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INTERACTIONS OF CARBON AND NITROGEN METABOLISM WITH CHANGING LIGHT INTENSITY IN NATURAL POPULATIONS AND CULTURES OF PLANKTONIC BLUE-GREEN ALGAE

By

Amelia Kay Ward

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1978

DISCLAIMER

ABSTRACT

INTERACTION OF CARBON AND NITROGEN METABOLISM WITH CHANGING LIGHT INTENSITY IN NATURAL POPULATIONS AND CULTURES OF PLANKTONIC BLUE-GREEN ALGAE

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This study dealt with the factors contributing to the occurrence of blue-green algae in the plankton of lakes. Blue-green algal populations were examined in two different aquatic systems, moderately productive Lawrence Lake and hypereutrophic Wintergreen Lake, with regard to inorganic nitrogen source, light intensity and regime, and species of blue-green algae present. Unicellular and colonial blue-green algae in Lawrence Lake occurred with depth under continuously low light conditions with NO_3-N or NH_4-N as a nitrogen source; whereas, filamentous nitrogen-fixing species in Wintergreen Lake occupied the upper water strata and were exposed to variable light intensities over a diurnal period, when combined, inorganic nitrogen sources were low.

In order to understand the relationship between light and nitrogen source better among natural populations, representative species of blue-green algae, including isolates of Aphanizomenon flos-aquae, Microcystis aeruginosa, and Anabaena flos-aquae, were grown in laboratory cultures under continuously high, variable, and continuously low light at intensities similar to those in the lakes. Of three inorganic nitrogen sources utilized (NH_4-N, NO_3-N, and N_2-N), NH_4-N always resulted in highest

growth rates with all light regimes. However, intermittent exposure to high and low light intensities over a diurnal period had several advantages for N_2-fixing cultures compared to those grown with NO_3-N or NH_4-N. Growth could not be maintained at continuously low light for N_2-N cultures, and cultures transferred to continuously low light from a higher light intensity decreased in photosynthetic rates within 4 to 8 hours. Cultures grown with NO_3-N or NH_4-N maintained a more constant photosynthetic rate over this period. Furthermore, periodic exposure to low light ameliorated inhibition of photosynthesis in cultures grown under high light conditions. Because nitrogen fixation saturated at lower light intensities than carbon fixation, nitrogen-fixing cultures showed a greater disparity in cellular carbon and nitrogen content than NO_3-N or NH_4-N grown cultures when exposed to continuously high versus continuously low light intensities. Hence, a more uniform cellular carbon and nitrogen content could be maintained with nitrogen-fixing cultures exposed to an array of light intensities daily. Regardless of nitrogen source, periodic exposure to high light intensities was necessary to keep cultures from becoming low-light adapted.

The molecular weight of dissolved organic carbon released from axenic cultures was dependent on nitrogen source and light intensity. From 36 to 70 per cent of released dissolved organic carbon was in the molecular weight range of less than 500 Daltons.

Natural populations of blue-green algae from Lawrence and Wintergreen lakes appeared particularly well adapted to the habitats in which

they occurred. Maximum growth rates for non-nitrogen-fixing populations in Lawrence Lake subjected to a continuously low light regime could be maintained with NH_4-N as a nitrogen source. High light adapted nitrogen-fixing populations in Wintergreen Lake exposed to a gradient of light intensities could respond maximally to high light intensities when exposed, survive brief periods of low light intensities as well as maintain a balanced C:N ratio.

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This work is dedicated to my parents,
Ralph and Ruthelle Jones, who first
encouraged me to ask questions.

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INTRODUCTION

Certain members of the blue-green algae are unique among primary producers in that they are capable of assimilating elemental nitrogen under extracellularly aerobic conditions. This ability extends the range of ecologically important inorganic nitrogen sources to include N_2-N as well as NO_3-N and NH_4-N. As prokaryotes, these organisms have many physiological characteristics more similar to bacteria than algae. However, in a majority of their natural habitats, they function as primary producers, incorporating inorganic carbon photosynthetically, which is then available to the rest of the system in the form of particulate or dissolved organic carbon. From a functional standpoint, then, blue-green algae seem more closely aligned to other algal groups than bacteria. However, those bacterial characteristics which make blue-green algae unique among algal groups should give them a competitive advantage under certain conditions in their natural habitat. Among the characteristics which distinguish blue-green algae from other algal groups are the ability to fix atmospheric nitrogen and the possession of gas vacuoles, whereby some members can alter their position within a light or nutrient gradient.

Occurrence

Planktonic blue-green algae occur in several different habitats. Frequently, these algae are associated with surface "blooms" in

eutrophic lakewaters during periods when measurable inorganic nitrogen of the water strata are low (e.g., Duong, 1972; Ganf and Horne, 1975; Horne, 1970; Horne and Goldman, 1972; Horne et al., 1972). Nitrogen-fixing species are usually dominant in these systems, but uptake of combined inorganic nitrogen, in the form of NO_3-N and NH_4-N concomitant with N_2-N has been reported among phytoplanktonic assemblages almost exclusively composed of blue-green algae (Dugdale and Dugdale, 1965; Billaud, 1969). Populations of blue-green algae can also become successfully established in deeper, more oligotrophic waters. Particularly well-documented examples of this type include accounts of species of Oscillatoria occurring within the metalimnion during summer stratification (e.g., Wetzel, 1966; Baker et al., 1969; Saunders, 1972; Klemer, 1976). Other groups besides Oscillatoria, however, have also been reported in this type of low-light environment (Eberly, 1959; Baker and Brook, 1971).

Various hypotheses have been advanced to explain the success of blue-green algal populations in these physiologically divergent habitats. Several factors must be considered in relation to those populations which develop at great depth in less productive lakes as opposed to surface populations common to eutrophic conditions. First, the temperature-density characteristics of the metalimnion may be more suitable for maintenance of buoyancy than the warmer, less dense water of the epilimnion in that populations may exist poised in the lower water strata of the photic zone (Fogg, 1969). Second, there is some evidence that the resulting low-light conditions found at this depth are more favorable to growth of blue-green algae than other groups of algae, for example green

algae (Mur et al., 1977). Third, existence in this deeper zone is closer to accumulated minerals in the commonly reduced conditions of the hypolimnion during summer stratification (Wetzel, 1975; Klemer, 1976).

Maintenance within the photic zone, although important in oligotrophic lakes, becomes increasingly critical in very productive eutrophic systems, where light can be attenuated rapidly over a short distance because of biogenic turbidity. Synthesis and collapse of gas vascuoles, which are unique to certain prokaryotes (Walsby, 1972), provide potentially powerful mechanisms for maintaining buoyancy within the photic zone (Dinsdale and Walsby, 1972). When attenuation of light becomes extreme, photosynthetic maxima are increasingly displaced and compressed toward the surface of lakes (Wetzel, 1966; 1975). Hence, gas vacuoles provide a means by which rapid movement in response to changing light conditions could be attained over a relatively short distance.

Nitrogen Sources

Superimposed on effects of light and nutrients in general, are the more specific effects of nitrogen source on the success of different types of blue-green algal populations. From an energetic standpoint, preference of nitrogen source should follow the order NH_4-N > NO_3-N > N_2-N. However, studies with natural populations and cultures of blue-green algae indicate the effect of nitrogen source on growth is considerably more complex. Although blue-green algae grow well on NO_3-N and NH_4-N as well as N_2-N (in N_2-fixing species), NO_3-N has been suggested as the "preferred" source in culture media (Fogg, 1973), as well as the better

nitrogen source for growth (Wetzel, 1975, p. 198). Reservations concerning the use of N_2-N and NH_4-N probably stem from culture studies in which combined nitrogen supported better growth than N_2-N (Kratz and Myers, 1955) although this is not always the case (Allen and Arnon, 1955; Singh and Srivastava, 1968), and undesirable side-effects can result from use of NH_4-N in culture. For example, the use of NH_4-N in culture can cause a drop in pH to inhibitory levels (Singh and Srivastava, 1968), cell lysis (Pintner and Provasoli, 1958), and toxicity at high pH (Stewart, 1964). Paradoxically, while N_2-N and NH_4-N can present problems as nitrogen sources in culture, natural populations of blue-green algae are frequently N_2-fixers or populations which occur in systems with potentially high turnover rates of NH_4-N (as in eutrophic systems or in the metalimnion of less productive systems). With non-nitrogen-fixing populations of Oscillatoria agardhii in Lake Deming, Minnesota, Klemer (1976) found that enrichment with NH_4-N resulted in increased filament numbers; whereas, enrichment with NO_3-N or a combination of NO_3-N and PO_4-P did not. Klemer concluded that Oscillatoria agardhii was likely restricted to metalimnetic water strata because of nutrient limitations in the epilimnion (specifically NH_4-N) rather than an inherent oligothermy. Thus, nitrogen source varies considerably among planktonic blue-green populations depending upon other environmental conditions.

Interaction of Light, Nitrogen Source, and Photosynthesis

The combination of light and nitrogen source could be determining factors in the success of blue-green algal populations in that a specific

light regime may be required to maintain optimal growth on a given nitrogen source. All processes of inorganic nitrogen assimilation in primarily autotrophic organisms are dependent upon photosynthesis for carbon for the incorporation of end products of nitrogen reduction. In addition, nitrate reduction, nitrogen fixation, and to some extent, ammonia assimilation are light-stimulated events in blue-green algae (Fogg and Than-Tun, 1960; Hattori, 1962; Healey, 1977). Nitrogen fixation appears specifically associated with Photosystem I (Lex and Stewart, 1973) and to a lesser extent on Photosystem II, depending on the physiological state of the cell (Cox and Fay, 1969) and rates of photorespiration (Lex et al., 1970). The association of nitrate reduction with the photosystems is less well-defined, with nitrite reductase apparently more closely associated with Photosystem II (Fujita and Hattori, 1963; Wolk, 1973) than is nitrate reductase (Stevens and Van Baalan, 1973).

Of potential importance to natural populations of blue-green algae are the different effects of light on nitrogen assimilation and photosynthetic carbon fixation. Cobb and Myers (1964) established differences in response to light intensity between nitrogen fixation and carbon fixation in Anabaena cylindrica; that is, nitrogen fixation rates tended to saturate at lower light intensities than carbon fixation rates. They hypothesized that Anabaena cylindrica possessed a mechanism whereby the cellular C:N ratio played a role in other cellular events, specifically heterocyst formation. Also, a positive correlation was reported by Kulasooriya et al. (1972) between C:N ratio and heterocyst development in Anabaena cylindrica. Therefore, differences in assimilation rates in response to light between nitrogen fixation and carbon

fixation affect the cellular C:N ratio, which in turn may affect other aspects of metabolism as specific as heterocyst development or more generally to "more balanced growth" (Peterson et al., 1977). Less is known of the relative rates of NH_4-N and NO_3-N assimilation in relation to photosynthetic carbon fixation and light intensity among representatives of natural populations of blue-green algae.

In summary, planktonic blue-green algae are capable of utilizing a number of inorganic nitrogen sources. Their presence in surface waters of eutrophic lakes and at depths of low light intensities in more oligotrophic systems indicates a competitive advantage in these habitats, which differ considerably in nitrogen source, light intensity, and light regime. Therefore, among the many factors which contribute to the success of blue-green algae in lakes, the interactions of light, nitrogen source and photosynthesis offer particular potential.

Objectives

This research focused on the dynamics of two different types of blue-green algal populations in lakes of differing trophic status. The first part of the investigation was devoted to a study of various physical-chemical and biological factors of the lake systems. Of particular interest were 1) source of nitrogen, 2) light regime, and 3) species of blue-green algae present. The second part of the investigation was directed at elucidating mechanisms operative in the establishment of blue-green populations by using unialgal or axenic laboratory cultures of representative species. Isolates of Microcystis aeruginosa,

Aphanizomenon flos-aquae, and Anabaena flos-aquae were employed to examine growth rates, cellular carbon and nitrogen content, N_2-fixation rates and carbon fixation rates under conditions designed to simulate those found in situ in the two main types of observed habitat.

Although Rodhe (1948) recognized a "time of exposure" factor with regard to phytoplankton response to light and temperature, no experimental studies have incorporated, in a systematic manner, differences in light intensity and light regime as they occur in natural systems. Of emphasis in this study were the effects of continuous light of one intensity versus variability of light intensity in the context of the lake populations under investigation, and how these effects would be manifested in the parameters listed above.

The presence of dissolved organic carbon and particularly dissolved organic nitrogen is intimately associated with natural populations of blue-green algae (e.g., Pearsall, 1932; Fogg, 1971) and is potentially of great importance to the detrital dynamics of aquatic ecosystems (Rich and Wetzel, 1978). In certain strata of lakes where concentrations of combined, inorganic nitrogen sources are low, dissolved organic nitrogen released from the phytoplankton populations can represent a significant input of nitrogen to that portion of the system. Besides providing a substrate for the bacterial component of the community, these organic nitrogen compounds, upon mineralization, may provide an inorganic nitrogen source in the form of NH_4-N for the algal component. Of particular interest in this study were the qualitative differences among dissolved organic compounds released by blue-green algae under different conditions of light and nitrogen source.

MATERIALS AND METHODS

Field Procedures

General:

Sampling on Lawrence and Wintergreen lakes began in April, 1975, shortly after ice-off and continued through October, 1975, before autumnal circulation. Water samples were collected with an opaque Van Dorn water sampler from the central depression of each lake (Figures 1 and 2) every two weeks at four depths (2, 4, 6, and 10 meters in Lawrence; 0.5, 2.0, 3.0, and 4.0 meters in Wintergreen). Physical-chemical parameters which were monitored included temperature, light penetration, pH, alkalinity, and concentrations of NO_3-N and NH_4-N. In situ rates of photosynthesis and nitrogen fixation were also measured.

Physical-Chemical:

Temperature and Light. Temperature measurements were made with a Yellow Springs Instrument Tele-thermometer (Model 43TB). Light penetration as percentage of surface light was determined using an underwater photometer (Rich and Wetzel, 1969).

pH and Alkalinity. Measurements of pH were made utilizing either a Beckman Expandomatic (Model 76A) or Coleman (Model 38A) pH meter. Alkalinity in meq l^{-1} was determined by titrating 50 milliliter water samples with 0.02 N H_2SO_4 to pH 4.46 to 4.48 indicated by a mixture of brom-cresol red and methyl orange (Amer. Publ. Health Assoc., 1976).

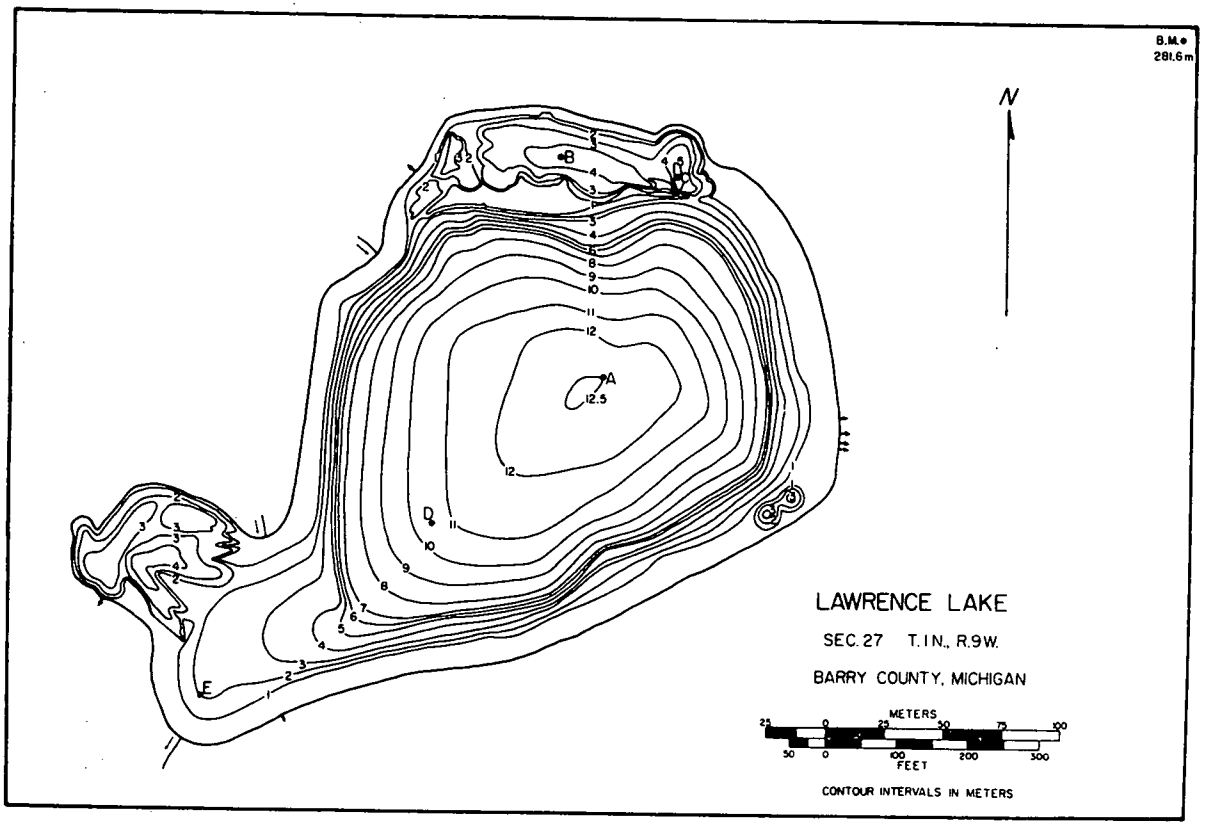

Figure 1. Bathymetric map of Lawrence Lake, Barry County, Michigan.

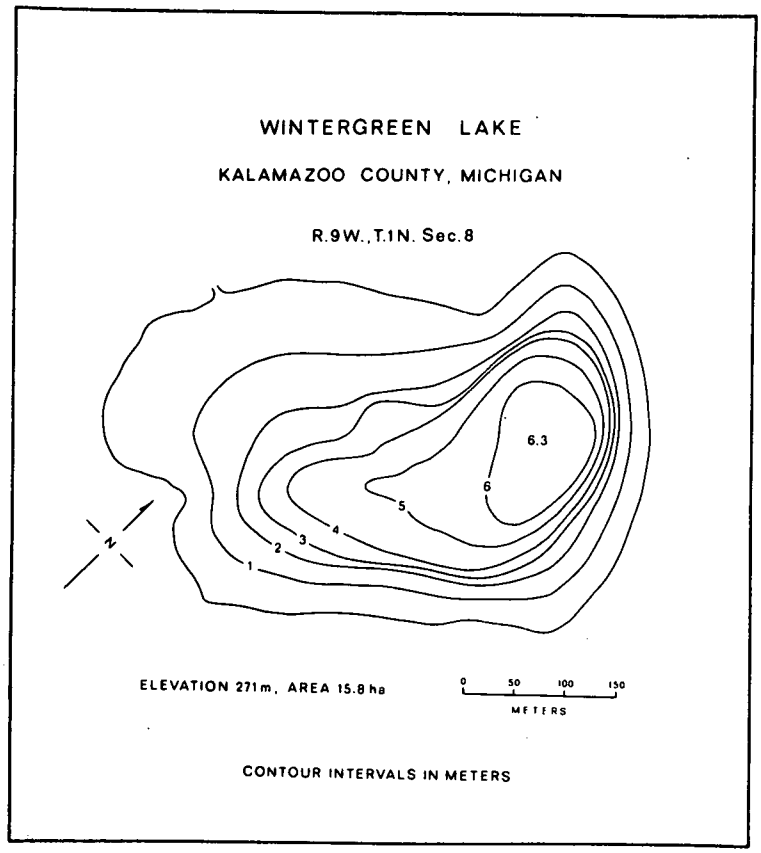

Figure 2. Bathymetric map of Wintergreen Lake, Kalamazoo County, Michigan.

Nitrate and Ammonia. Water samples were filtered through pre-combusted (525 C for 45 minutes), glass-fiber filters (Reeve-Angel, 984H) and analyzed for NH_4-N by the procedures of Harwood and Kuhn (1970), and for NO_3-N by the cadmium reduction technique (Wood et al., 1967).

Biological:

Primary Productivity. In situ rates of photosynthesis were obtained by incubation over an approximately four hour period with $NaH^{14}CO_3$ (Strickland, 1960). One milliliter of $NaH^{14}CO_3$ was added to a water sample in 125-ml stoppered glass Pyrex bottles and incubated at depths from which the samples had been collected. After incubation, aliquots of either 50 ml (Lawrence Lake) or 25 ml (Wintergreen Lake) were filtered from the bottles through Millipore HA filters (0.45 μm pore size). Filters were desiccated, fumed with HCl (Wetzel, 1965), and analyzed by Geiger-Müller radioassay (Nuclear-Chicago D-47 of known counting efficiency). Results were reported as mg $C/m^3/hr$.

Nitrogen Fixation (Acetylene Reduction). The procedure for estimating in situ rates of nitrogen fixation followed closely those described previously by Ward and Wetzel (1975). However, the vials were not flushed with a nitrogen-free gas prior to incubation in order to provide a more natural atmosphere above the samples and an extra set of controls for determining ethylene transformation (Flett et al., 1975) was included. The procedure for detecting ethylene transformation was as follows: a known amount of purified ethylene (Matheson Gas Products, Joliet, Illinois) was injected into killed (0.2 ml 2% $HgCl_2$) and

unkilled samples prior to incubation. Ethylene from these vials was measured concurrently with regular samples. A significant decrease in ethylene concentration in the head-space of unkilled samples compared to that in killed samples would indicate ethylene transformation. No significant decrease was noted, therefore ethylene transformation was assumed absent. Gas analyses were performed on a flame-ionization Varian Aerograph gas chromatograph (Model 600-D) equipped with a stainless steel column (3 mm x 2 meters) packed with Porapak-N (80-100 mesh).

Pigments. Concentrations of chlorophyll a (corrected for pheopigments) were determined by filtering known volumes of lakewater through Millipore AA (0.8 µm pore size) filters. Pigments were extracted from the filters in 90% basic, aqueous acetone and absorption of the supernatant measured by a Hitachi-Perkin Elmer spectrophotometer (Model UV-VIS 139). Calculations were those of Parsons and Strickland (1963), Westlake (1969), and Wetzel and Westlake (1969).

Phytoplankton. Algal samples were preserved with Lugol's solution (Ward and Whipple, 1959, p. 2000) and examined by the sedimentation technique in settling chambers with a Wild inverted microscope.

Laboratory Procedures

Culturing Techniques:

Representatives of blue-green species found in Lawrence and Wintergreen lakes were grown in cultures in the laboratory. Isolates of Microcystis aeruginosa Kütz emend. Elenkin, clone NRC-1 (SS-17), and Aphanizomenon flos-aquae (L.) Ralfs), isolate NRC-566, were used most

frequently in experimental work. Isolates of Anabaena flos-aquae (s-29-f-6), Anabaena flos-aquae (A-113-s-q-a), and Anabaena flos-aquae (A-52) were used periodically. These strains were isolated by Drs. Wayne Carmichael and Paul Gorham (Carmichael and Gorham, 1975) from the phytoplankton of hardwater lakes in Canada. In addition, a culture of Anabaena flos-aquae (G-R) was provided by Dr. G-Y Rhee, New York State Department of Health, isolated from Lake Erie. Samples from cultures of Microcystis aeruginosa (SS-17) and Anabaena flos-aquae (G-R), periodically streaked on Plate-Count agar (Difco) and examined microscopically (1000 X, phase contrast, Zeiss microscope) showed no bacterial growth. All other cultures were unialgal, but bacterized.

Algae were maintained in batch culture on modified Moss medium (1972). The concentration of nutrients was based approximately on world averages for fresh waters and hence was less than concentrations of nutrients employed in other algal media (e.g., ASM-1; Carmichael and Gorham, 1975). The following modifications were made: $NaSiO_3 \cdot 9H_2O$ and the vitamin mixture were deleted; $NaHCO_3$ concentrations were doubled (4.0 g/1); Tricine* (Sigma Chem. Co.) was included as a buffering agent (130 mg/1); and the pH before autoclaving was adjusted (0.1 N NaOH) to 7.3-7.5 to yield a final pH after autoclaving of 8.0 ± 0.1. In preliminary experiments no difference in algal growth was found between $Ca(NO_3)_2 \cdot 4H_2O$ and KNO_3 as a NO_3-N source. Thereafter, KNO_3 was substituted as a NO_3-N source. Concentrations of NH_4Cl and KNO_3 were adjusted

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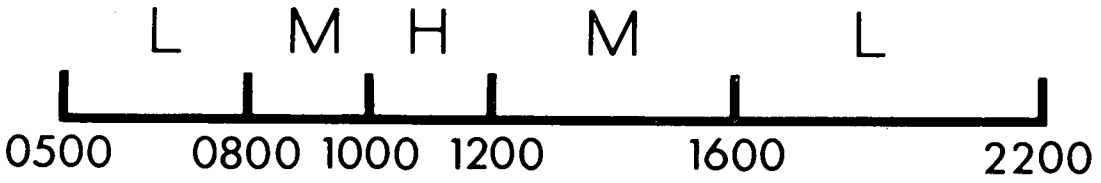
*(N-Tris(hydroxymethyl)methyl glycine).

to yield a final concentration of 5.0 mg N/l in experimental media. The medium was buffered rigorously; therefore, little fluctuation in pH occurred regardless of nitrogen source. A maximum increase of pH to 8.3-8.5 occurred in certain late log phase cultures.

Stock and experimental cultures were grown in Sherer growth chambers at a temperature of 23 $\pm$ 1.0 C. Light was supplied by Vita-lite fluorescent bulbs (Luxor) at an intensity of 150-200 Lux, variable (Figure 3), or 1500-1800 Lux (high) on a light regime of 17 hours light: 7 hours dark. Light intensities simulated those found *in situ* during mid-morning in July between a depth of 0.5 meters and 2.0 meters in Wintergreen Lake and 6.0 meters in August in Lawrence Lake. Surface light intensities were not used in order to avoid confounding effects of photoinhibition. The spectral composition of light from Vita-lite bulbs, determined utilizing a scanning spectroradiometer (Model SR, Instrumentation Specialties Company, Inc.), was found to be similar in quality to that at 2.0 meters in Gull Lake (Kalamazoo County, Michigan), a hard-water lake similar in chemical composition to Lawrence and Wintergreen lakes (Figure 4).

Cellular Carbon and Nitrogen:

A known volume of algal culture was filtered onto 13-mm diameter pre-combusted (525 C for 45 minutes) glass fiber filters (Reeve-Angel 984H). Filters were rinsed with glass distilled water to remove dissolved carbon and nitrogen contaminants left on the filter from the culture medium, wrapped in tin, and pelletized. Pellets were combusted in a Carlo-Erba elemental analyzer (Model 1104), interfaced with an



L = 200 LUX
M = 800 LUX
H = 1500 LUX

Figure 3. Light regime for cultures grown under variable light intensities.

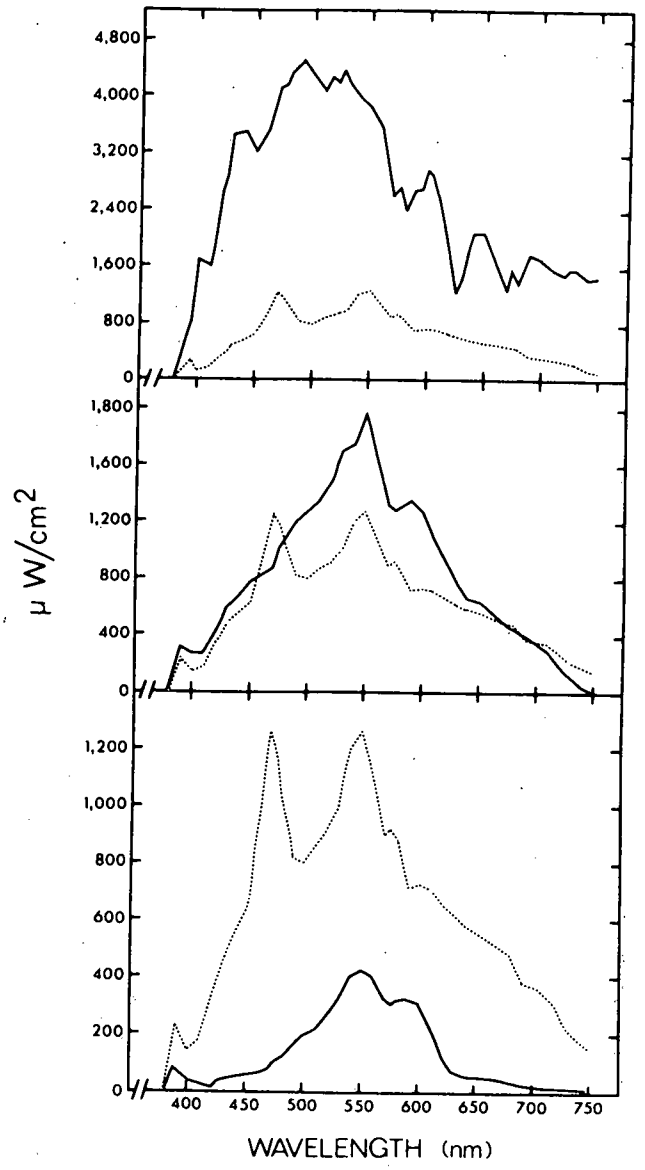


Figure 4. Spectral composition of Vita-lite bulbs compared to three depths in Gull Lake: —— Gull Lake at 0 (upper), 2.0 (middle), and 6.0 meters (lower); ···· Vita-lite lamps.

automatic digital integrator (Columbia Model CSI-208) and Monroe (Model 1305) digital print-out. Two blanks (glass fiber filter and tin) and three standards (Cicloesanone-2,4-dinitrofenilidrazone, Carlo-Erba) were assayed with every 18 samples. Cellular carbon values were corrected for carbon contamination (1-5% of sample values) in the calculations. Nitrogen contamination was undetectable.

^{14}Carbon Fixation:

Rates of photosynthesis were measured by incubating 50-ml aliquots from experimental cultures with 0.25 ml of $Na^{14}CO_3$ (specific activity, 5.12 μCi/ml) for 30-60 minutes. Smaller amounts (5-10 ml) were filtered onto Millipore HA (0.45 μm pore size) filters for radioassay.

Molecular Weight Fractionation of Released Dissolved Organic Carbon:

Dissolved organic carbon released from blue-green algal cultures was obtained by incubating cultures with $NaH^{14}CO_3$ of high specific activity (approximately 100 μCi added to 300 ml of culture) under various light intensities. After approximately 4 hours of incubation, cultures were filtered through Reeve-Angel glass fiber filters (984H). The resulting filtrate was the "Total" fraction and aliquots (25 ml, 2 determinations) from this fraction were passed through Amicon membranes of the following designation and nominal molecular weight cut-off: PM 30 = 30,000 Daltons; PM 10 = 10,000 Daltons; UM 2 = 1000 Daltons; and UM 05 = 500 Daltons. These fractions were acidified to pH 3 with H_3PO_4, purged for 10 minutes with CO_2 to remove residual inorganic carbon, and then

subjected to lyophilization and scintillation radioassay by the techniques of McKinley et al. (1976). Results were reported as the percentage disintegrations per minute (DPM) of the "Total" fraction.

OVERVIEW OF STUDY SITES

Lawrence and Wintergreen lakes are small, hardwater lakes located in southwestern Michigan. However, whereas Lawrence Lake is considered oligo- to mesotrophic, Wintergreen Lake is hypereutrophic. These lakes have been and continue to be the subject of intensive limnological research and considerable information on the physical-chemical and biological interactions within these systems has been accrued from previous studies (e.g., Wetzel et al., 1972; Manny, 1973).

In summary, Lawrence Lake, located in a small depression of land and bounded on several sides by emergent macrophytes, has a rather small surface area to volume ratio (maximum depth = 12.6 m., surface area = 4.9 ha.). Suppression of primary productivity within the open-water portion of the basin is directly related to the chemistry of the lakewater, which is strongly alkaline and carbonate-rich. Nutrients, such as phosphorus, and trace metals tend to precipitate out, complex with $CaCO_3$, or are otherwise made physiologically unavailable to the phytoplankton (Wetzel, 1972; Wetzel and Manny, 1978). Because of the interactions of basin morphometry and chemical characteristics of the water as well as aspects of the watershed in general, Lawrence Lake retains a considerable capacity to withstand rapid change. The system is chemically well-buffered and this capacity is reflected in various biological parameters of the system as well. For example, although extensive

seasonal variations exist among many parameters (e.g., primary production, oxygen concentration and pH), there are not marked day to day or, in some case, week to week fluctuations (cf. review by Wetzel, 1975).

Although of similar geological background to Lawrence Lake, Wintergreen Lake has a much larger surface area to volume ratio (maximum depth = 6.3 m., surface area = 15.8 ha). The higher percentage of shallow water has encouraged greater macrophyte production over the years in relation to Lawrence Lake and has been one factor contributing to the hypereutrophy of the lake (Manny et al., 1978). However, the establishment of the W. K. Kellogg Bird Sanctuary at this site in the recent past has greatly accelerated this condition. The organic input derived from 4600 kg dry weight of waterfowl feces annually (Manny et al., 1975) has eroded the buffering capacity of the system still inherent in Lawrence Lake, by means of a complex of interacting factors (cf. Wetzel and Allen, 1970). As a result, Wintergreen Lake exhibits frequent and violent oscillations in annual biological and chemical properties.

RESULTS

Lawrence Lake

Occurrence of Blue-green Algae:

Lawrence Lake exhibited temperature characteristics typical of a dimictic, north temperate lake (Figure 5). During summer stratification, the depth of the epilimnion extended to about 6 meters by August. Typically, the blue-green populations occurred within or just above the metalimnion and were made up predominantly of colonial and unicellular forms such as Microcystis spp., Gomphosphaeria spp., Coelosphaerium spp., Chroococcus spp., and Aphanocapsa spp. Blue-green algae are often associated with eutrophic systems; however, even in moderately productive Lawrence Lake, productivity rates associated with the blue-green algal populations in summer were higher than at any other time of the year (Figure 6). This late summer metalimnetic blue-green algal association has developed similarly each year for 11 years of continuous analysis (Wetzel, unpublished data).

Sources of Nitrogen:

Concentrations of NH_4-N in Lawrence Lake were consistently low in the upper water strata (Figure 7), ranging from 25 to 100 µg NH_4-N/l, however in the hypolimnion, NH_4-N accumulated, increasing as the period of summer stratification progressed. At the time of the strong

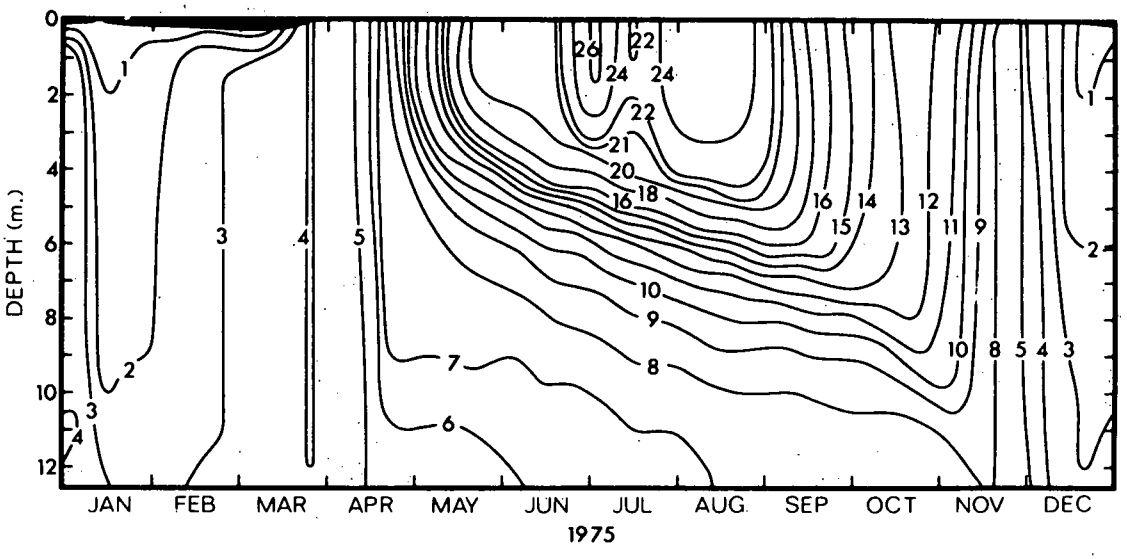


Figure 5. Depth-time diagram of isopleths of temperature (^{o}C) in Lawrence Lake, Michigan, 1975.

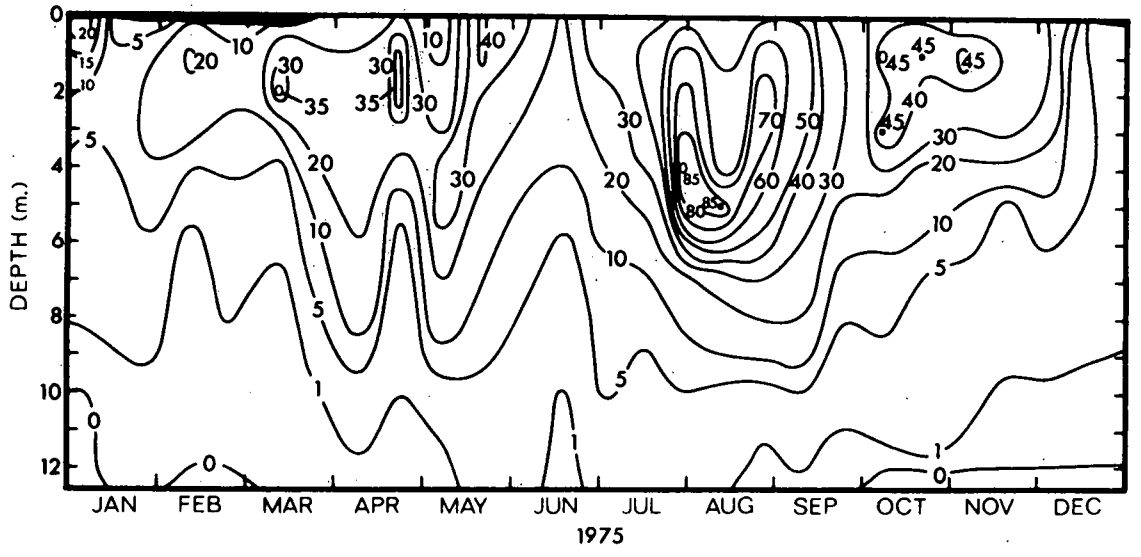


Figure 6. Depth-time distribution of *in situ* rates of primary production in mg C m^{-3} day^{-1}, Lawrence Lake, Michigan, 1975.

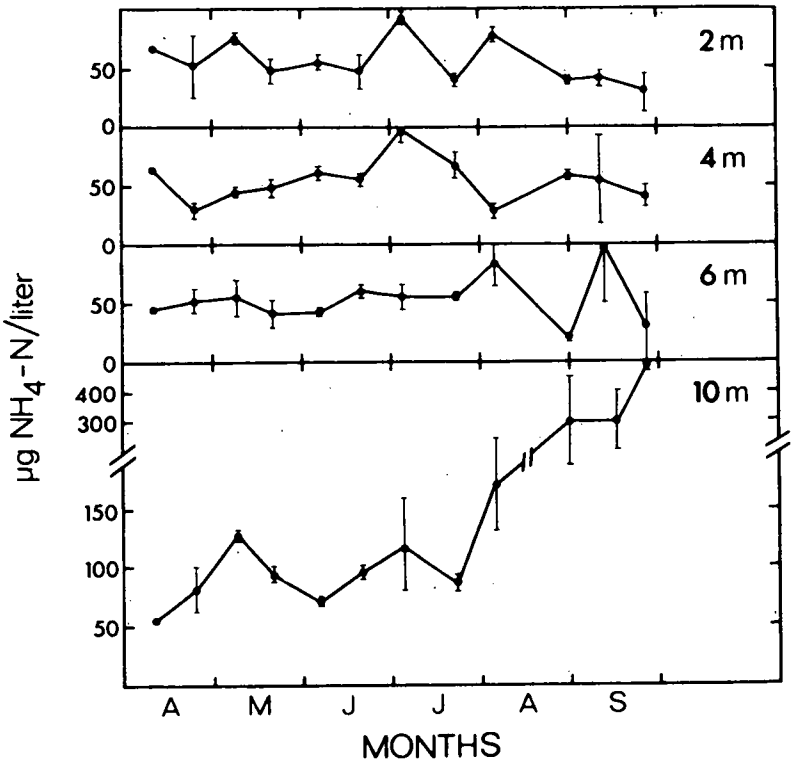


Figure 7. Concentrations of NH_4-N ($\mu g\ l^{-1}$, $\pm$ S.D.) at four depths in Lawrence Lake, Michigan, 1975.

development of blue-green algal populations in August, a reservoir of NH_4-N was available in the hypolimnion. A significant decrease in NH_4-N concentrations at 6 meters indicated utilization of that nitrogen source at that depth, presumably by blue-green algae. Because of the close physical association of the bacterial and the algal components, it is not possible to distinguish the relative importance of each to the uptake of NH_4-N. However, the utilization of NH_4-N by bacteria, e.g., nitrifying bacteria, would not preclude concomitant utilization by the blue-green algae or vice versa. The problem could only be resolved directly by microautoradiography, assuming a suitable radioactive nitrogen source. The occurrence of the blue-green populations was correlated with low levels of NH_4-N in the epilimnion (2 and 4 meters), a depletion of NH_4-N at 6 meters and accumulation of NH_4-N at depth. Concentrations of NO_3-N were measured periodically and were much higher than NH_4-N concentrations, in the range of 2-5 mg NO_3-N/l (cf. Wetzel, 1975, p. 204).

Light Regime:

Blue-green populations occurring in Lawrence Lake were exposed to a continuously low-light regime. Figure 8 illustrates a representative light profile taken during mid-August with the arrows indicating the depths occupied by blue-green algae. Light in this water stratum had been attenuated to between 9-11% surface light intensity, corresponding to approximately 180-240 Lux. Measurements of diurnal productivity rates during August indicate little, if any, movement above this stratum daily (McKinley and Wetzel, 1978). Samples from populations exposed to light intensities varying from 150 to 2400 Lux were low-light adapted in

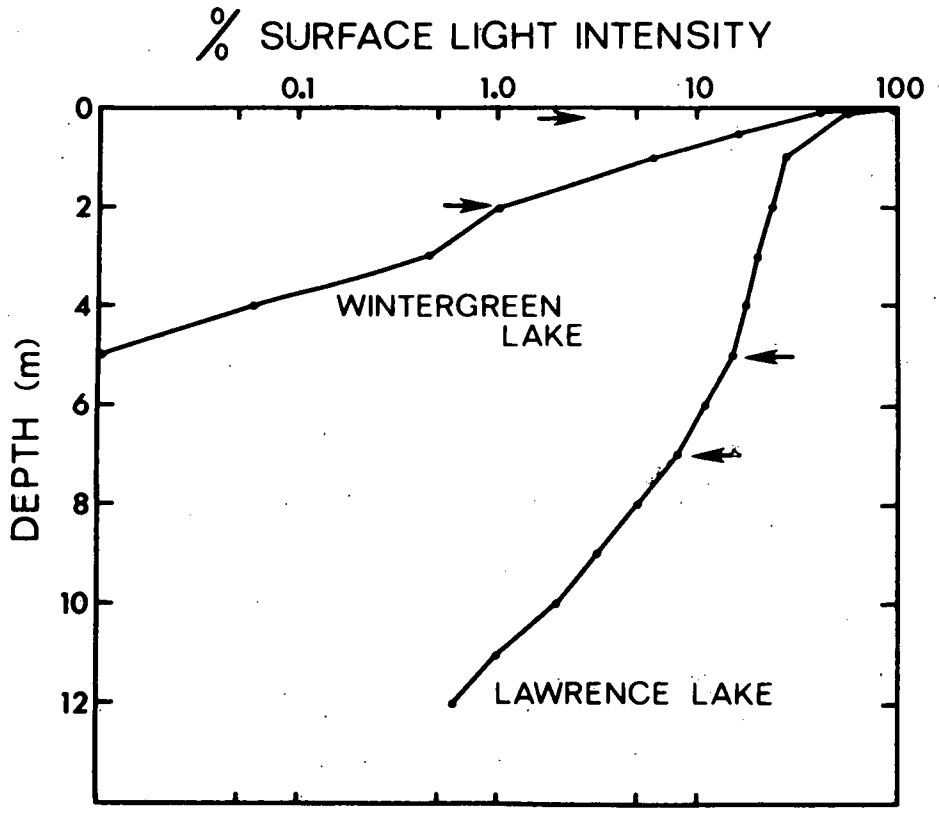


Figure 8. Light penetration in Lawrence and Wintergreen lakes as percentage surface light intensity during maximum development of summer blue-green algal populations. Arrows delineate strata occupied by blue-green algae.

that photosynthetic rates saturated between 800 and 1400 Lux (Figure 9). Frequently, these populations are more efficient than others occurring higher in the water column at other times of the year in terms of carbon fixed/unit chlorophyll a/photosynthetically active light (cf. Wetzel, 1975, p. 343).

Wintergreen Lake

Occurrence of Blue-green Algae:

The temperature profile of Wintergreen Lake, like that of Lawrence, was characteristic of a dimictic, north temperate lake (Figure 10). However, unlike Lawrence Lake, the epilimnion was much narrower, approximately 2-3 meters. Populations occurring within the epilimnion were somewhat appressed to the surface as a result. Blue-green algal populations within this system occurred in the epilimnion from late June through July and until about mid-August. During 1975, the dominant form present was Aphanizomenon flos-aquae intermingled with Microcystis aeruginosa (identified as Aphanizomenon flos-aquae (L.) Ralfs and Microcystis aeruginosa Kütz. emend. Elenkin by Moss, 1973). Other years, however, species of Anabaena have been the dominant nitrogen-fixing algae (cf. Duong, 1972).

Sources of Nitrogen:

Concentrations of NH_4-N in Wintergreen Lake were much higher and more dynamic than in Lawrence, ranging from negligibly measurable amounts to over 1500 µg NH_4-N/l in the upper strata (Figure 11).

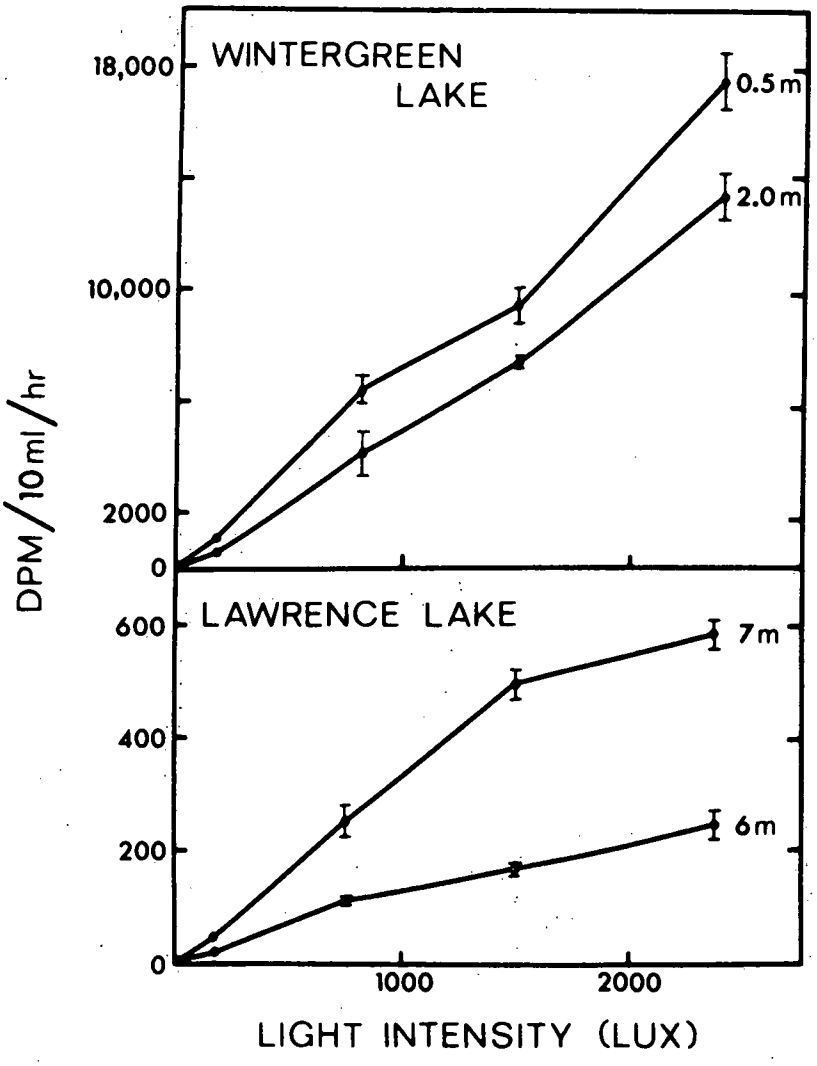


Figure 9. Saturation curves for photosynthesis for blue-green algal populations in Lawrence and Wintergreen lakes (± S.D.).

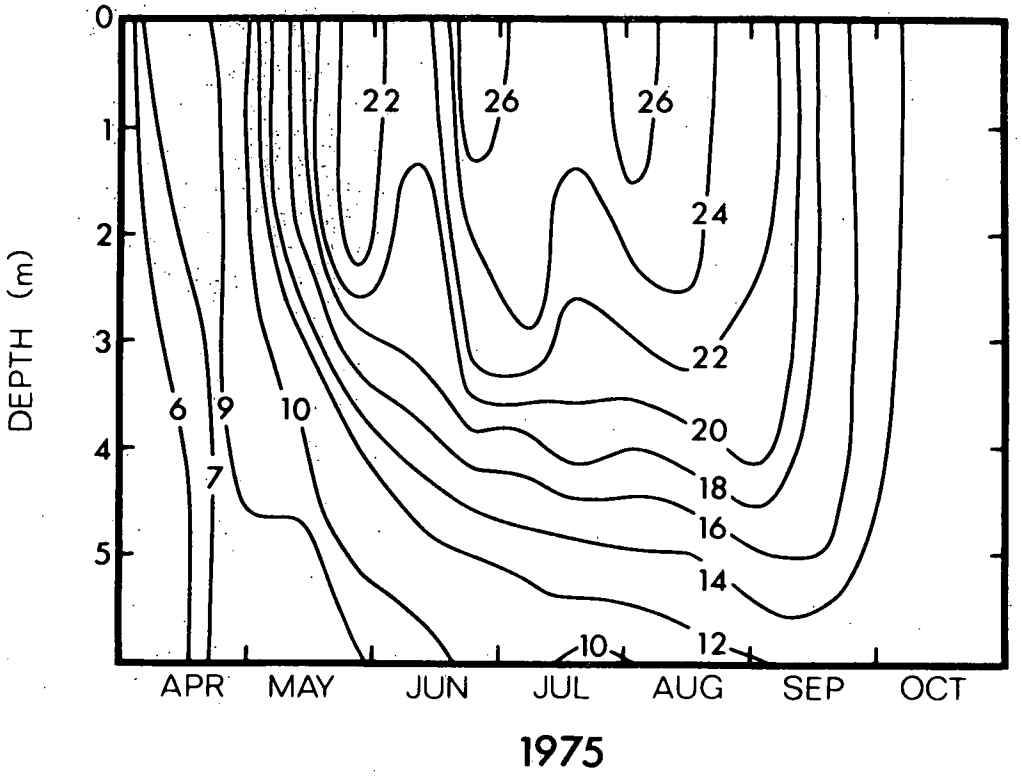


Figure 10. Depth-time diagram of isopleths of temperature (^{o}C) in Wintergreen Lake, Michigan, 1975.

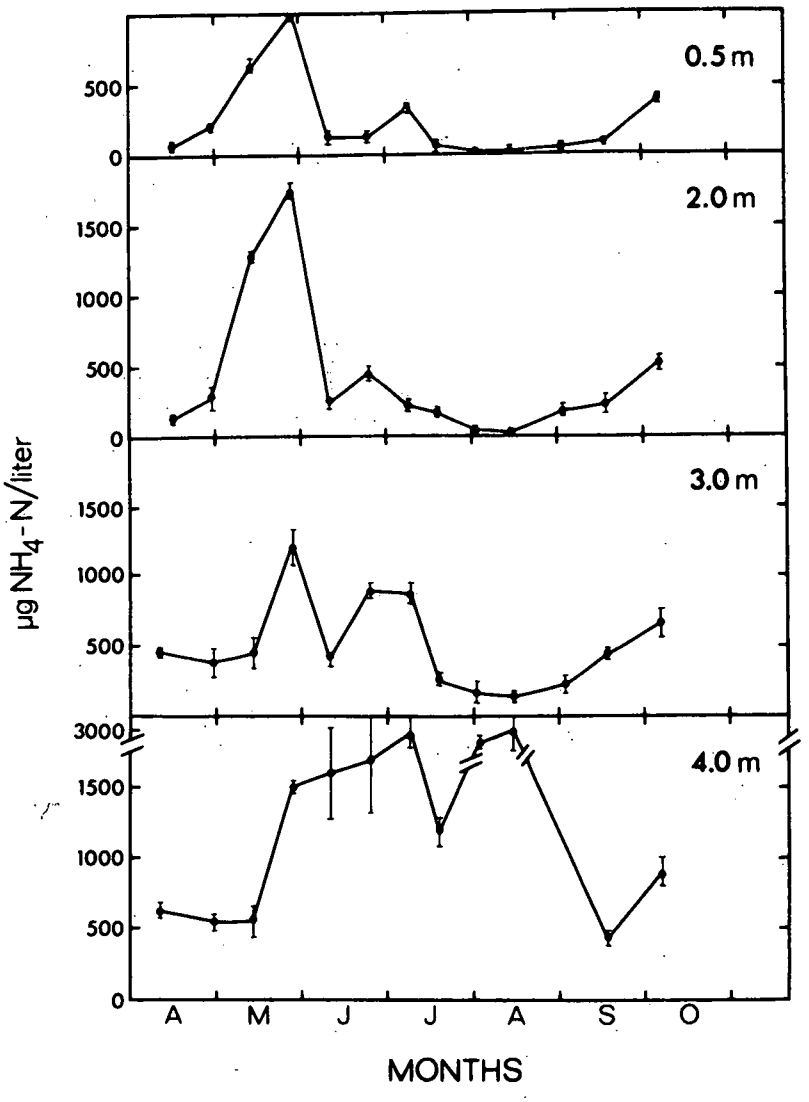


Figure 11. Concentrations of NH_4-N (µg/l, ± S.D.) at four depths in Wintergreen Lake, Michigan, 1975.

Ammonium concentrations remained high just below the epilimnion from early May through mid-September with maximum values of 3000 µg/l during the latter part of July and August, probably due to decomposing blue-green algal populations sedimenting out of the upper strata. Rates of primary production were generally inversely proportional to NH_4-N concentrations in the upper two meters (cf. Figures 11 and 12). The first values on the graph were obtained from samples taken in April about one week after ice-off. The depletion of ammonium between ice-off and the first sampling date was correlated with a dense diatom population. The diatoms decreased precipitously and were immediately followed by a population of predominately unicellular green algae in late April which was correlated with a decrease in NO_3-N concentrations. By early May, NO_3-N had become immeasurable in the water column and the predominant combined inorganic nitrogen source in the system was NH_4-N. The increase in NH_4-N in May at all depths occurred simultaneously with low productivity rates and presumably was a result of the rapid decomposition of the previous green algal populations and mineralization of algal protein. Shortly thereafter, other populations of predominantly green algae (Schroederia spp. and Scenedesmus spp.) became established in late May and early June. By June 20, Aphanizomenon flos-aquae was present in the phytoplankton; however, it was not until July that maximum blue-green populations occurred, concomitant with high nitrogen fixation rates (Figure 12) and low levels of NH_4-N.

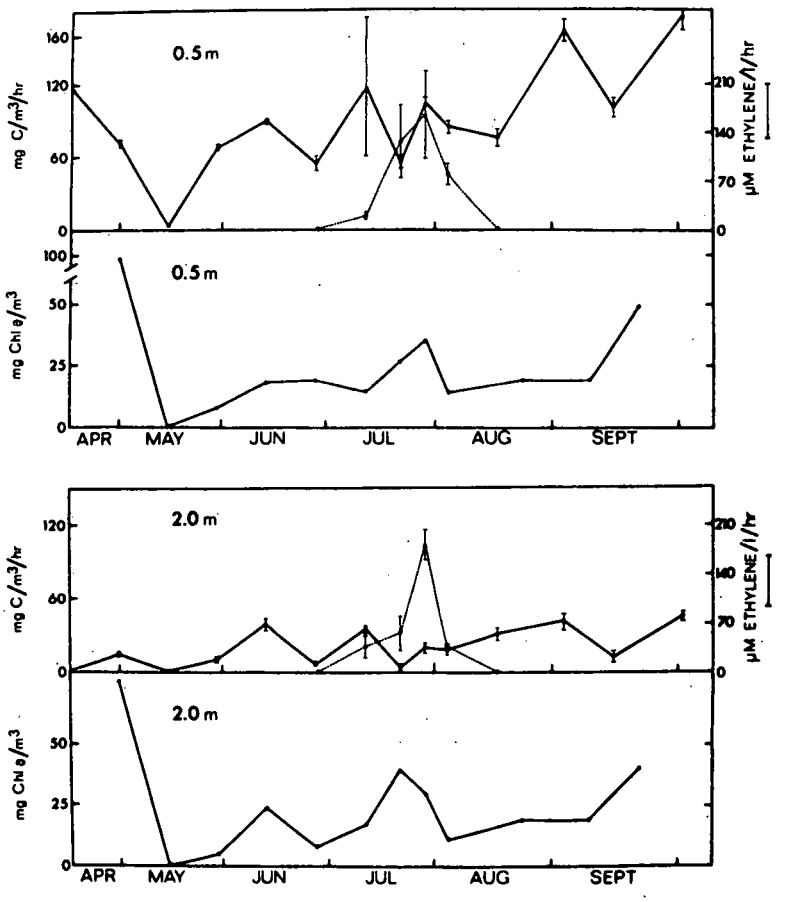


Figure 12. Concentrations of chlorophyll a (mg/m^3) in situ rates of primary production (mg $C/m^3/hr$, ± S.D.), and rates of acetylene reduction (µM ethylene/l/hr., ± S.D.) at 0.5 and 2.0 meters in Wintergreen Lake, 1975.

Light Regime:

Unlike the blue-green populations in Lawrence Lake, those in Wintergreen were located in a water stratum appressed to the surface of the lake. In this manner the populations were exposed to an array of light intensities ranging from surface light intensities to less than 1.0% surface light intensity during peak bloom periods (Figure 8). Chlorophyll a concentrations and temperature did not differ markedly between 0.5 and 2.0 meters, indicating that the water and phytoplankton within this stratum were well-mixed, probably from wind-generated turbulence. In addition, samples taken from the upper and lower portions of the epilimnion were both high light adapted (Figure 9), indicating that the phytoplanktonic community within these upper strata were not exposed to low light intensities for prolonged periods of time.

Growth Rates, Nitrogen Source, and Light Intensity

In studies designed to simulate various in situ light regimes, Aphanizomenon flos-aquae and Microcystis aeruginosa were inoculated into medium with different nitrogen sources and grown in batch culture under continuously high, variable, and continuously low light. With Aphanizomenon flos-aquae, inocula from N_2-fixing cultures were transferred to experimental flasks with N_2-N or combined inorganic nitrogen (NO_3-N or NH_4-N). With Microcystis aeruginosa, inocula for experimental flasks were from a stock culture grown with NO_3-N. In this manner, slower growth rates with N_2-N or NO_3-N grown cultures could not be attributed to lag time necessary for the synthesis of nitrogenase or

nitrate reductase. Growth rates, k (doubling of cell carbon/day), were calculated from the following equation (Stein, 1973):

$$k = \frac{\log N_1/N_0}{t_1 - t_0} \cdot 3.322$$

At all light intensities and with both species, NH_4-N resulted in higher k values than NO_3-N or N_2-N; Microcystis aeruginosa had higher k values on a given nitrogen source than Aphanizomenon flos-aquae (Table 1). Nitrate as a nitrogen source resulted in intermediate k values and N_2-N the lowest. With Aphanizomenon flos-aquae, no growth occurred at continuously low light with N_2-N as the only nitrogen source. This characteristic was variable among the nitrogen-fixing species tested. Cultures of isolates of Anabaena flos-aquae grew very slowly at continuously low-light levels. Nitrate and ammonium always elicited higher photosynthetic rates/unit biomass at continuously low light than N_2-N.

During log phase growth, NH_4-N grown cells resulted in higher photosynthetic rates/unit biomass (DPM/mg cell C/hr) than cells grown with other nitrogen sources (Figures 13 and 14). With Microcystis aeruginosa, this increase became apparent within one to two hours after NO_3-N grown cells were inoculated into medium with NH_4-N. The photosynthetic rates/unit biomass of Aphanizomenon flos-aquae increased markedly throughout log phase growth under all light regimes. With Microcystis aeruginosa, photosynthetic rates were more variable over the growth phase at high and variable light, although growth with NH_4-N at continuously low light resulted in slight increases (Figure 14).

Table 1. Growth rates (k)* of Aphanizomenon flos-aquae and Microcystis aeruginosa grown with three nitrogen sources and under three light regimes: High (continuously 1500-1800 Lux), Variable (see Figure 3), and Low (continuously 150-200 Lux).

Species	Light Regime	Nitrogen Source	k
Aphanizomenon flos-aquae	High	N_2-N	0.42
		NO_3-N	0.76
		NH_4-N	0.81
	Variable	N_2-N	0.32
		NO_3-N	0.44
		NH_4-N	0.46
	Low	N_2-N	0.0
		NH_3-N	0.16
		NH_4-N	0.23
Microcystis aeruginosa	High	NO_3-N	0.89
		NH_4-N	0.91
	Variable	NO_3-N	0.50
		NH_4-N	0.51
	Low	NO_3-N	0.22
		NH_4-N	0.24

* k = doublings of cell carbon/day
See Results for equation.

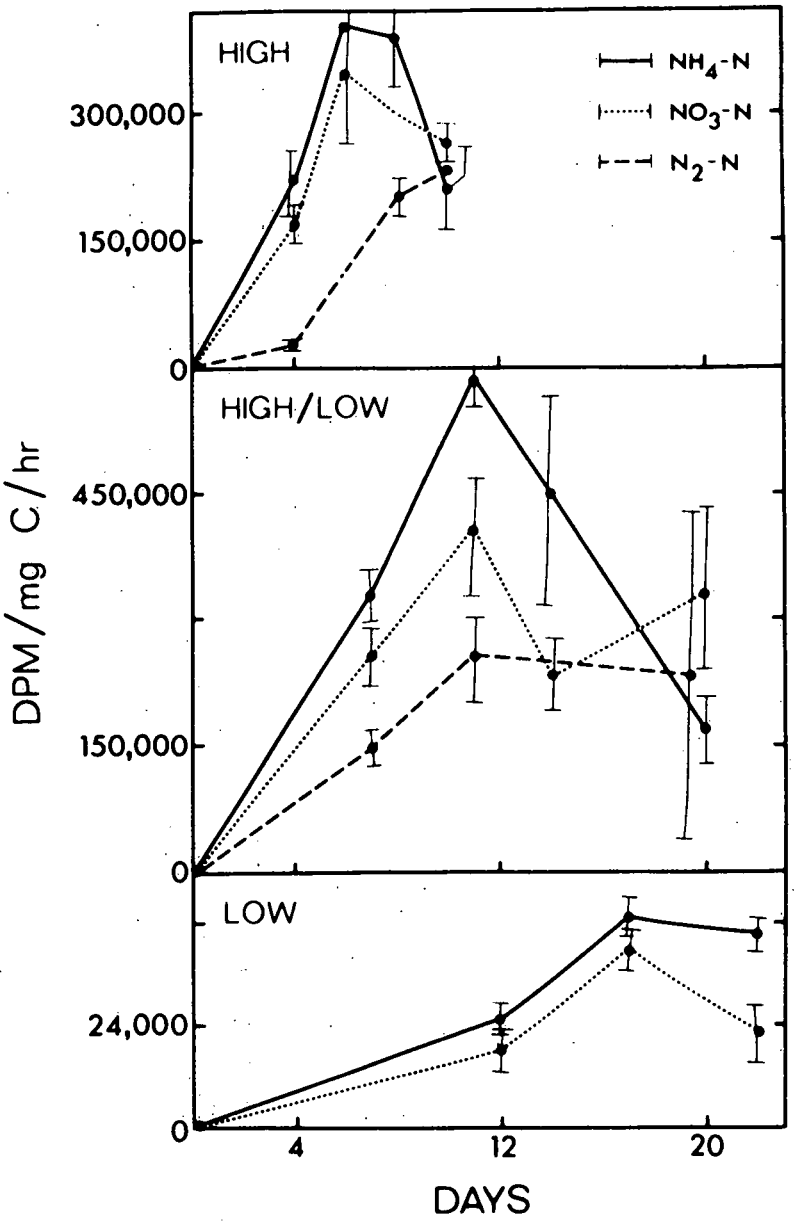


Figure 13. Changes in photosynthetic activity/unit biomass (DPM/mg C/hr) with growth for cultures of Aphanizomenon flos-aquae grown with N_2-N, NO_3-N, and NH_4-N at continuously high, variable and continuously low light intensities (± S.D.).

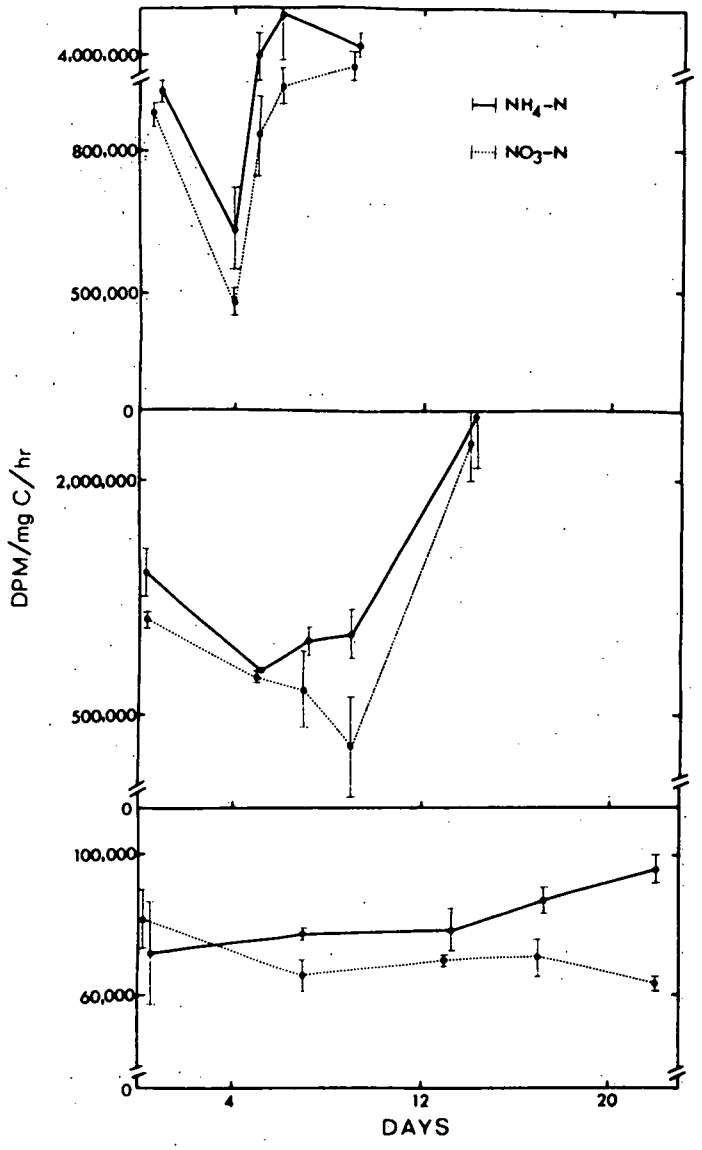


Figure 14. Changes in photosynthetic activity/unit biomass (DPM/mg C/hr) with growth for cultures of Microcystis aeruginosa grown with NO_3-N and NH_4-N at continuously high, variable and continuously low light intensities (± S.D.).

Although NH_4-N resulted in higher growth rates and photosynthetic rates, less cumulative cellular carbon was produced at continuously high and variable light regimes. This effect probably resulted from a greater decrease in photosynthetic rates after cessation of log phase growth among NH_4-N grown cultures.

C:N Ratios, Nitrogen Source, and Light Intensity

Differences in carbon and nitrogen contents of cells from cultures used in the growth experiments are presented as differences in C:N ratios (Table 2). A significant difference among C:N ratios was not evident with changing light regime from samples taken early in the morning (in contrast to changes in C:N ratios as the day progressed with cultures grown under different light regimes; see below). However, nitrogen source did affect the C:N ratio in that a higher nitrogen content, reflected in a lower C:N ratio, resulted from growth on NH_4-N as compared to NO_3-N and N_2-N with both species and at all light intensities. A general trend of decreasing C:N ratio was also evident as growth progressed through log phase, particularly with Aphanizomenon flos-aquae.

Changes in Rates of Assimilation of Carbon and Nitrogen with Changes in Light Intensity

Nitrogen fixation (acetylene reduction) saturated at lower light intensities than photosynthesis (Figure 15) with cultures of Aphanizomenon flos-aquae. Data from Figure 12, presented as assimilation ratios of carbon fixation/nitrogen fixation (acetylene reduction),

Table 2. Changes in C:N ratios throughout growth with Aphanizomenon flos-aquae and Microcystis aeruginosa grown with three nitrogen sources and under three light regimes: High (continuously 1500-1800 Lux), Variable (see Figure 3), and Low (continuously 150-200).

		Aphanizomenon flos-aquae C:N			Microcystis aeruginosa C:N	
		N_2-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N
High	T_2	7.5	5.1	4.9	4.8	4.6
	T_3	4.8	4.4	4.2	4.9	4.6
	T_4	5.0	4.7	4.3	4.7	4.3
	T_5	5.2	4.8	4.2	4.9	4.9
	$\bar{x}$ =	5.6	4.8	4.4	$\bar{x}$ = 4.8	4.6
Variable	T_2	7.5	6.0	6.7	4.7	4.0
	T_3	7.4	5.5	4.7	4.9	4.7
	T_4	-	5.5	5.0	5.2	4.5
	T_5	5.9	4.9	4.7	4.4	4.8
	$\bar{x}$ =	6.9	5.5	5.3	$\bar{x}$ = 5.8	4.5
Low	T_2	-	6.6	6.0	4.3	4.5
	T_3	-	5.3	4.9	5.0	4.8
	T_4	-	5.7	4.9	4.5	4.4
	T_5	-	-	-	4.8	4.6
	$\bar{x}$ =	-	5.9	5.2	$\bar{x}$ = 4.6	4.5

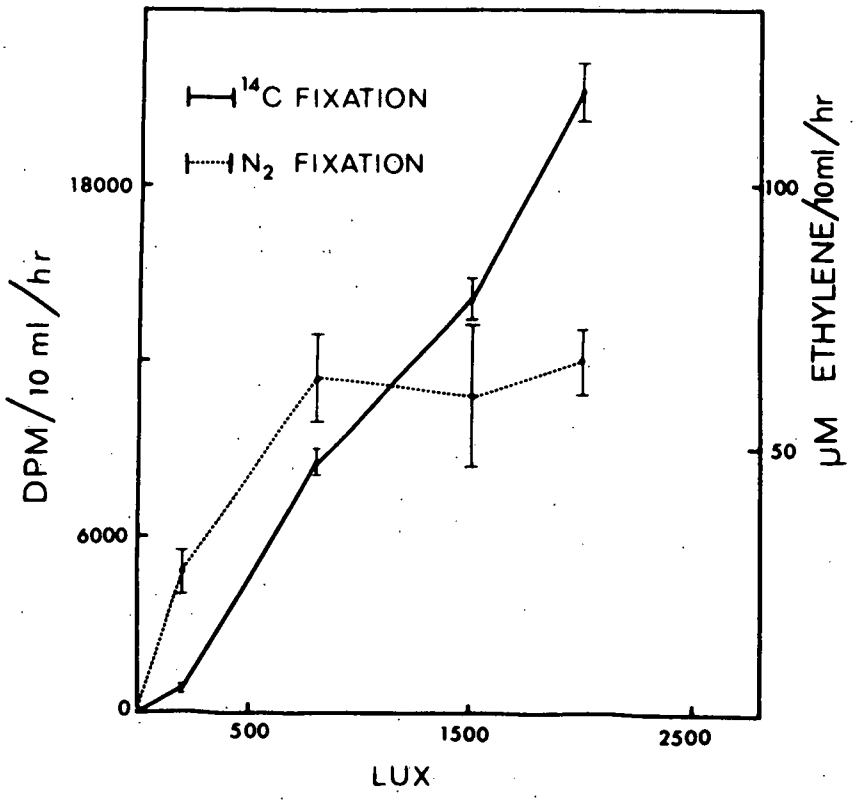


Figure 15. Increase in relative rates of carbon fixation (DPM/10 ml/hr, ± S.D.) and N_2-fixation (µM ethylene/10 ml/hr ± S.D.) with increasing light intensities in cultures of Aphanizomenon flos-aquae.

indicated a similar relationship with natural populations of Aphanizomenon flos-aquae with depth in Wintergreen Lake (Figure 16). That is, because of different responses to light intensities, carbon fixation rates decreased more rapidly than nitrogen fixation rates.

Cultures of N_2-fixing Aphanizomenon flos-aquae and Anabaena flos-aquae kept in the dark overnight and then exposed to four different light intensities reflected this difference in assimilation ratios by a greater increase in cellular carbon relative to cellular nitrogen at higher light intensities (Table 3). This effect resulted in striking differences in C:N ratios among the light treatments (Figure 17). Differences in C:N ratios became apparent after 4 hours and continued for the duration of the experiment, approximately 10 hours. The increasing C:N ratios with exposure to higher light was the result of increasing cellular carbon relative to increasing cellular nitrogen, rather than a decrease in cellular nitrogen. With Aphanizomenon flos-aquae, a decrease in the C:N ratio occurred after approximately 8 hours exposure to low light intensities due to decreases in cellular carbon rather than an increase in cellular nitrogen (Table 3).

Cultures of Microcystis aeruginosa, grown with NO_3-N, reflected differences in assimilation ratios with changing light intensities similar to that of the nitrogen-fixing species (Table 3). The similar disparity in C:N ratios resulted between cultures exposed continuously to low and high light intensities (Figure 18). This disparity was not as great as that with N_2-N grown cultures, and Microcystis grown on NO_3-N continued to increase in cellular carbon at the lowest light intensity; whereas, N_2-fixing cultures did not.

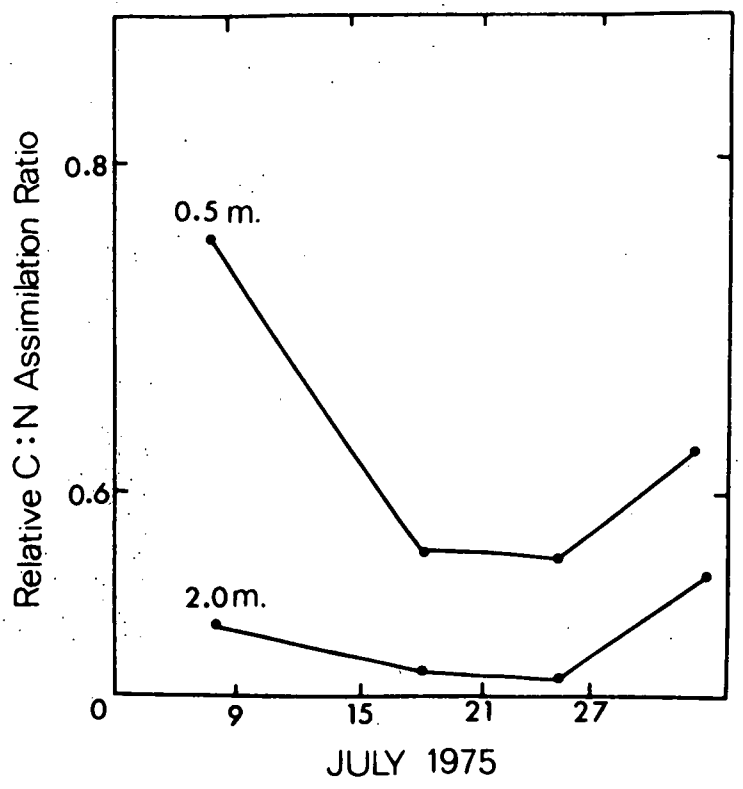


Figure 16. Photosynthetic carbon fixation/nitrogen fixation assimilation ratios at 0.5 and 2.0 meters in Wintergreen Lake. Values were calculated from data presented in Figure 12.

Table 3. Changes in cellular carbon and nitrogen content in N_2-fixing cultures of Aphanizomenon flos-aquae and Anabaena flos-aquae exposed to continuous light of four different intensities (L_1 = 2000 Lux; L_2 = 1300 Lux; L_3 = 800 Lux; L_4 = 150 Lux). Changes in cellular carbon and nitrogen content in cultures of Microcystis aeruginosa grown NO_3-N and NH_4-N and exposed to continuous light of two different intensities (L_1 = 2000 Lux; L_4 = 150 Lux). T_1 = initial time of exposure; T_2 = time after 10 hours of exposure with Aphanizomenon flos-aquae and Anabaena flos-aquae; and after 12 hours exposure with Microcystis aeruginosa.

Species	$\bar{x}$ T_1 mg C/l n=4	$\bar{x}$ T_1 mg N/l n=4	$\bar{x}$ T_2 mg C/l n=4	$\bar{x}$ T_2 mg N/l n=4
Aphanizomenon flos-aquae (N_2-N)	L_1 2.10 L_2 2.29 L_3 2.14 L_4 2.18	L_1 0.37 L_2 0.42 L_3 0.38 L_4 0.39	L_1 3.02 L_2 3.25 L_3 2.62 L_4 2.06	L_1 0.45 L_2 0.51 L_3 0.46 L_4 0.38
Anabaena flos-aquae (N_2-N)	L_1 4.58 L_2 4.57 L_3 4.47 L_4 4.67	L_1 0.96 L_2 0.95 L_3 0.91 L_4 0.95	L_1 6.34 L_2 5.95 L_3 5.15 L_4 4.86	L_1 0.97 L_2 0.96 L_3 0.93 L_4 0.96
Microcystis aeruginosa (NO_3-N)	L_1 0.78 L_4 0.46	L_1 0.15 L_4 0.08	L_1 1.50 L_4 0.78	L_1 0.22 L_4 0.14
Microcystis aeruginosa (NH_4-N)	L_1 1.02 L_4 1.03	L_1 0.21 L_4 0.20	L_1 1.94 L_4 1.28	L_1 0.37 L_2 0.27

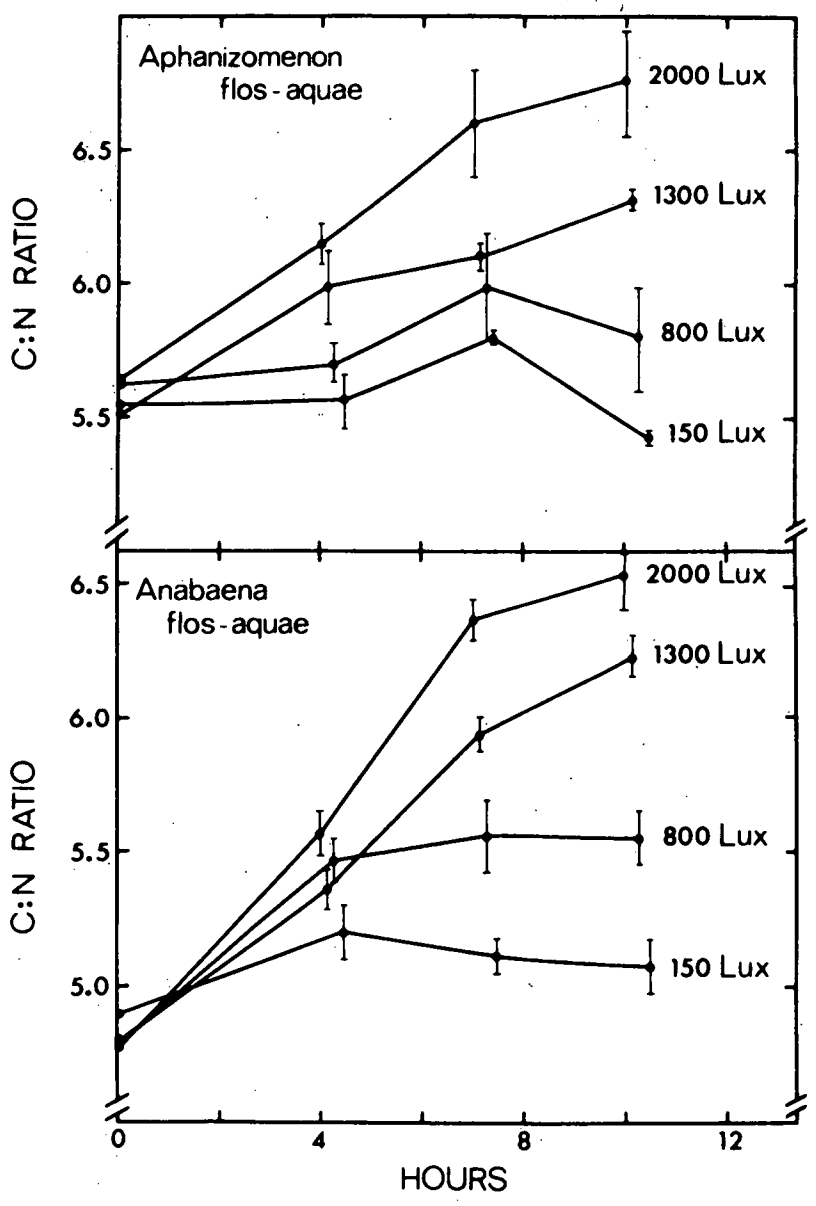


Figure 17. Changes in C:N ratios of N_2-fixing cultures of Aphanizomenon flos-aquae and Anabaena flos-aquae exposed to continuous light of four different intensities (± S.D.). Cultures were sampled during early log phase growth.

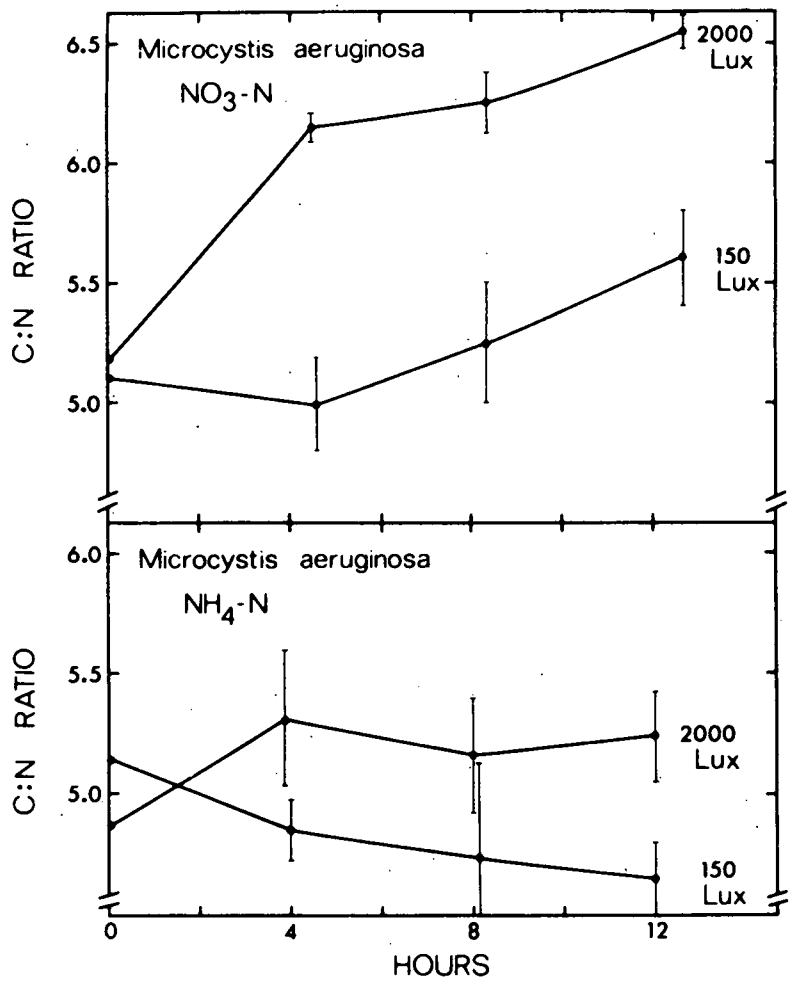


Figure 18. Changes in C:N ratios in cultures of Microcystis aeruginosa grown with NO_3-N and NH_4-N and exposed to continuous light of two different intensities (± S.D.). Cultures were sampled during early log phase growth.

In contrast to results with N_2-N and NO_3-N, Microcystis grown on NH_4-N displayed much less of a disparity between carbon and nitrogen assimilation at high and low light intensities as reflected in C:N ratios (Figure 18). Hence, the carbon and nitrogen content remained more uniform with changes in light intensity. The decrease in C:N ratio at low light with NH_4-N grown cultures, unlike the N_2-fixing cultures, was the result of a greater increase in cellular nitrogen relative to cellular carbon at that light intensity (Table 3).

Photosynthetic rates/unit cell carbon differed with nitrogen source at continuously low light (Figure 19). Cultures of Microcystis aeruginosa grown with NH_4-N resulted in higher photosynthetic rates than those grown with NO_3-N. Further, rates of carbon fixation did not decrease over an 8 hour period with these nitrogen sources. However, N_2-fixing cultures of Aphanizomenon flos-aquae and Anabaena flos-aquae transferred from higher light intensities to low light showed an immediate decrease in photosynthetic rates, undoubtedly a factor in the decrease in cellular carbon over the same period.

When exposed to continuously high light, however, carbon fixation rates of N_2-fixing cultures of Aphanizomenon flos-aquae and Anabaena flos-aquae decreased over an 8 hour period (Figure 20). The decrease in photosynthetic rates was more pronounced with the denser cultures of Anabaena flos-aquae (4-5 mg cell carbon/l) compared to the cultures of Aphanizomenon flos-aquae (2-3 mg cell carbon/l). The decrease in photosynthetic rates at continuously high light was ameliorated to some extent if cultures were exposed intermittently to a lower light intensity

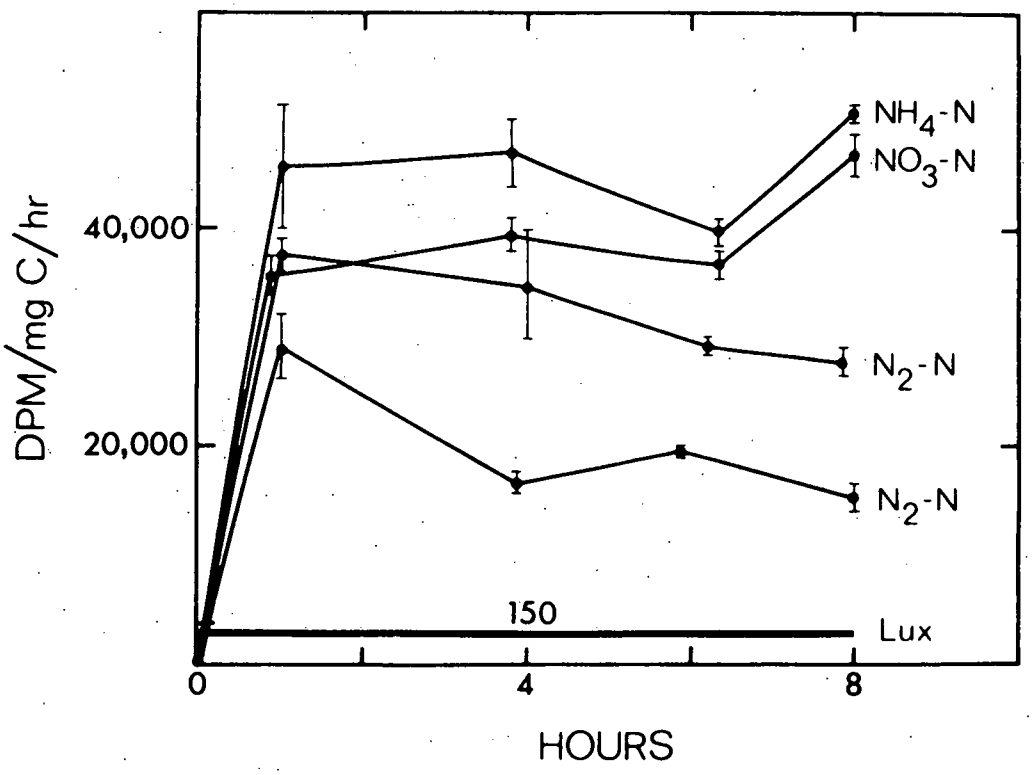


Figure 19. Changes in photosynthetic rates/unit biomass (DPM/mg C/hr, ± S.D.) with NO_3-N and NH_4-N grown cultures of Microcystis aeruginosa (upper two lines) and N_2-fixing cultures of Aphanizomenon flos-aquae (third line) and Anabaena flos-aquae (fourth line) when exposed to continuously low light (150 Lux). Cultures were sampled during early log phase growth.

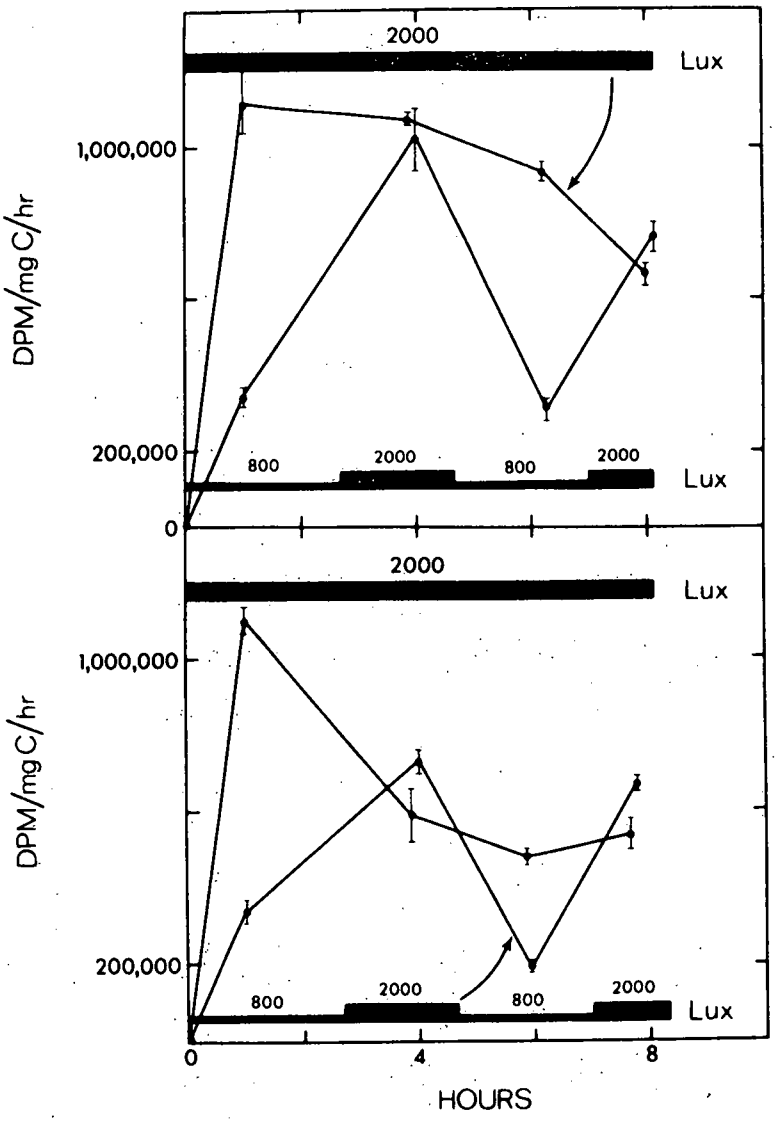


Figure 20. Changes in photosynthetic rates/unit biomass (DPM/mg C/hr, ± S.D.) with N_2-fixing cultures of Aphanizomenon flos-aquae (upper) and Anabaena flos-aquae (lower) when exposed to continuously high vs. intermittent light intensities (2000 and 800 Lux). Cultures were sampled during early log phase growth.

(Figure 20), as would theoretically be the case in circulating epilimnetic water. This alternation was particularly effective with the denser cultures of Anabaena flos-aquae.

Differences in Photosynthetic Response with Changing Light Regime

Photosynthesis of these species of blue-green algae became saturated at a lower light intensity (800-1000 Lux) when grown at continuously low light than those grown at variable or continuously high light (Figures 21, 22, and 23). Cultures of Aphanizomenon flos-aquae and Microcystis aeruginosa, grown under the variable light regime saturated at intensities similar to those of cultures grown under continuously high light (cf. Figure 22 with Figures 23 and 24).

Cultures of Microcystis aeruginosa, once adapted to a high or low light regime, would not re-adapt rapidly whether grown with NO_3-N or NH_4-N (Figures 23 and 24). In Figure 23 (upper portion), cultures of Microcystis were grown under continuously high light prior to assays of photosynthetic response to different light intensities (arrow indicates light intensity at which cultures were grown). Cultures were then transferred to a continuously low light regime (lower portion of Figure 23; arrow indicates the light intensity to which cultures were transferred). Re-examination of photosynthetic response after 47 hours (including two dark cycles of 14 hours) indicated a similar saturation curve, although fixation rates/unit cell carbon had increased markedly. Similarly, cultures grown at continuously low light (T_1, Figure 24) and then

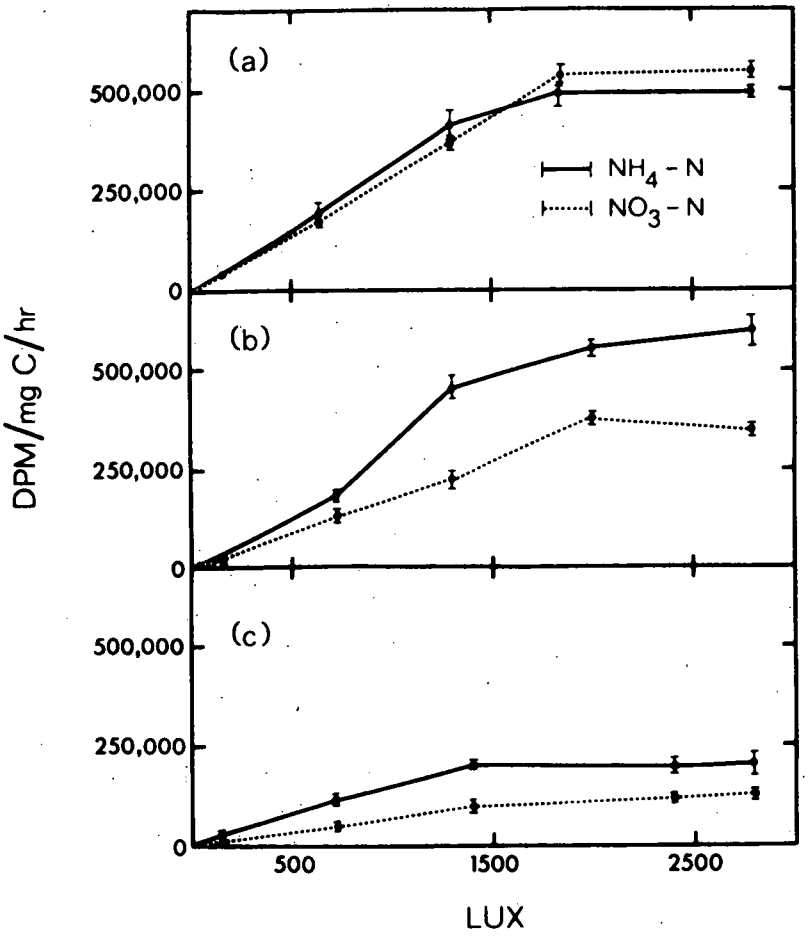


Figure 21. Saturation curves for photosynthesis for NO_3-N and NH_4-N grown cultures grown at continuously low light intensities (150-180 Lux) for a) Aphanizomenon flos-aquae, b) Anabaena flos-aquae (A-52), and c) Anabaena flos-aquae (A-113-s-q-a) (± S.D.). Cultures were sampled during early log phase growth.

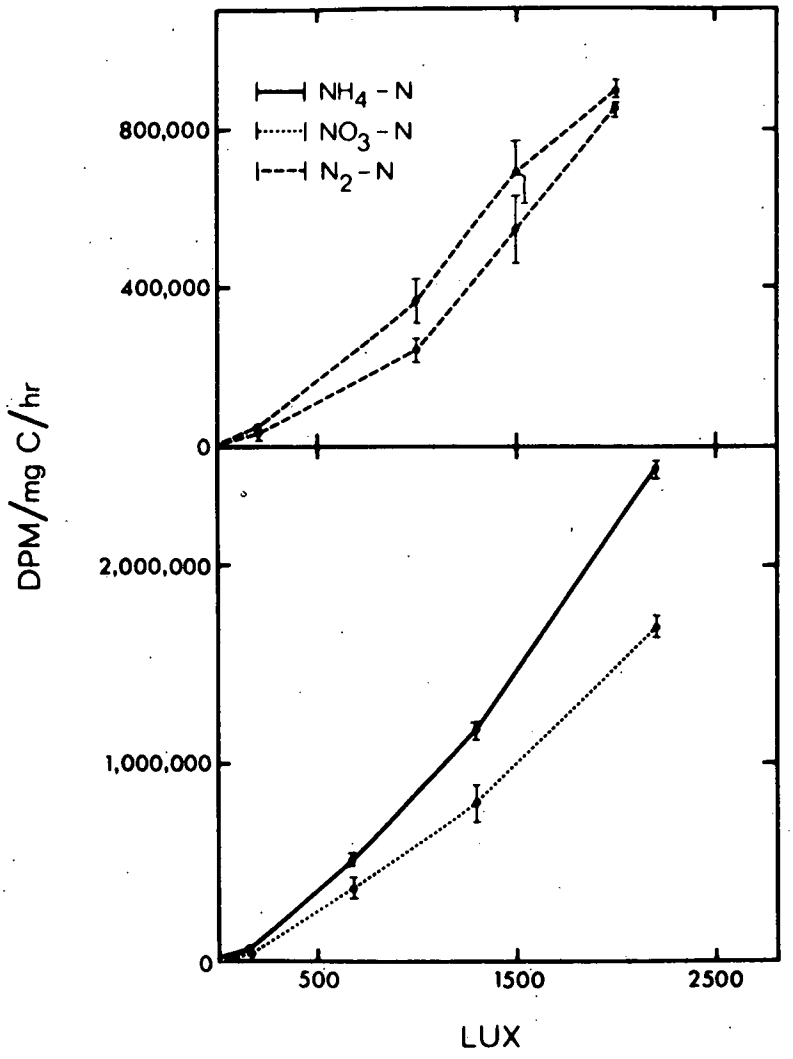


Figure 22. Saturation curves for photosynthesis for N_2-N grown cultures of Aphanizomenon flos-aquae (upper graph) grown under variable (top line) or continuously high light (lower line) and for NO_3-N and NH_4-N grown cultures of Microcystis aeruginosa (lower graph) grown under variable light intensities (± S.D.). Cultures were sampled during early log phase growth.

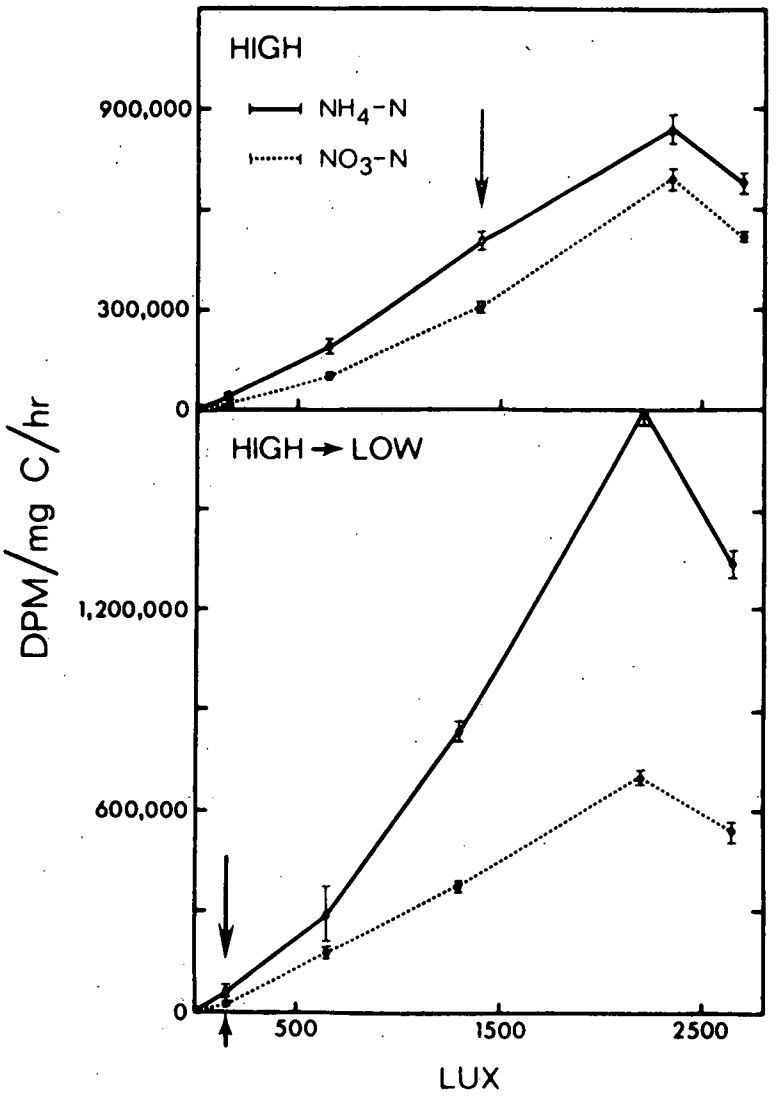


Figure 23. Saturation curves for photosynthesis for NO_3-N and NH_4-N grown cultures of Microcystis aeruginosa grown under continuously high light (upper graph; arrow indicates light intensity cultures grown under) and after 47 hours of exposure to continuously low light (lower graph; arrow indicates light intensity to which cultures were transferred) (±S.D.). Cultures were sampled during early log phase growth.

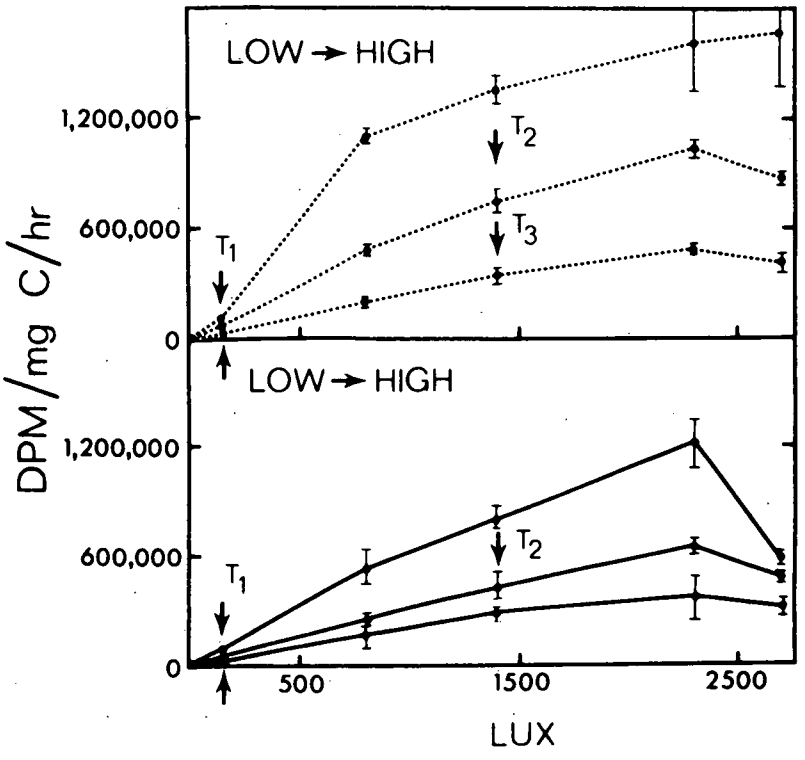


Figure 24. Saturation curves for photosynthesis for NO_3-N and NH_4-N grown cultures of Microcystis aeruginosa grown under continuously low light (T_1; arrow indicates light intensity under which cultures were grown); after 27 hours of exposure to a high light regime (T_2; arrow indicates light intensity to which cultures were transferred), and after 47 hours of exposure to a high light regime (T_3) ($\pm$ S.D.). Cultures were sampled during early log phase growth.

transferred to continuously high light showed little modification in saturation curves when examined after 27 hours (corresponding to T_2 on the graph and including one 7 hour dark period) and 47 hours (corresponding to T_3 on the graph and including two 7 hour dark periods). However, there was a significant decrease in photosynthetic rate/unit cell carbon.

Molecular Weight Fractionation of Released Dissolved Organic Carbon

From 36 to 70% of released dissolved organic carbon from axenic cultures of Microcystis aeruginosa and Anabaena flos-aquae (G-R) were filterable through Amicon UM 05 membranes and, hence, in the molecular weight range of less than 500 Daltons (Tables 4 and 5). Filtrates from NO_3-N-grown cultures exposed to high light intensities were of lower molecular weight than those grown at low light. In addition, NH_4-N-grown cultures produced a larger percentage of lower molecular weight compounds than NO_3-N-grown cultures. Nitrogen-fixing cultures of Anabaena flos-aquae (G-R), in contrast, tended to produce a larger percentage of lower molecular weight compounds when exposed to low-light intensities.

Table 4. Molecular weight fractionation of dissolved organic carbon released from cultures of Microcystis aeruginosa grown with NO_3-N and NH_4-N and exposed to high (1500-1800 Lux) and low (150-200 Lux) light intensities. % Total ^{14}C refers to the percentage DPM of total dissolved organic carbon filtered through glass fiber filters. C.V. = coefficient of variance.

Nitrogen Source	Light Intensity	Fraction	% Total	(C.V.)
NO_3-N	High	PM 30	72.5	(27.1)
		PM 10	70.5	(27.7)
		UM 2	54.9	(17.6)
		UM 05	47.9	(22.8)
NH_4-N	High	PM 30	87.5	(1.8)
		PM 10	84.4	(1.2)
		UM 2	73.3	(0.4)
		UM 05	69.6	(-)
NO_3-N	Low	PM 30	87.1	(13.2)
		PM 10	91.1	(11.4)
		UM 2	54.0	(14.9)
		UM 05	41.9	(5.0)
NH_4-N	Low	PM 30	96.7	(4.2)
		PM 10	90.3	(3.5)
		UM 2	55.1	(10.3)
		UM 05	46.9	(27.1)

Table 5. Molecular weight fractionation of dissolved organic carbon released from cultures of Anabaena flos-aquae (G-R) grown with N_2-N and exposed to high (1500-1800 Lux), medium (800 Lux) and low (150-200 Lux) light intensities. % Total refers to the percentage DPM of total dissolved organic ^{14}carbon filtered through glass fiber filters. C.V. = coefficient of variance.

Nitrogen Source	Light Intensity	Fraction	% Total	(C.V.)
N_2-N	High	PM 30	52.5	(4.6)
		PM 10	33.3	(5.6)
		UM 2	38.3	(14.9)
		UM 05	35.8	(2.2)
N_2-N	Medium	PM 30	53.4	(4.5)
		PM 10	55.7	(5.4)
		UM 2	38.7	(6.1)
		UM 05	37.1	(6.5)
N_2-N	Low	PM 30	70.7	(2.9)
		PM 10	76.3	(7.6)
		UM 2	52.7	(10.1)
		UM 05	48.9	(0.3)

DISCUSSION

Overview

Highest growth rates and photosynthetic rates/unit biomass resulted with all isolates tested when grown with NH_4-N in batch culture at either continuously high, variable, or continuously low light intensities. Growth rates with NO_3-N and N_2-N were significantly less. It is not surprising, then, that blue-green algae are frequently found in close proximity to relatively high concentrations of NH_4-N as with the metalimnetic populations in Lawrence Lake or where turnover rates of NH_4-N would be rapid as in eutrophic systems such as Wintergreen Lake. Nitrate could serve as an alternative nitrogen source to NH_4-N in the metalimnion of Lawrence Lake, since adequate growth rates were maintained at continuously low light with all isolates tested in culture. The absence of large assemblages of blue-green algae in the NO_3-N rich, but NH_4-N poor upper strata in Lawrence Lake is less easily explained, but could be related to a competitive disadvantage under conditions of higher light with NO_3-N as the main nitrogen source as compared to other algal groups. In addition, other nutrients, besides NH_4-N, could be depleted in the upper strata of the lake by late summer. For example, alkaline phosphatase activity is very high in these upper strata (Wetzel, unpublished data), indicating a phosphorus demand. In that context, one could predict that if the epilimnion became more nutrient rich at that

time of year in Lawrence Lake, a substantially larger volume of the lake would be occupied by blue-green algae.

With the depletion of both NO_3-N and NH_4-N in the photic zone of Wintergreen Lake, nitrogen-fixing species have a competitive advantage over other algal groups. The proximity of these populations to the surface could be very important in maintaining growth, since periodic exposure to high light intensities was necessary for some N_2-fixing isolates. The narrow nature of the epilimnion as well as the large surface area in Wintergreen Lake (conducive to rapid mixing), are ideal for maintenance of the N_2-fixing populations within an array of light intensities daily. Presence of gas vacuoles in this circumstance may serve more to maintain populations within the general upper strata, thereby preventing sinking into the hypolimnion, than aligning cells to a specific light gradient. During calmer periods, however, populations may become more appressed to the surface and, hence, be exposed to continuously high light intensities.

Rapid turnover rates of NH_4-N in the epilimnion of Wintergreen Lake, associated with the decomposition and mineralization of dissolved and particulate matter of high protein content, could provide the nitrogen source for the non-nitrogen fixing species such as Microcystis aeruginosa, which occur concomitantly (but in fewer numbers) with the nitrogen-fixing forms. Examination of K_m values for NH_4-N for Microcystis aeruginosa and other species offers a potentially fruitful area of future research in this regard.

Nitrogen Source and Growth Rates

The above hypothetical overview of events deserves closer scrutiny in the context of other studies. Kapp et al. (1975), in comparing 60 organic and inorganic nitrogen sources, found that NH_4Cl supported highest growth rates with Agmenellum quadruplicatum, a marine blue-green alga, under conditions of pH and NH_4-N concentrations similar to this study (pH 8.2, 9.6 mg N/l). Therefore, toxicity or poor growth reported previously may, in some cases, be an artifact of culture conditions in which NH_4-N was supplied at artificially high concentrations (Winkenbach et al., 1972), the medium was not sufficiently buffered, or when high cellular yields produced harmful side-effects under culture conditions. Although use of laboratory cultures is frequently subject to criticism for engendering artificial conditions not applicable to natural populations, it is relevant in this study that NH_4-N was supplied at concentrations lower than in many culture studies at 5.0 mg NH_4-N/l. Also, pH of the medium was rigorously buffered to simulate the well-buffered properties of the lakewater in which natural populations occurred. Furthermore, conditions of growth resulted in cell yields during log phase growth within range of that found in some lakewaters (1.0 to 4.0 mg cellular carbon/l in cultures compared to 5.0 to 6.0 mg particulate carbon/l in Wintergreen Lake during periods of blue-green predominance). Conditions within the euphotic zone in lakes during summer stratification resemble aspects of continuous cultures and batch cultures. For example, Healey and Hendzel (1976) found that certain cellular constituents in natural populations of Aphanizomenon flos-aquae fluctuated in

a manner more similar to batch cultures than continuous cultures. However, short-term culture experiments were performed during early log phase growth in this study, when nutrients would not be limiting. For example, cellular nitrogen content of cells (mg N/l) during early log phase growth was always well below inorganic nitrogen concentrations (mg N/l) available in the growth medium.

The phenomenon of better growth with NH_4-N as a nitrogen source is not universal among algal groups, although generally NH_4-N will be utilized preferentially to NO_3-N (Morris, 1974). Moss (1973) found that only 4 out of 13 freshwater species of non-blue-green algae grew better (doublings/day) with NH_4-N than NO_3-N. Two of the four, isolates of Chlamydomonas reinhardii and Euglena gracilis (eutrophic forms), grew only with NH_4-N and not at all with NO_3-N. Moss' study makes a particularly useful comparison to this one since similar growth media were employed. Therefore, blue-green algae as well as eutrophic forms of non-blue-green algae seem particularly well adapted to growth with NH_4-N. Variations in growth rate in response to different nitrogen sources have also been reported with marine phytoplankton (Antia et al., 1975). Urea and ammonium generally resulted in best growth in 25 isolates tested.

An additional reason for slower growth rates with NO_3-N could be related to apparent blue-light inhibition of induction of nitrate reductase (Stevens and Van Baalan, 1974). The action spectrum for nitrite production (i.e., nitrate reductase) indicated a peak at 680 nm (Stevens and Van Baalan, 1973). However, induction of nitrite production was inhibited in the range of 430-480 nm, although nitrite production would

continue at a high rate if induction had occurred previously in cells exposed to white light. Reports of a decrease in nitrate reductase activity with depth in ocean systems (e.g., Eppley et al., 1970) could be related to the selective attenuation of red light and consequent predominance of blue light with depth. Predominant light with depth in hardwater Gull Lake, however, was in the green range (540-560 nm), as was that supplied by Vita-lite bulbs in culture experiments (Figure 4). The spectral composition of Vita-lite bulbs resembled most closely that at 2.0 meters in Gull Lake with some selective absorption of red light occurring relative to surface light. Further absorption of light in the red and blue range would occur in deeper water. Therefore, the differences in growth rates between NO_3-N and NH_4-N grown cultures in this study may represent minimum differences in that further attenuation of red light could result in inhibition of induction of nitrate reductase in deeper strata, where populations would not be exposed periodically to light of similar spectral composition to surface light.

Lower growth rates with N_2-N as opposed to NO_3-N or NH_4-N may be somewhat dependent on the isolate. Gentile and Maloney (1969) reported maximum growth with a nitrogen fixing isolate of Aphanizomenon flos-aquae; however, Healey and Hendzel (1976) found an apparent nitrogen deficiency among some natural populations of Aphanizomenon flos-aquae, which they attributed to insufficient phosphorus supply (Stewart et al., 1970; Stewart and Alexander, 1971). Raising concentrations of phosphorus, iron, EDTA, molybdenum, and including vitamins (Moss, 1972) did not enhance growth of N_2-fixing isolates in this study. Further, whereas

cultures of Aphanizomenon flos-aquae tended to have higher C:N ratios than that of Microcystis aeruginosa, there was some overlap of range between the two species. Also, C:N ratios of Anabaena flos-aquae and Aphanizomenon flos-aquae were lower than that reported necessary to induce heterocyst formation in Anabaena cylindrica, that is, approximately 8:1 (Kulisooriya, 1972). Therefore, although growth rates were lower with N_2-N, C:N ratios did not reflect a nitrogen deficiency.

Light Regime and Nitrogen Source

The effect of lower growth with N_2-N as a nitrogen source was most striking at continuously low light, where isolates of Aphanizomenon flos-aquae could not maintain growth. Some N_2-fixing isolates of Anabaena flos-aquae, however, would grow at this light intensity, but only very slowly. The lower limit of growth at continuously low light could be an important factor in determining which blue-green algae dominate within a system. That is, it is generally believed that both epilimnetic and metalimnetic blue-green populations develop at their respective depths in a system (Brook et al., 1971; Reynolds and Rogers, 1976) as opposed to developing within the surficial strata and "settling" down through the water column. Hence, given an equal inoculum of several varieties or species of blue-green algae among depth strata in a lake, the interactions of light intensity (directly related to depth of the epilimnion), nitrogen source, and onset of thermal stratification could determine which species or sub-species predominate. For example, in Wintergreen Lake the shallow nature of the epilimnion as well as the

lake as a whole would guarantee rather rapid exposure to higher light intensities. The distance between the nutrient-rich, but poorly lighted hypolimnetic water, and the higher light regime of the surficial waters is relatively short. This relationship may facilitate the predominance of Aphanizomenon flos-aquae in this lake during most years.

Once established, populations of blue-green algae in Wintergreen Lake are exposed to an array of light intensities diurnally. The effect of variable light intensity diurnally in cultures of Aphanizomenon flos-aquae was a lowered growth rate compared to those exposed to continuously high light. However, rates of photosynthesis/unit cell carbon measured between 1000-1200 hours at high light intensities for cultures grown under a variable light regime (cf. Figure 3) were greater than cultures grown with continuously high intensities during the same time period during the day. In addition, although 9 out of 17 hours of light supplied daily to cultures grown under a variable light regime were of low intensity, periodic exposure to high or medium intensities resulted in positive growth among N_2-fixing cultures not able to grow under continuously low light. Therefore, with natural populations, mixing within the upper water strata could result in enhanced photosynthetic response during periods of exposure to high light intensity as well as a carbon and energy supply during brief periods of exposure to low intensities. This general hypothesis is supported by the photosynthetic response of N_2-fixing cultures of Aphanizomenon flos-aquae exposed for several hours to continuously high, variable, or continuously low light.

An increase in the rates of photosynthesis/unit cell carbon during log phase growth was a characteristic of Aphanizomenon flos-aquae, but not of Microcystis aeruginosa. Rates of photosynthesis/unit cell carbon were highly variable during growth for Microcystis grown under continuously high or variable light intensities and uniform for cultures grown under continuously low light. These data agree with those of Fallon (personal communication) and Konopka and Brock (in press) for changes in photosynthetic efficiencies of natural populations of Aphanizomenon flos-aquae and Microcystis aeruginosa during bloom conditions in Lake Mendota. Although intriguing, the consequences to success or sequence of blue-green populations are, for the most part, unexplained.

As in Lake Deming (Brook et al., 1971), the metalimnetic blue-green populations in Lawrence Lake are active photosynthesizers rather than senescent populations settling out of the epilimnion. Among other factors, the higher concentrations of inorganic nitrogen as well as the greater depth of the epilimnion contribute to inhibit the development of nitrogen-fixing populations. The development of colonial and unicellular forms in Lawrence Lake as opposed to non-nitrogen-fixing filamentous forms is more problematic, but could be related to differences in the concentration and availability of nutrients in the hypolimnion, with higher concentrations supporting the filamentous forms such as Oscillatoria. In that case colonial and unicellular forms would reflect a less productive system, as is the case of Lawrence Lake.

Assimilation Rates of Carbon and Nitrogen with Changing Light Intensity

Aside from growth rates and photosynthetic responses, a further consideration with respect to light regime and nitrogen source is the difference between assimilation rates of carbon and nitrogen with changing light intensities. This difference would be most applicable with the N_2-fixing populations of Wintergreen Lake, since cultures of Aphanizomenon flos-aquae and Anabaena flos-aquae grown with N_2-N showed a greater discrepancy between carbon and nitrogen content with changing light intensity than did cultures grown with either NO_3-N or NH_4-N. Vertical migration through a gradient of light intensities daily may result in more balanced growth for N_2-fixing populations (Peterson et al., 1977), although the term "balanced" is not physiologically explicit. However, in this study, cultures of blue-green algae had a generally lower C:N ratio during the most rapid period of growth as was also the case with natural populations of Aphanizomenon flos-aquae (Healey and Hendzel, 1977).

Adaptation to High and Low Light Intensities

All species grown in culture became low light adapted when grown at continuously low light and exhibited saturation curves similar to those of natural populations in Lawrence Lake. Cultures grown under variable or continuously high light regime exhibited saturation curves similar to the high-light adapted populations in Wintergreen Lake.

The saturation curves for low- and high-light adapted cultures appeared to resemble the "Cyclotella" adaptive type described by Jørgensen (1969) and Steemann-Nielsen and Jørgensen (1968) in that initial slopes of the curves were alike, but the light-saturated rate for high-light adapted cells was higher than that for low light adapted ones. Adaptation in the "Cyclotella" type was, then, primarily via enzymatic processes. However, unlike the "Cyclotella" type, cultures of Microcystis aeruginosa did not adjust rapidly (up to 47 hours) to other light regimes, once adapted to either high or low light. Because of this effect, the blue-green algal populations in Lawrence Lake would not be expected to respond in the same manner as high-light adapted cells, even if they were exposed to light intensities encountered in the upper strata in the lake. Similarly, periodic exposure to high light may enable populations in Wintergreen Lake to remain high-light adapted and, hence, able to respond to higher light intensities with considerably higher rates of photosynthesis than low-light adapted populations.

Molecular Weight Fractionation of Released Dissolved Organic Carbon

Fractionation by Amicon ultrafiltration of dissolved organic carbon released by cultures of Anabaena flos-aquae (G-R) and Microcystis aeruginosa produced intriguing results in that light intensity as well as nitrogen source affected the molecular weight of the released compounds. Besides providing a substrate for aquatic bacterial populations, there is increasing evidence that released organic substances from

blue-green algal populations affect the succession of algal populations as well (Keating, 1977, 1978). Further qualification of the nature of these substances offers another relevant and potentially fruitful area for future investigation.

CONCLUSIONS

Populations of blue-green algae in Lawrence and Wintergreen lakes are particularly well-adapted to the light and nitrogen conditions which occur in these two divergent habitats. Presumptive evidence is presented that metalimnetic populations of blue-green algae in Lawrence Lake utilize NH_4-N as a nitrogen source, although NO_3-N is also available in abundant supply. In this manner, these populations are able to maintain maximum growth rates under conditions of continuously low light. Among other factors, the morphometry of the lake basin and the general trophic status of the entire Lawrence Lake system interact to restrict populations to this stratum, hence suppressing potential productivity of these populations. Since productivity rates associated with the metalimnetic populations already contribute significantly to the annual phytoplanktonic productivity, enhancement of growth of the planktonic blue-green algae by increased rates of decomposition in the hypolimnion or enrichment of the epilimnion could markedly increase the annual primary productivity of the entire open water portion of the system.

In highly productive Wintergreen Lake, on the other hand, high-light adapted nitrogen-fixing populations can be maintained in the surficial waters. Because of the shallow nature of the epilimnion, phytoplankton are well-mixed in the upper strata and, hence, are exposed to an array of light intensities during growth. The consequences of

intermittent exposure to high and low light intensities are advantageous in maintaining a more uniform carbon and nitrogen content within the cell, which is more important to N_2-fixing populations exposed to different light intensities than to NO_3-N or NH_4-N utilizing populations. In addition, in spite of rapid attenuation of light with depth, populations can remain high-light adapted, hence responding maximally to higher light intensities when intermittently exposed. In this context, the populations could be viewed as opportunistic, which undoubtedly is a competitive advantage in a habitat subject to rapid change such as Wintergreen Lake.

The nature of the dissolved organic compounds released by phytoplanktonic populations is of particular interest, since these compounds are a direct link between the primary producers and the bacterial-detrital component and, hence, the ecosystem as a whole. All evidence from these culture studies indicate a substantial portion of the dissolved organic compounds released, with all nitrogen sources and at all light intensities, is of low molecular weight and probably readily utilizable by bacteria. This finding was particularly true of organic carbon released from cultures grown with NH_4-N and that from N_2-fixing cultures exposed to low light intensities.

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