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Changes in Photosynthetic Rate and Pigment Content of Blue-Green Algae in Lake Mendota

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Blue-green algal blooms were present in Lake Mendota (Dane County, Wis.) from June to November 1976. Concentrations of total algal biomass and of particular algal species were monitored and compared with the pigment contents (chlorophyll a and phycocyanin) and photosynthetic rate of the algal populations. The specific photosynthetic rate (micrograms of C fixed per microgram of chlorophyll a per hour) was a good measure of the physiological state of the algae because this quantity increased just before each population increase and decreased before algal densities diminished. Since the quantity of light in the epilimnion which was available for photosynthesis by algal cells decreased in summer when the high algal densities attenuated incoming radiation, we investigated the possibility that the organisms would utilize lower light intensities more efficiently by increasing their pigment contents. Although some evidence of enhanced utilization of low light levels was found in the period from July to October, this result was not due to increasing chlorophyll and phycocyanin contents. There was a decrease in the phycocyanin content of the algae during this period, perhaps related to the availability of inorganic nitrogen.

Although many studies have been done on the physical and chemical conditions that lead to the formation of blue-green algal blooms, the physiology of natural populations has rarely been investigated (6, 7, 11). Physiological parameters can be measured and interpreted if algal blooms are predominantly of one species, a situation that was often found in Lake Mendota (Dane County, Wis.) from June to November 1976.

Lake Mendota is a dimictic, eutrophic lake in which blue-green algae are the dominant phytoplankton from June to November. The large algal biomass that is present in the summer depletes dissolved inorganic phosphorus and nitrogen in the epilimnion and also causes a decrease in the depth of the euphotic zone such that it is much less than the mixed-zone depth. The lake is subject to wind stress so that, periodically, the epilimnion is homogeneously mixed (R. E. Stauffer, Ph.D. thesis, University of Wisconsin, Madison, 1974). Incident light is so strongly attenuated in the summer that the daily amount of light to which algae are exposed is less in midsummer (in spite of the shallower mixed zone and increased daily radiation) than in the spring.

The present investigation was undertaken to determine whether the algae responded to the decrease in light quantity and inorganic nutrient concentration by altering their pigment contents or by using lower light intensities more efficiently. Changes in pigment content were monitored not only as indicators of low light utilization (1, 8, 12), but also as a measure of nitrogen availability (2). Although complete determinations of photosynthesis as a function of light intensity (20) were not made, measurement of photosynthesis at three light intensities was used to monitor seasonal changes in photosynthetic rate and utilization of low light quantities.

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MATERIALS AND METHODS

Study site and sampling procedure. Lake Mendota 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 from a station located above the deepest part of the lake with a 3.2- or 10-liter Van Dorn bottle (Wildco Supply Co., Saginaw, Mich.). Exposure of subsurface samples to direct sunlight was avoided. Thermal stratification was determined with a temperature/oxygen probe (model 54, Yellow Springs Instruments, Yellow Springs, Ohio), and light penetration was measured with a Whitney photometer (Chipman Instruments, Madison, Wis.) that had maximum sensitivity at 510 μm. Meterological data were obtained from the U.S. Weather Bureau at Truax Field, Madison, Wis.

Biochemical measurements. Algae were quantitatively filtered from lake water with glass-fiber filters (Whatman GFC). Chlorophyll was extracted in 90% acetone and determined spectrophotometrically by

the method of Lorenzen (13). On a separate sample, phycocyanin was extracted in distilled water (adjusted to pH 8) by grinding the filter containing the algae with a mortar and pestle. Glass fibers and cell debris were removed by centrifugation (20 min, at 4°C, 27,000 × *g*), and the absorbance of the supernatant at 625 nm was determined. An extinction coefficient of 7.5 ml mg^{-1} cm^{-1} for phycocyanin was used (4).

Algal protein was determined by heating the filter containing the algae to 100°C for 10 min in 1 N NaOH and then assaying by the method of Lowry et al. (14).

Direct microscopic counts of algae. Biomass volume was determined by the method of Brock (Limnol. Oceanogr., in press) in which algae are identified and enumerated by epifluorescence microscopy.

Determination of photosynthetic rate. The rate of photosynthesis was determined by adding 1 μCi of $NaH^{14}CO_3$ (10 μg/μCi; New England Nuclear Corp., Boston, Mass.) to 30 ml of lake water in a 32-ml serum vial. Each of three vials was incubated at 1,300, 2,500, or 6,500 lx and at a temperature within 2°C of the temperature in situ. The experiments were carried out in a laboratory incubator with fluorescent illumination. Two vials, one 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 incorporation of $^{14}CO_2$ increased linearly with time for 7 h.) The contents were filtered through a 0.45-μm membrane filter (Gelman GN-6); the filter was washed, placed in fuming HCl for 12 to 18 h, and counted in a toluene-based scintillation cocktail {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. In calculating the amount of $^{12}CO_2$ fixed from the radioisotopic data, a factor of 1.06 was used to correct for the preferential use of ^{12}C over ^{14}C in photosynthesis. The level of dissolved inorganic carbon in the lake water was determined with a gas chromatograph system described by Nelson and Zeikus (18). Microautoradiograms of selected samples were prepared by the method of Brock and Brock (3).

RESULTS

Blue-green algae first appeared in Lake Mendota in early June and were responsible for a 30-fold increase that month in the chlorophyll a content of the lake (Fig. 1). *Aphanizomenon flos-aquae* was the dominant alga in June and July, comprising 80 to 95% of the biomass (Fig. 2). This population declined at the end of July and was replaced by *Microcystis aeruginosa* in the first half of August. From mid-August to late September, significant amounts of *A. flos-aquae, M. aeruginosa,* and *Anabaena circinalis* were found in the water column. *Aphanizomenon* again predominated in October and persisted in significant amounts through November, although the diatom *Stephanodiscus* represented 20 to 40% of the phytoplankton biomass in early November.

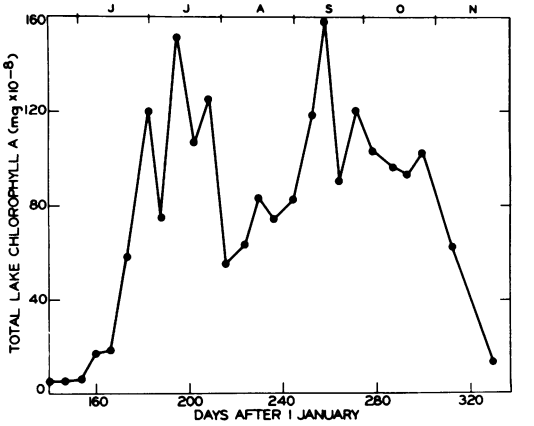

FIG. 1. *Chlorophyll a concentration of Lake Mendota from May to November 1976. Samples were collected at seven depths from each of eight stations throughout the lake. The chlorophyll concentration in each sample was determined, and the eight values for each depth were averaged. From this distribution of chlorophyll, the amount per square meter was determined and converted to the amount in the lake by considering the surface area of the lake. A 10^{10}-mg amount is equivalent to an epilimnetic concentration of ca. 20 μg/liter.*

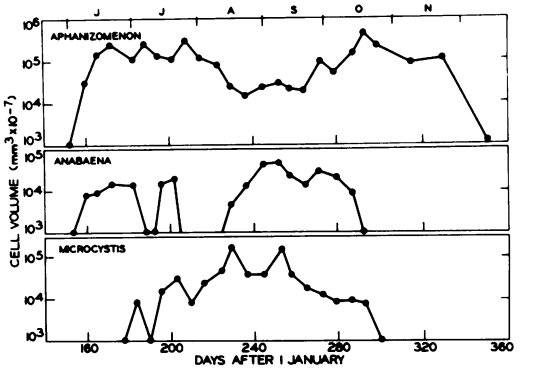

FIG. 2. *Cell volumes of the three predominant blue-green algae in Lake Mendota, May to November 1976. Cell numbers or filament length were determined by direct microscopic count and used to determine volumes by considering cell shape and dimensions. An algal volume of 10^{12} mm^3 for the entire lake is equivalent to an epilimnetic concentration of 5 mm^3/liter.*

Figure 3a illustrates the changes in the ratio of chlorophyll to protein that occurred during the year in Lake Mendota. Three major peaks in the ratio were observed: the first peak (day 171) just preceded the *Aphanizomenon* population increase, and the second (day 229) occurred as *Microcystis* was becoming the dominant alga. No increase in the chlorophyll-to-protein ratio was seen in early September when the chlorophyll content of the lake increased to its

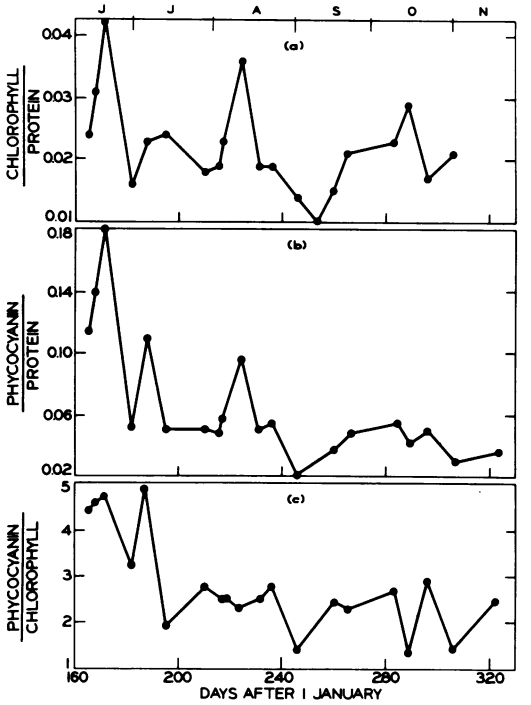

FIG. 3. *Changes in relative amounts of (a) chlorophyll to protein, (b) phycocyanin to protein, and (c) phycocyanin to chlorophyll of Lake Mendota algae collected from the surface in the period from May to November 1976. Chlorophyll and phycocyanin were measured spectrophotometrically in 90% acetone and water extracts, respectively. Protein was determined by the method of Lowry et al. (14).*

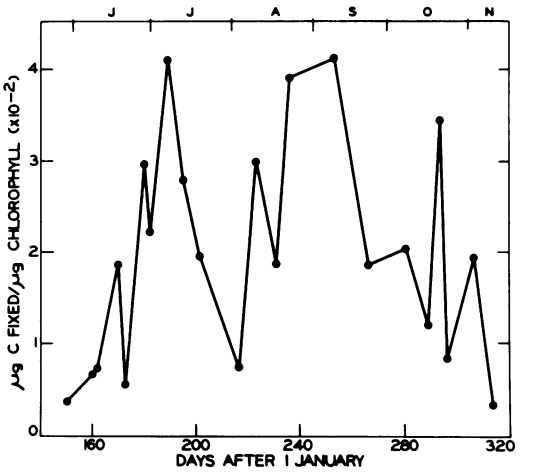

FIG. 4. *Photosynthetic rate of Lake Mendota algae, May to November 1976. Photosynthesis in surface samples was measured by adding 1 μCi of $NaH^{14}CO_3$ to 30 ml of lake water and incubating for 5 h at about the in situ temperature at 2,500 lx. Light-dependent incorporation of radioactivity was converted to micrograms of carbon incorporated per microgram of chlorophyll a.*

maximum value, but the ratio did increase at the time of lake turnover (day 290) when the *Aphanizomenon* population density was at a peak.

The variation in the phycocyanin/protein ratio in the algae (Fig. 3b) was similar to the changes seen in the chlorophyll/protein ratio, except that the phycocyanin content did not increase in September. The phycocyanin/protein ratio tended to decrease in the late summer, as did the phycocyanin/chlorophyll ratio (Fig. 3c). This may indicate that, although nitrogen was not growth-limiting (nitrogen-fixing algae were present), cellular surpluses had been depleted. Dissolved inorganic nitrogen and phosphorus were undetectable in the epilimnion in July and August (unpublished data).

The specific photosynthetic rate (i.e., the amount of light-dependent carbon fixation per microgram of chlorophyll a of natural samples incubated at 2,500 lx for 5 h) was related to the chlorophyll a concentration of the lake (cf. Fig. 4 and Fig. 1). The rate increased in June, when the chlorophyll a concentration increased because of the growth of *Aphanizomenon*. Although this organism maintained a total lake population of ca. 150,000 × 10^7 mm^3 through July, the specific photosynthetic rate declined in the last half of the month. It again increased in mid-August when a *Microcystis* population developed, and reached its maximum level in late August and early September when a mixed bloom of three blue-green algae was present. The specific photosynthetic rate declined in late September and October, although the *Aphanizomenon* population increased 5- to 15-fold in this time period (Fig. 2). The pigment contents of the algae did not appear to be correlated with the specific photosynthetic rate of the population.

Examination of microautoradiograms (3) prepared from samples incubated with $NaH^{14}CO_3$ in the light indicated that the majority of the organisms in a population were photosynthetically active and that the rate of photosynthesis of different species in mixed algal blooms was approximately the same.

Since the large blue-green algal biomass increased the attenuation of light, the algae might have physiologically adjusted to the use of lower light intensities in late summer. The ratio of $NaH^{14}CO_3$ incorporation at 1,300 lx (at which point photosynthesis is light limited) to incorporation at 6,500 lx (at which point light is not limiting) was used as a simple measure of low-light utilization and is called the saturation coefficient. This ratio indicates the degree to which

photosynthesis at 1,300 lx approaches the maximum level. Efficient utilization of low light levels was not directly related to light penetration (Fig. 5) but was inversely related to the *Aphanizomenon* population density in June and September. While the *Aphanizomenon* cell density increased, the saturation coefficient was high, but when the population peaked the saturation coefficient decreased. Although the proportion of different algal species fluctuated in August and September, the saturation coefficient steadily increased from 8 July until 6 October, which is consistent with better utilization of lower light intensities. The saturation coefficient was low during and after fall overturn.

DISCUSSION

In the present investigation, the pigment contents and photosynthetic rate of blue-green algae were compared with the algal biomass concentration to determine whether physiological changes could be measured during the onset and decline of algal blooms. Measurement of these changes may provide a better insight into the factors responsible for algal blooms, since standing crop measurements are a simplification of the dynamic processes involved in the growth, maintenance, and decline of a population. Furthermore, the rise and decline each season of several algal blooms may be due to various factors such as light, temperature, or nutrient availability; any physiological adaptations are comparable to laboratory phenomena whose causes are known.

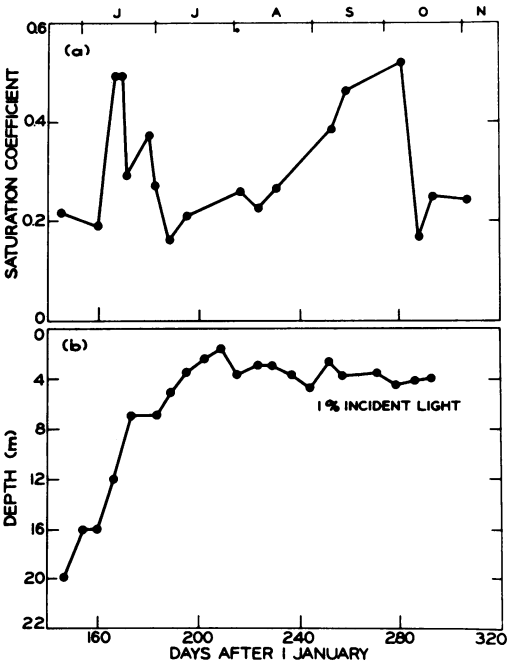

FIG. 5. *Changes in (a) saturation coefficient of photosynthesis and (b) light penetration in Lake Mendota. Saturation coefficient is the ratio of photosynthesis at a light-limiting intensity (1,300 lx) to that at a saturating intensity (6,500 lx). The depth at which incident light had been attentuated to 1% of the surface intensity was determined with a Whitney photometer. Water samples for (a) were collected from a depth of 0.5 m.*

The pigment contents of algae sampled through the summer were normalized to the amount of protein, since there is some evidence that the cellular protein content is less variable than that of other macromolecules when an organism grows at various rates (15). The protein in Lake Mendota is primarily algal; bacteria (estimated by epifluorescent microscopic counting) accounted for no more than 10 to 20% of the biomass during the period studied (C. Pedro-Alios, personal communication). Paerl et al. (19) estimated that the bacterial biomass of several lakes in the western United States does not exceed 5 to 10% of the total biomass.

Increases in algal biomass in June and August were preceded by increases in both chlorophyll a and phycocyanin (Fig. 3). However, these increases were transient, and the amounts of both pigments decreased. The algal biomass either continued to increase or maintained a high density (Fig. 2). Also, the pigment levels were not related to the photosynthetic rate (Fig. 4). Increases in the pigment content could precede periods of algal growth, but this was not always the case, as shown by the very low cellular pigment contents in September when *Aphanizomenon* was increasing.

Of the factors studied, the specific photosynthetic rate (micrograms of C fixed per microgram of chlorophyll) was most directly related to the chlorophyll a concentration of the lake (Fig. 1 and 4). This result was unexpected because high algal densities could have severely reduced nutrient levels, thus limiting photosynthesis. One explanation is that these experiments did not distinguish incorporation of $^{14}CO_2$ into polysaccharide from its incorporation into protein, lipids, or nucleic acids, so that even under nutrient-limited conditions the cell could have appeared metabolically active. The decline of *Aphanizomenon* in July and *Microcystis* in August was preceded by decreases in specific photosynthetic rate, suggesting that this parameter was a useful physiological indicator. The *Aphanizomenon* population persisted for 2 to 3 weeks after the decrease in specific photosynthetic rate, which may indicate that either a lesser degree of photosynthesis was sufficient to maintain the high

algal density, or the algae had synthesized sufficient reserve material to maintain themselves for that period of time.

The biliprotein phycocyanin represents a large percentage of the cellular protein in certain blue-green algae (2, 17). Allen (2) showed that it is unstable under conditions of nitrogen limitation. Thus, the phycocyanin content might be an indicator of nitrogen levels in blue-green algae. The phycocyanin/protein and the phycocyanin/chlorophyll ratios decreased from May to October (Fig. 3b and 3c), a fact that is consistent with decreases in the nitrogen content of the algae; however, phycocyanin content cannot be used as a direct measure of nitrogen content, since other factors also influence its concentration (1, 5, 8, 9). Our results probably reflect storage of nitrogen by blue-green algae in June (when dissolved inorganic nitrogen was present) and depletion of these reserves by August (when dissolved inorganic nitrogen was undetectable and the organisms depended upon nitrogen fixation).

The $NaH^{14}CO_3$ incorporation observed at different light intensities suggested that the algal populations had become adapted to the use of lower light intensities in the period from 8 July to 6 October. Several investigators have examined changes in pigment content of blue-green algae when cultures were grown at various light intensities. The levels of chlorophyll a and phycocyanin in cultures of *Anacystis nidulans* and *Anabaena* (10) were inversely proportional to the light intensity at which they were cultivated, but the ratio of phycocyanin to chlorophyll a did not change. Waaland et al. (21) found that the ratio of phycoerythrin to chlorophyll a in the red alga *Griffithia pacifica* decreased as the ambient light intensity increased. Natural populations of *Synechococcus* in a hot spring also adapted to light intensities that had been reduced experimentally (16). Although the amount of chlorophyll a did not change, the color of the algae changed markedly during light adaptation, and this was presumed to be the result of increased phycocyanin content. It was also found that the specific photosynthetic rate increased in light-adapted populations. The pigment content of the blue-green algal populations in Lake Mendota did not increase between 8 July and 6 October (Fig. 3), but the saturation coefficient did. It is possible that these blue-green algae used a different mechanism to adapt to low light intensities. For example, the diatom *Cyclotella* increased the concentration of CO_2-fixing enzymes when grown at higher light intensities (12). Since activities of carbon-fixing enzymes of Lake Mendota algae were not measured, the role of these enzymes in the apparent light adaptation of the organisms remains unknown.

Yentsch and Lee (22) have criticized the use of changes in I_k, the intensity at which photosynthetic rate is not light limited, as a measure of light adaptation, because shifts in I_k can result from physiological stress of the algae. Their criticism applies to our study, in that we measured photosynthesis at only three light intensities. Two were light limited and one was a nonlimiting intensity that indicated the maximum rate of photosynthesis. It was difficult to separate the effects of physiological stress from those of light adaptation in the increase in the saturation coefficient of surface populations from July to October (Fig. 5), because the photosynthetic rate varied from 80 to 400 μg of C per μg of chlorophyll during this time. Since the coefficient increased during periods of high photosynthetic activity, adaptation to lower light quantities probably occurred in this period. The changes in saturation coefficient observed during unialgal blooms of *Aphanizomenon* were probably more related to physiological stress than to light adaptation, since the saturation coefficient increased when the photosynthetic rate of the alga decreased.

In summary, the pigment content of Lake Mendota algae was not a good indicator of their physiological state. The phycocyanin content may have been related to inorganic nitrogen concentrations of the lake, but the relationship was not as dramatic as might be inferred from laboratory studies. The specific photosynthetic rate (per μg of chlorphyll a) was a useful physiological measure because it increased during algal growth and decreased before the decline of a population. Some indication of adaptation to utilization of low light intensities was found, but the putative adaptation was not due to increased amounts of photosynthetic pigments of the algae.

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