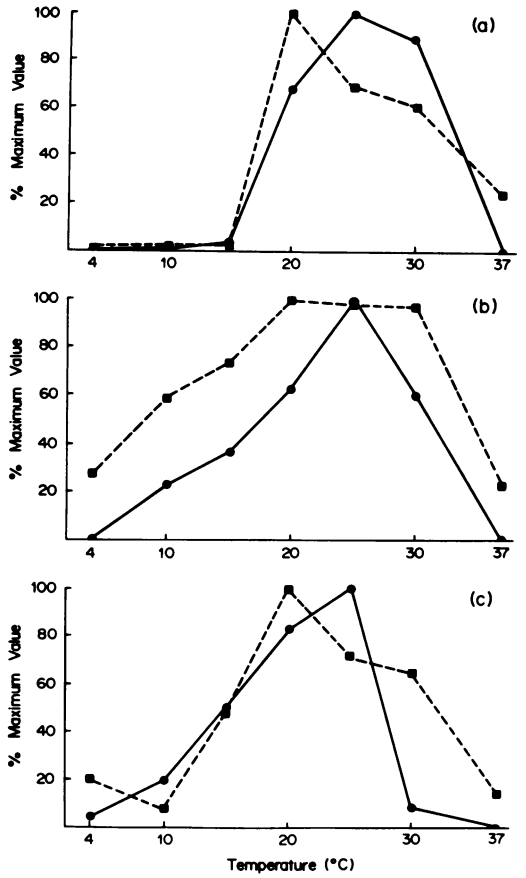

FIG. 3. *Temperature range for growth (●) and photosynthesis (■) of cultures of (a) Microcystis, (b) Anabaena, and (c) Aphanizomenon isolated from Lake Mendota. Both parameters were measured by incubation at 2,500 lux. Growth was monitored by determination of increases in protein in Gerloff-Skoog (a and b) or ASM-1 (c) culture medium after 7 days of incubation. Photosynthesis was measured by light-dependent incorporation of $NaH^{14}CO_3$ into cellular material. The maximum amounts of growth and photosynthesis observed in the experiments were (a) 26.1 μg of protein per ml and 43,400 cpm/ml per 5 h for Microcystis, (b) 10.8 μg of protein per ml and 75,400 cpm/ml per 5 h for Anabaena, and (c) 5.6 μg of protein per ml and 44,600 cpm/ml per 5 h for Aphanizomenon.*

not necessarily direct, because several physical, chemical, and biological parameters changed at this time. Incident solar radiation had increased, thermal stratification was beginning, and the green algal population was very low, presumably due to grazing by the large number of zooplankton that were present. Any one or several of these factors might provide blue-green algae with a competitive advantage, but temperature is a parameter that can be easily manipulated and whose effect can be easily studied both in natural samples and in cultures.

Although one wishes to know the effect of temperature on growth rate, it is impractical to measure algal growth of natural samples in a short period of time. Thus, the photosynthetic rate was used as a measure of growth. Other work has shown that this measurement is a good indicator of the physiological state of the algae (12). The validity of using photosynthesis as a measure of growth was tested with laboratory cultures (Fig. 3). The two measurements agreed well below 30°C, especially for *Microcystis* and *Aphanizomenon*. Because most of the natural samples tested contained predominantly *Aphanizomenon*, measurement of photosynthesis was a valid indicator of growth.

The results of the experiments with natural samples indicate that temperatures below 15°C do not limit blue-green algal growth. Even at 4°C, the amount of photosynthesis was usually 50% of that at the optimal temperature. Autoradiography indicated that the incorporation of $^{14}CO_2$ at low temperatures was due to the blue-green algae and not to other algal types present at very low concentrations. This relative photosynthetic rate at 4°C is higher than that observed for benthic algae in two other Wisconsin lakes (2, 5) and epilithic algae in the Firehole River in Wyoming (1). Thus, the springtime lake temperature per se does not explain the absence of blue-green algae in this period.

These experiments were conducted at relatively low light intensity (2,500 lux) compared with the incident solar radiation at the surface of the lake (80,000 lux). There were two reasons for this choice. As mentioned above, the three laboratory cultures had the same growth characteristics at 10,000 lux as at 2,500 lux. Furthermore, growth of cultures was inhibited at light intensities above 15,000 lux (unpublished data). Second, because of the physical characteristics of Lake Mendota, it is unlikely that algae are exposed to high light intensities for long periods of time. The photic zone of the lake is 2 to 4 m deep in summer, yet the epilimnion is 10 m deep (12), and the lake is exposed to sufficient wind power to homogeneously mix the epilimnion for much of the summer. Thus, the algae are subject to darkness a great deal of the time even during daylight hours, and the average light intensity to which the organisms are exposed is much lower than the surface intensity. Although a decrease in temperature optimum was found in samples incubated at extremely low light intensity (Fig. 2), we feel that the results obtained at 2,500 lux are also valid for higher light intensities, because the growth characteristics of algal cultures were the same at 2,500 and 10,000 lux.

The fact that these experiments were con-

ducted with lake water in which blue-green algae could grow is worth consideration. The difference in temperature optima of samples incubated at different light intensities illustrates that interaction between different factors is important in the response of an organism to these factors. (The basis for this interaction was not investigated in this study.) The interaction between the chemical environment and temperature is illustrated by the temperature optima of photosynthesis of laboratory cultures compared with natural samples. Very little photosynthesis was observed in two of three cultures at low temperatures. Laboratory culture media are quite artificial environments in comparison to lake water. Perhaps photosynthesis could not occur in the chemical environment of the growth medium at low temperature, whereas it could in the different chemical environment present in midsummer lake water. Interactions between nutrients and temperature have been found to affect the optimal temperatures for growth of *Ochromonas malhamensis* (11) and *Nitschia closterium* and *Tetraselmis* sp. (14). Another possibility is that a variety of strains of each genus exists in the lake and that the laboratory cultures represent strains adapted to warmer temperatures.

The optimal temperature for photosynthesis of Lake Mendota algae was lower in autumn than in early summer, but was usually higher than the environmental temperature. Thus, although there appears to be slight adaptation to lower temperatures by these algae, they do not become optimally adapted to their habitat. Similar results were found in two other Wisconsin lakes (2, 5). Even in winter, the temperature optima of periphytic algae and diatoms in Lake Monona and diatoms in Lake Wingra were 25°C, although the environmental temperatures were 0 to 11°C. The optimal temperature in these lakes was related to the midsummer environmental temperature, because the organism must be able to survive this temperature to remain in the habitat. This relationship was clearly shown in the Firehole River, which has been subject to thermal inputs for hundreds of years (1). A thermal gradient occurred along the river, and the temperature optimum of epilithic algae increased as the water temperature increased, but the optimum was always about 3°C higher than the midsummer environmental temperature. Boylen and Brock (1) reasoned that the optimum temperature of the algae was slightly higher than the environmental temperature to protect against transient heating to several degrees above normal. Because the optimum temperature of microorgansms is quite near the maximum, exposure to superoptimal temperatures could result in extinction.

In summary, the blue-green algae in Lake Mendota do not become optimally adapted to the temperature of their habitat. The optimum temperature for photosynthesis was usually a few degrees greater than the in situ temperature. Significant photosynthesis also occurred at low incubation temperatures. This suggests that low temperature per se does not prevent growth of blue-green algae in the spring in temperate lakes.

ACKNOWLEDGMENT

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