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Influence of Temperature, Oxygen, and pH on a Metalimnetic Population of *Oscillatoria rubescens*

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Planktonic *Oscillatoria* spp. often inhabit depths of thermally stratified lakes in which gradients of physical and chemical factors occur. Measurements of photosynthetic rate or photosynthetic carbon metabolism were used to evaluate the importance of vertical gradients of temperature, oxygen, and pH upon *Oscillatoria rubescens* in Crooked Lake, Ind. At the low light intensities experienced in situ, neither photosynthetic rate nor relative incorporation of carbon dioxide into low-molecular-weight compounds, polysaccharide, or protein was affected by temperature. At a 10-fold-higher light intensity, the photosynthetic rate increased as temperature increased; most of the additional carbon accumulated as polysaccharide. Polysaccharide which was synthesized at high light intensity and temperature was respired when the organisms were placed in the dark, but was not used for protein biosynthesis. When *O. rubescens* was shifted from high light to low light, a fraction of the polysaccharide was metabolized into protein. Adaptation to growth at lower temperatures by *O. rubescens* cultures resulted in a decrease in the maximum photosynthetic rate. Oxygen inhibited photosynthesis by only 10 to 15% at concentrations typically found in the lake. The photosynthetic rates at pH values which occurred in Crooked Lake were all near the maximum. Thus, gradients of temperature, oxygen, or pH are not likely to significantly affect the distribution of *O. rubescens* in Crooked Lake, given the low light intensity at which *O. rubescens* grows and the range of values for those factors in the lake.

Planktonic *Oscillatoria* spp. commonly stratify in the metalimnia of lakes. In these regions, there are gradients of physical and chemical factors which influence the vertical stratification of these organisms. The most recent evidence suggested that the distribution of *Oscillatoria aghardii* was determined by light intensity and nutrient concentration (12). In this report, three factors which exhibit gradients in the metalimnion of Crooked Lake (Noble County, Ind.) were studied to determine their effect upon a natural population of *Oscillatoria rubescens* in the lake. These factors were temperature, oxygen concentration, and pH.

Low temperatures commonly are found in habitats occupied by planktonic *Oscillatoria* spp. Observations of natural populations have led to speculation that they grow best at low temperature (6, 24); however, laboratory studies indicated that cultures of *O. rubescens* could grow at temperatures as high as 30°C (34). Oxygen concentrations in metalimnetic layers of *Oscillatoria* spp. are often greater than saturation concentrations and have been reported to be as high as 36 mg/liter (5). However, high oxygen concentrations inhibit photosynthetic carbon fixation in cyanobacteria (29), most probably as a result of photorespiration (31). Hydrogen ion concentration can also affect the growth of cyanobacteria; the organisms prefer alkaline conditions (7) and do not grow at a pH of 5 or below (2, 25).

The effect of these factors upon natural populations of *O. rubescens* was determined by exposing samples to various levels of temperature, oxygen, or pH and monitoring changes in the rate of photosynthesis or in the macromolecular products of photosynthesis. The results indicated that the influence of these factors upon the distribution of *O. rubescens* in Crooked Lake was small compared with that of light intensity and nutrient concentration.

MATERIALS AND METHODS

The study was conducted on Crooked Lake (Noble County, Ind.). Temperature and oxygen measurements at various depths of the lake were made with a YSI model 54 temperature/oxygen probe (Yellow Springs Instruments, Kettering, Ohio). Light penetration was determined with a Li-Cor quantum sensor (Lambda Instruments, Lincoln, Nebr.). Water samples were collected with a Van Dorn bottle (Wildco Supply

Co., Saginaw, Mich.). The location of the *O. rubescens* layer was determined from chlorophyll *a* measurements of discrete samples from the water column. Chlorophyll concentrations were determined from the absorbance at 663 nm of dimethyl sulfoxide-acetone extracts (40:60) of organisms filtered onto glass fiber filters (26). Biomass measurements were made by direct cell counts of samples preserved in 4% Formalin; the algae were observed by epifluorescence microscopy (3). The pH of water samples were determined with a Corning model 12 pH meter. Samples for photosynthesis measurements were collected at the depth where *O. rubescens* had the greatest concentration. Within 10 min, the samples were taken to the Crooked Lake Biological Station laboratory and the experiments were begun.

Photosynthesis was determined by the uptake of $NaH^{14}CO_3$ into particulate material. One μCi of ^{14}C was added to 25 ml of lake water in a screw-cap test tube. In the laboratory, tubes were incubated in triplicate at appropriate temperatures and light intensities (provided by cool white fluorescent bulbs) for 2 h. At the end of the incubation period, samples were filtered through glass fiber filters (Reeve Angel 984 H), rinsed with distilled water, exposed to HCl fumes for 20 min, dried, and counted in a Beckman 100-S scintillation counter by employing a toluene-based scintillation cocktail with 4 g of 2,5-diphenyloxazole and 0.1 g of *p*-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene per liter. Some samples were processed to separate macromolecular fractions (14). After filtration of the sample, the filter was placed in 3 ml of 80% ethanol and incubated for 5 min at 70°C. The liquid was refiltered; the filtrate represented the low-molecular-weight fraction. Particulate material was added to 3 ml of 10% trichloroacetic acid, heated at 100°C for 10 min, and again filtered. The filtrate contained polysaccharide; particulate material contained protein. The fractions were counted by liquid scintillation techniques; the particulate fraction was counted in the cocktail described above, and the liquid fractions were added to a similar fluid that contained BioSolv BBS-3 (Beckman Instruments). Counting efficiencies ranged from 76 to 82% and were determined by the addition of an internal standard ([^{14}C]toluene) to selected samples. Samples incubated in the dark or with Formalin added at the start of the incubation were used as controls. The concentration of dissolved inorganic carbon was determined from measurements of pH and alkalinity (30). In radioisotopic pulse-chase experiments, radioactive CO_2 was recovered from the culture filtrate by acidifying the liquid to pH 2, bubbling with air for 10 min while trapping the CO_2 in phenethylamine. This preparation was counted by liquid scintillation.

Cultures of *O. rubescens* were grown in MAME medium (14) at irradiances of 20 or 80 $\mu E \cdot m^{-2} \cdot s^{-1}$ provided by cool white fluorescent lights.

RESULTS

Description of the habitat. *O. rubescens* formed a layer in the lower metalimnion of Crooked Lake throughout the periods of thermal stratification in 1979 and 1980. The depth of the layer fluctuated from 8 to 11 m during 1979 and 6 to 8 m in 1980. The light intensities at these depths were low; the seasonal averages were 1.5% of the intensity at the lake surface in 1979 and 2.0% in 1980. Changes in temperature, oxygen concentration, and pH were found to occur primarily in the metalimnion (Fig. 1). The range of temperatures in the epilimnion was 14 to 26°C; the changes were due to seasonal heating and cooling. Temperature at the depth of the *O. rubescens* layer ranged from 7 to 13°C; the variation was due to differences in the depth at which *O. rubescens* stratified. The highest concentration of dissolved oxygen in the water column occurred in the metalimnion. The maximum oxygen concentrations in the metalimnion were always in excess of the saturation concentration at atmospheric pressure; the largest observed concentration was 22 mg/liter (200% saturation) on 23 July 1979. Note the sharp decrease in oxygen concentration in the *Oscillatoria* spp. layer (Fig. 1) where light intensities were extremely low. The pH of the lake water decreased from 8.3 in the epilimnion to 7.0 to 7.4 in the hypolimnion. The change in pH was greatest in the region where oxygen decreased; in this

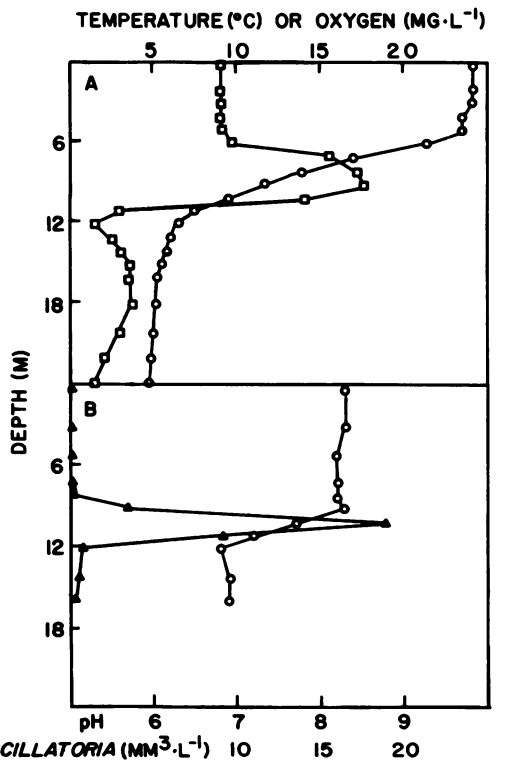

FIG. 1. *Vertical profiles of (A) temperature* (○) *or oxygen concentration* (□) *and (B) biovolume of Oscillatoria rubescens* (△) *or pH* (○) *from Crooked Lake on 6 September 1979.*

zone there is a transition from organic carbon production to organic carbon mineralization.

Samples were obtained from the *O. rubescens* layer and the specific photosynthetic rate (μg of C fixed per μg of chlorophyll per h) was determined for samples incubated at 10, 17, 24, or 30°C. Measurements were made at the in situ light intensity (20 $\mu E \cdot m^{-2} \cdot s^{-1}$) or 180 $\mu E \cdot m^{-2} \cdot s^{-1}$. In samples incubated at low light intensity, the average photosynthetic rate determined from five experiments (conducted during the summer of 1979) was about the same at all temperatures, whereas the average photosynthetic rate increased as temperature was increased for samples incubated at 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Table 1). Although there was significant variation in the photosynthetic rates obtained in different experiments (e.g., the range of photosynthetic rates at 24°C and 180 $\mu gE \cdot m^{-2} \cdot s^{-1}$ light intensity was 2.0 to 3.9 μg of C per μg of cholorophyll per h), the trends that appear in the average values were also observed within individual experiments. A two-way analysis of variance of the data, in which a randomized complete block design was employed, indicated that there was significant variance in the photosynthetic rates obtained on different dates at both 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity ($F_{(4,12)} = 21.9$, $P < 0.01$) and 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ ($F_{(4,12)} = 24.0$, $P < 0.01$). Variation in photosynthetic rate due to temperature was significant at the high light intensity ($F_{(3,12)} = 18.0$, $P < 0.025$), but not at 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ ($F_{(3,12)} = 2.0$, not significant).

The effects of temperature and light intensity upon photosynthetic carbon metabolism were analyzed by determining the proportion of CO_2 that was incorporated into low-molecular-weight compounds, polysaccharide, and protein (Table 2). Incubation temperature did not influence the relative proportions of photosynthetic products obtained at low light intensity. However, in samples incubated at 180 $\mu E \cdot m^{-2} \cdot s^{-1}$, relative incorporation into polysaccharide increased as incubation temperature increased; the decrease in relative protein incorporation was not significant. The data in Table 2 indicated that most of the increase in photosynthetic carbon fixation found at higher temperatures was due to polysaccharide accumulation. The amount of carbon incorporated into polysaccharide doubled as the incubation temperature was raised from 10 to 24°C, whereas protein incorporation only increased by 30 to 50% (Table 3).

TABLE 1. *Photosynthetic rate of natural populations of O. rubescens at various combinations of light intensity and temperature*

Temp (°C)	Photosynthetic rate at light intensity of[a]:	
	20 $\mu E \cdot m^{-2} \cdot s^{-1}$	180 $\mu E \cdot m^{-2} \cdot s^{-1}$
10	0.87	1.63
17	0.87	2.32
24	0.79	2.70
30	0.70	2.64

[a] Pooled standard deviation was 0.12 for samples at 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 0.26 for samples at 180 $\mu E \cdot m^{-2} \cdot s^{-1}$. Data are the means of five experiments and are expressed as micrograms of C per microgram of chlorophyll per hour.

TABLE 2. *Relative carbon fixation into photosynthetic products by O. rubescens populations incubated under various temperature and light conditions*

Temp (°C)	% of total carbon fixed at light intensity of[a]:					
	20 $\mu E \cdot m^{-2} \cdot s^{-1}$			180 $\mu E \cdot m^{-2} \cdot s^{-1}$		
	Low MW	Polysaccharide	Protein	Low MW	Polysaccharide	Protein
10	15	66	19	20	60	20
17	12	64	24	12	67	21
24	17	64	19	10	73	17
30	16	65	19	8	76	16

[a] Data are the means of five experiments. Low MW, Low-molecular-weight fraction.

Polysaccharide metabolism was investigated by labeling cells with $NaH^{14}CO_3$ for 1 h at high light intensity (180 $\mu E \cdot m^{-2} \cdot s^{-1}$) and then resuspending the organisms in filtered lake water without ^{14}C. The distribution of ^{14}C in macromolecules was determined periodically for samples incubated in the dark (Fig. 2A) or the light at 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 2B). Under either condition, the radioactivity in the polysaccharide fraction decreased; after 4 h, only 5% of the counts incorporated into polysaccharide during the labeling period remained in samples incubated in the dark, whereas 65% remained in samples incubated in the light. Polysaccharide radioactivity did not decrease further during the next 6 h in samples exposed to light. Radioactivity in the protein fraction did not change significantly over an 18-h incubation in the dark. In the light, protein radioactivity increased; this increase accounted for 35% of the loss from the polysaccharide fraction. In a similar experiment with a laboratory culture of *O. rubescens*, 86% of the radioactivity lost from particulate material during the dark incubation could be recovered in the medium as carbon dioxide. Radioactivity in dissolved organic compounds accounted for only 2 to 4% of the total uptake during the incubations in the light or dark.

Adaptation to the lower temperature characteristic of the metalimnion was investigated in a laboratory culture of *O. rubescens* isolated from

TABLE 3. *Absolute incorporation of CO_2 into macromolecules at 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity*

Temp (°C)	μg of C per μg of chlorophyll per h incorporated into:		
	Low MW[a]	Polysaccharide	Protein
10	0.32	0.98	0.33
17	0.28	1.55	0.49
24	0.28	1.97	0.46
30	0.20	2.01	0.43

[a] Low MW, Low-molecular-weight fraction.

the habitat. The maximum photosynthetic rate of batch cultures grown at 15 or 25°C at a light intensity of 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ was determined at several times during the exponential growth phase. The maximum rate was always greater for the culture grown at 25°C than the one grown at 15°C when measurements were conducted at either 25°C (3.7 versus 1.7 μg of C per μg of chlorophyll per h) or 15°C (2.9 versus 1.1).

The specific photosynthetic rate was measured in samples in which the oxygen content of the lake water was manipulated by bubbling with air, N_2, or O_2. In samples incubated at a range of oxygen concentrations typically found in Crooked Lake (5 to 16 mg/liter) at 25°C and 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity, the photosynthetic rate was at least 87% of the rate found under semianaerobic conditions. At initial oxygen concentrations of 0, 5, 10, 16, and 40 mg/liter, the relative photosynthetic rates were 100, 98, 96, 87, and 72%, respectively. The photosynthetic rate at 0 mg of oxygen per liter ranged from 3.4 to 4.6 μg of C per μg of chlorophyll per h in separate experiments. When samples were purged with pure oxygen gas to obtain a concentration of 40 mg/liter, the photosynthetic rate decreased by 30%. The manipulation of oxygen concentration by bubbling did not itself affect the photosynthetic rate, because samples in which oxygen was removed by purging with nitrogen and then reintroduced to its original concentration had the same photosynthetic rate as untreated samples.

Photosynthetic rates of natural populations of *O. rubescens* were measured at different pH values. The highest rates were found in samples with a pH between 6.5 and 8.5 (Fig. 3). If the pH was less than 6 or greater than 9, the photosynthetic rate was less than 50% of the rate at the optimal pH. The pH of Crooked Lake was always found to be in the range of 7.0 to 8.3.

DISCUSSION

The physical and chemical environment in the metalimnion of Crooked Lake differed from that experienced by phytoplankton in the epilimnion. Because of the stability of the water column in this region, sharp gradients of environmental factors occurred (Fig. 1). Some of these were due to the presence of *Oscillatoria* spp.; the oxygen maximum was due to photosynthetic oxygen evolution in the metalimnion and the decreases in oxygen and pH in the bottom portion of the *Oscillatoria* spp. layer resulted from light attenuation by the population such that net photosynthetic production could not occur and respiration processes consumed oxygen and produced carbon dioxide.

Light intensity and nutrient concentration were important factors controlling the buoyancy and vertical stratification of *Oscillatoria aghardii* in Deming Lake (12, 33). Light intensity has been shown to affect photosynthetic carbon metabolism in natural populations of *O. rubescens* (14) and buoyancy is regulated in response to light and nutrient availability (Konopka, manuscript in preparation). However, because temperature, oxygen, and pH have been re-

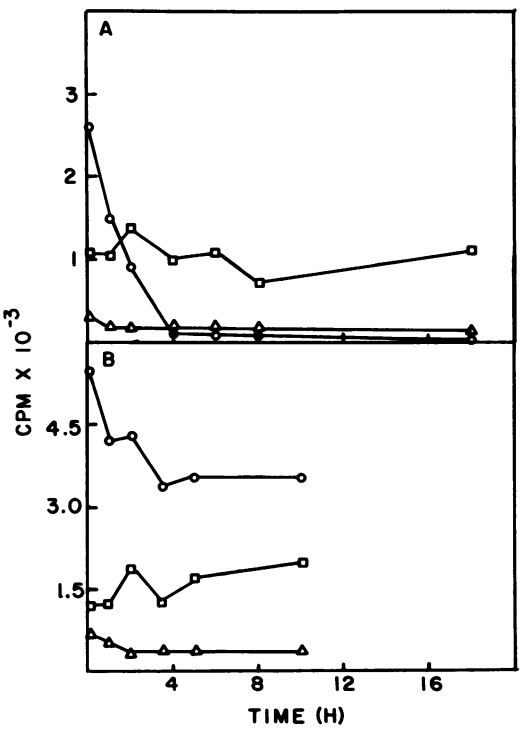

FIG. 2. *Polysaccharide metabolism of O. rubescens (A) in the dark or (B) at an irradiance of 20 $\mu E \cdot m^{-2} \cdot s^{-1}$. A natural sample was labeled for 1 h with $NaH^{14}CO_3$ at 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity, and after removal of $NaH^{14}CO_3$, the fate of label in low-molecular-weight* (△), *polysaccharide* (○), *and protein* (□) *fractions was monitored.*

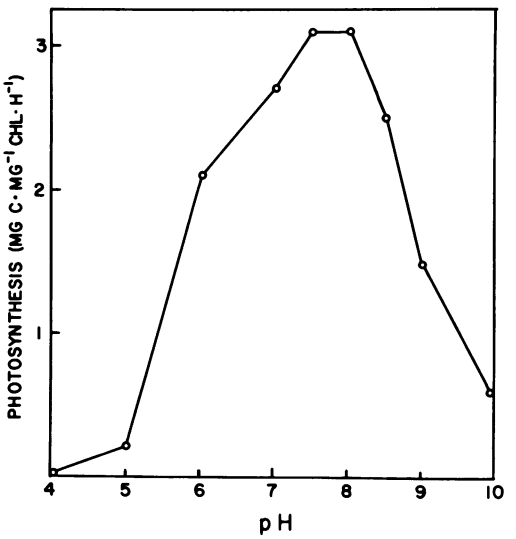

FIG. 3. *The effect of pH on the photosynthetic rate of natural samples of O. rubescens.*

ported to affect the growth and metabolism of cyanobacteria, and vertical gradients of these factors occurred in Crooked Lake, their influence upon the activity of a natural population was determined.

Photosynthetic rate was used to monitor the effect of these environmental factors upon the cyanobacteria. Earlier work had indicated that the temperature ranges for growth and photosynthesis were correlated for cultures of planktonic cyanobacteria, although relative photosynthesis sometimes exceeded growth rate at the extremes of the range (13). Short-term photosynthetic rates per unit dry weight have been found to exceed growth rates when phototrophs adapted to low irradiance levels were exposed to high light intensity (21). However, when photosynthetic rates are normalized to chlorophyll content (as in this study), the photosynthetic capacity of low-light-adapted cells is less than or equal to that of high-light-adapted cultures (10). Also, the relative photosynthetic rate of *Phaeodactylum tricornutum* grown at different temperatures had the same relationship to growth rate irrespective of the temperature at which photosynthesis was determined (19). Although growth rates cannot be directly calculated from photosynthetic rates because physiological adaptations alter photosynthetic capacity, conditions that result in the highest relative photosynthetic rates are presumed to be those most favorable for growth.

The effect of temperature upon photosynthesis was evaluated at two light intensities: 20 $\mu E \cdot m^{-2} \cdot s^{-1}$ (approximately the in situ intensity) and 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ (an intensity at which the maximum photosynthetic rate was obtained [14]). Both were necessary because the interaction between temperature and light intensity can significantly affect the photosynthetic rate (4, 18, 28). Under in situ conditions, photosynthetic rate was limited by incident light intensity and not by temperature (Table 1), because increasing the incubation temperature did not increase the photosynthetic rate at a light intensity of 20 $\mu E \cdot m^{-2} \cdot s^{-1}$. Thus, the low temperature of the metalimnion would not limit the productivity of the *Oscillatoria* spp. layer. Temperature would affect productivity of *Oscillatoria* spp. at a shallower depth where the light intensity was higher.

These experiments with natural populations of *O. rubescens* are consistent with physiological studies of temperature and light interactions in several eucaryotic algae (17, 27, 28). At low light intensities, the rate of photosynthesis depends upon the amount of light which hits the cells, and this is not affected by temperature. Temperature does affect the rates of dark reactions (e.g., the enzymes involved in CO_2 fixation), and it is these rates which determine the maximum photosynthetic rate. As temperature increases, both the maximum photosynthetic rate and the light intensity necessary for maximum photosynthesis increase (4).

Organisms can also physiologically adapt to growth at different temperatures. Although Jorgensen (9) reported that the maximum photosynthetic rate of *Skeletonema costatum* cultures increased as the growth temperature decreased, more recent evidence (19) suggested that this was primarily true in stationary-phase cells. During exponential growth in batch culture, growth at lower temperature resulted in a decreased maximum photosynthetic rate for several eucaryotic algae. A similar result was obtained with a culture of *O. rubescens* isolated from Crooked Lake. Thus, adaptation to growth in situ at low temperatures would depress the photosynthetic potential of the population; however, that potential is not expressed in situ because the incident light intensity is so low.

Although the highest photosynthetic rates were obtained at the highest temperatures and light intensity tested, growth of *Oscillatoria* spp. might not continue under these conditions because of low nutrient flux. Thus, it was of interest to determine whether the additional carbon fixed at high temperature and light intensity was used to make catalytically active biomass (i.e., protein) or storage products. The data in Table 3 indicate that the amount of protein incorporation at 17 to 30°C increased by only 33 to 50% above that at 10°C, whereas polysaccharide in-

corporation increased by up to 100%. Thus, the bulk of the additional carbon fixed at high light and temperature did not immediately contribute to the synthesis of ribosomes or enzymes.

However, the accumulation of a large proportion of fixed carbon as polysaccharide would be useful if the storage material served as carbon skeletons for protein synthesis when excess light energy was not available (at night or at low light intensities). Polysaccharide carbon apparently was not used by *O. rubescens* to synthesize protein in the dark, but was respired, in agreement with experiments on natural marine phytoplankton populations (20). However, cells that were incubated at low light intensity converted some polysaccharide into protein. Polysaccharide could serve as a source of energy for maintenance in the dark; glycogen has been found to be metabolized through the oxidative pentose phosphate pathway in several cyanobacteria (23). In contrast to the results of this study, polysaccharide carbon could be metabolized to protein in the dark by cultures of *Oscillatoria* spp. that were grown on a light-dark cycle (32). In addition, glycogen was used to synthesize protein in cultures of *Anacystis nidulans* which were incubated in the light and deprived of CO_2 (16).

The dissolved oxygen concentrations within the metalimnia of lakes with *Oscillatoria* spp. can be two to three times as high as the calculated saturation concentration (5) and could potentially inhibit the photosynthetic rate according to previous studies (29). Oxygen is a competitive inhibitor of ribulose bisphosphate carboxylase; the apparent rate of photosynthetic carbon fixation is decreased at high oxygen concentrations because of photorespiration (1). However, the oxygen concentrations typically found in Crooked Lake were only slightly inhibitory for photosynthesis; even in an atmosphere purged with pure oxygen, the photosynthetic rate was 70% of that under semianaerobic conditions.

Oxygen inhibition of photosynthesis might be expected to be unimportant in the metalimnion because of the low light intensities found there. The rate of photorespiration increases as light intensity increases (31). The measurements in this study were done at 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ to stimulate the environment at the depth of the oxygen maximum (Fig. 1). Oxygen inhibition should be less significant where *O. rubescens* is densest, because light intensity is only 20 $\mu E \cdot m^{-2} \cdot s^{-1}$. High oxygen concentrations do affect the physiology of natural populations of cyanobacteria that are exposed to high light intensities and low CO_2 concentrations (22).

Cyanobacteria are thought to grow best in alkaline environments (8). They were not found in natural habitats with a pH less than 5 (2), and optimal growth of cultures occurred in the pH range 7.5 to 9.0 (15). Optimal growth of *Microcystis aeruginosa* was reported at pH 10 (7). Keenan (11) concluded that pH itself was the controlling factor, rather than the form of inorganic carbon that was present at different values of pH. The pH of the water column of Crooked Lake varied by 1 unit, and the change occurred within the *Oscillatoria* spp. layer because the rate of photosynthetic CO_2 uptake was less than respiratory CO_2 release. However, high photosynthetic rates were found at all pH values that occurred in the lake. At pH values outside the range found in the lake, the photosynthetic activity of the natural populations declined (Fig. 3).

Thus, within the range of these three factors in the lake relatively small effects on the photosynthetic activity of natural populations of *O. rubescens* were found. The absence of temperature and oxygen effects was due to the low incident light intensity in the layer. If *O. rubescens* stratified at a depth of higher light intensity, temperature would affect productivity (Table 1), and loss of photosynthetically fixed carbon due to photorespiration might be more significant. Therefore, the vertical stratification of *O. rubescens* in Crooked Lake appears to be determined primarily by light intensity (14), although nutrient availability may affect the position of the layer on the vertical light gradient.

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