error (Table 1), the corrections for any error would make only minor shifts in the position of our sea-level line (Fig. 1). After 22,000 years B.P. the counting error is rather small.

Figure 1 contrasts our proposed sea levels with previous curves. Our data indicate that at approximately 36,000 years B.P. sea level stood at the present-day shoreline (0 m). From 36,000 until 22,500 years B.P. there was a lowering of the sea to between −10 and −20 m. We lack data on the maximum low stand, which presumably occurred at 18,000 to 19,000 years B.P.

From 17,000 to 10,000 years B.P. sea level climbed from about −60 to −22 m in relation to present sea level. This transgression is much shallower than that indicated by other curves. Some of Curray's dates (*9*, pp. 254-255) support our interpretation (Table 1). Other data in the literature suggest a sea level as shallow as the one proposed. A salt-marsh sediment off Texas falls on our transgression line (*5*) and a curve used by Richards (*10*, p. 8) for North America coincides with the younger end of our line around 10,000 years B.P.

The proposed sea levels apply specifically to the South Carolina continental shelf area. Shelves in other areas may have had different histories, particularly shelves adjacent to glaciated areas. Our data do agree well, however, with fixed dates for materials obtained off Delaware, the west coast of Florida, and the Gulf of Mexico. The South Carolina shelf area has apparently been tectonically stable over the last 30,000 years, and the proposed sea levels should be applicable to the entire southeastern United States. Compared to other sea-level curves, our data indicate that substantially less ice was present from 17,000 to 10,000 years B.P. Our data strongly suggest that the late Wisconsinan maximum regression was not as profound as has been indicated in the literature. Within the time span represented by our sea-level data, a low stand as great as 100 m or more would require catastrophic rates of regression and transgression.

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Nitrogen-Fixing *Anabaena*: Physiological Adaptations Instrumental in Maintaining Surface Blooms

Abstract. *Both laboratory and in situ studies indicate that the nitrogen-fixing blue-green nuisance algae* Anabaena *spp. have developed adaptive means of dominating surface lake waters. During the dramatic diurnal shifts in surface light intensity and oxygen saturation accompanying blooms,* Anabaena *can overcome oxygen toxicity by sequential optimization of carbon dioxide and nitrogen fixation and by pigment alteration. These mechanisms allow optimal utilization of the radiant energy while minimizing competition for photoreductant between two main energy-demanding processes.*

Various hypotheses have been proposed to explain the dominance of blue-green algae in the surface waters of nutrient-rich lakes (*1*). The ability of some species to fix elemental N_2 while occupying surface waters that are rich in radiant energy, nitrogen-poor, and highly oxygenated is a distinct advantage over eucaryotic organisms. Occupying such a region, however, is not without its constraints: the processes of carbon (CO_2) and nitrogen (N_2) fixation are sensitive to O_2; high light intensity and O_2 concentrations can lead to photooxidative inactivation of photosynthetic pigments (*1*). We report here on a number of mechanisms, including temporal separation of CO_2 and N_2 fixation and pigment alteration, that various species of *Anabaena* use to cope with such constraints.

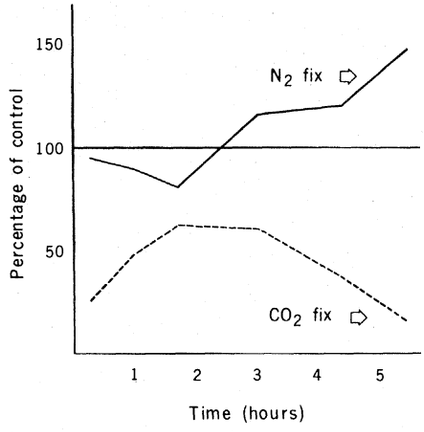

Fig. 1. Responses of N_2 fixation (acetylene reduction (——) and CO_2 fixation (-----) under O_2-supersaturated (pO_2 = 0.4 atm) conditions as percentages of the control (pO_2 = 0.2 atm) values. All O_2-supersaturated samples were prepared at the start of the experiment. At hourly intervals duplicate 30-minute acetylene reduction (nitrogenase activity) and $^{14}CO_2$ fixation assays were conducted on samples taken from the total pool of O_2-supersaturated and control samples.

In examining blue-green algal growth in eutrophic lakes, few studies have incorporated the vast amount of physiological information now available from laboratory studies. A majority of such studies have been carried out on axenic cultures of nonbloom-forming *Anabaena cylindrica* (*2*), rendering ecological interpretations of physiological mechanisms difficult. Nevertheless, among various species of *Anabaena*, the biochemical uniformity of N_2 and CO_2 fixation processes has become apparent (*3*).

In our studies we examined and compared several physiological responses of natural populations and axenic batch cultures of *Anabaena* to elevated O_2 concentrations and light levels. Our main objective was to ascertain how N_2 and CO_2 fixation in *Anabaena* respond to O_2-supersaturated conditions and if these responses promote the dominance of *Anabaena* in surface waters.

Results from studies in two small eutrophic lakes, Lake Rotongaio on the North Island of New Zealand and Thompson Lake near Toronto, Ontario,

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are discussed here. Both lakes are phosphorus-enriched and slightly alkaline (pH = 8.0 to 8.5) and both have strong thermal stratification throughout the summer months; these conditions are conducive to the growth of N_2-fixing blue-green algae (*2*). During sampling times, approximately 95 to 99 percent of the epilimnetic phytoplankton biomass was *Anabaena spiroides* in Thompson Lake, and *A. oscillarioides* and *A. circinalis* in separate blooms in Lake Rotongaio. Analysis of pigments for chlorophyll a, carotenoids, and phycocyanin was performed on GF/C filtered material. We used a dimethyl sulfoxide extraction (*4*) followed by spectrophotometric scans of chlorophyll a and carotenoids. We extracted phycocyanin from cold (4°C), sonicated cell material, using 0.1*M* phosphate buffer at pH 7.8 for 3 hours. We estimated the nitrogenase activity (NA) by the acetylene reduction method as described and modified by Flett *et al.* (*5*). On several occasions the ratios between acetylene reduction and actual N_2 fixation were determined by parallel $^{15}N_2$ uptake experiments. In experiments on CO_2 fixation, conducted concurrently with the acetylene reduction assays, we used the ^{14}C method (*6*). Since the pH values of lake water were high, we exposed filters containing ^{14}C-labeled algae to concentrated HCl fumes to remove any precipitated ^{14}C compounds. No loss of ^{14}C-labeled organic compounds occurred. We determined the biomass of phytoplankton by counting at least 400 filaments of *Anabaena* (*7*) on acetone-cleared Millipore filters, estimating cellular volume and converting to carbon by the regression method of Mullin *et al.* (*8*). During laboratory and in situ incubations, O_2 and temperature were monitored (Yellow Springs Instruments model 54 ARC DO). Photosynthetically active radiation was determined with a photometer-radiometer (Li-Cor LI-185). All in situ incubations were done on cloudless days.

We tested the effects of elevated oxygenation on N_2 and CO_2 fixation in the laboratory by displacing air with O_2-enriched air (partial pressure of oxygen, pO_2 = 0.4 atm) in the headspace of a 25-ml sealed serum bottle containing 15 ml of axenic *Anabaena oscillarioides* culture. We chose *A. oscillarioides* for these experiments because it is common in lakes and rivers and can be easily maintained in axenic culture (*9*). After headspace displacement, the O_2-enriched cultures showed 140 percent saturation while controls (air headspace) exhibited 100 percent saturation. Both saturated and supersaturated levels were commonly encountered during natural blooms. All laboratory cultures were incubated under "cool white" fluorescent lights at 2000 lux and 20°C.

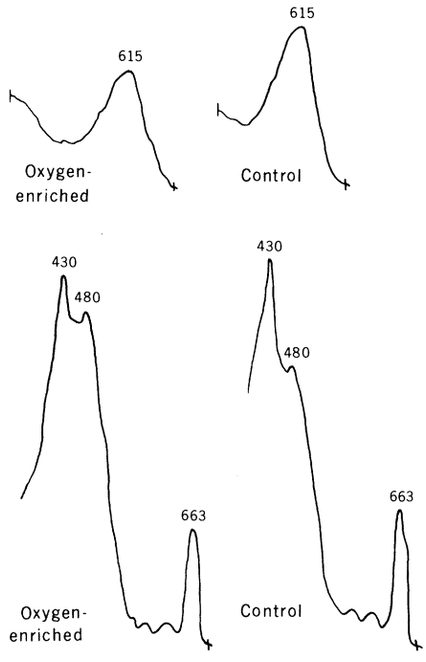

Fig. 2. Spectrophotometric scans of phycocyanin (615 nm) (top) and chlorophyll a (430 and 663 nm) and carotenoid (480 nm) (bottom) extracts from *Anabaena oscillarioides*. The peak heights of pigment absorbances are directly proportional. All samples were incubated under light for 12 hours. Control samples remained near 100 percent O_2 saturation while O_2-enriched samples remained near 150 percent O_2 saturation.

After O_2 enrichment in laboratory experiments, NA was initially depressed but eventually recovered to rates exceeding those of controls (Fig. 1) while CO_2 fixation remained depressed. During the latter stages of the experiment when NA recovered, CO_2 fixation decreased. The O_2 remained at the initial concentrations throughout the experiment.

Earlier work demonstrated that the ability of O_2-supersaturated *Anabaena* to restore NA after initial depression was light-mediated (*10*); dark incubation resulted in the sustained inhibition of N_2 fixation. Furthermore, neither $10^{-7}M$ or $10^{-6}M$ additions of DCMU [3(3,4-dichlorophenyl),1-dimethylurea], a specific photosystem II inhibitor, had little effect on NA recovery while CO_2 fixation under illumination was essentially blocked. When $10^{-7}M$ and $10^{-6}M$ DCMU were added to natural samples with 120 percent O_2 supersaturation and incubated in situ in Thompson Lake, CO_2 fixation was inhibited 63 and 98 percent, respectively, relative to that of controls; NA was not affected. The results implicate a photosystem I means of NA recovery. This recovery, often resulting in higher rates of NA than in controls, was sustained under continually elevated O_2 conditions for as long as 24 hours provided the cells were illuminated. Although *Anabaena* forms special cells called heterocysts to shield nitrogenase from O_2 inactivation (*2*), it is clear that these cells are not completely effective under O_2-supersaturated conditions. Hence, it appears necessary for this alga to use additional means to overcome long periods of O_2 supersaturation.

Consistent with functional changes in the photosystems, changes in pigment composition were also evident (Fig. 2). Phycocyanin, an accessory pigment associated with O_2-evolving photosystem II, decreases when O_2 supersaturation and concomitant inhibition of photosystem II activity are maintained. If O_2 elevation is sustained for at least 12 hours, the cellular carotenoid content increases while the chlorophyll a content remains unchanged. Carotenoids function primarily to transfer light energy to chlorophyll a but can also protect the cell from photooxidation which occurs under high O_2 conditions and high light intensity.

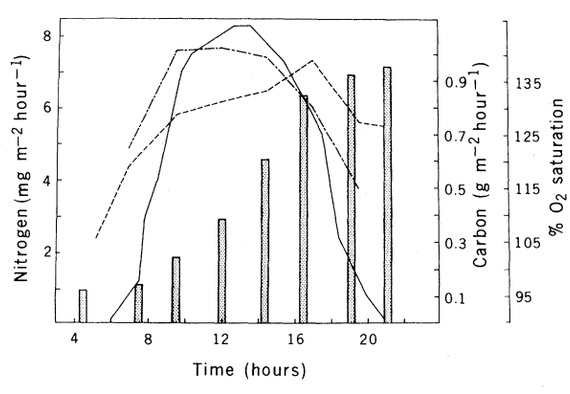

Fig. 3. Diurnal patterns of photosynthetic CO_2 fixation (·---·), photosynthetically available radiation (——), N_2 fixation (----), and O_2 saturation (bars) integrated per square meter in Thompson Lake. The curve for photosynthetically available radiation has been left dimensionless since it illustrates mainly the daily distribution of incoming solar radiation during CO_2 and N_2 fixation measurements. These measurements were conducted every 2 hours at four near-surface depths in the water column.

This protective mechanism has been demonstrated in the cyanophyte *Gloeocapsa* (*11*).

Our laboratory findings complement field observations made in Thompson Lake. As the bloom of *Anabaena spiroides* proceeds, accompanied by increased O_2 supersaturation, the ratio of carotenoid to chlorophyll a increases. The phycocyanin content tends to decrease, a phenomenon serving two purposes. (i) Phycocyanin has been shown to be a source of N_2 under conditions of nitrogen stress (*12*). (ii) The decrease in this pigment aids in minimizing photosynthetic O_2 evolution.

An additional mechanism by which *Anabaena* can maintain optimal CO_2 and N_2 fixation in O_2-supersaturated lake water is through a temporal separation of these two processes. In the field as in the laboratory, CO_2 fixation appears more sensitive to O_2 effects than N_2 fixation. If integrated under a square meter of lake surface, maximum CO_2 fixation rates consistently precede maximum N_2 fixation rates as well as maximum O_2 supersaturation (Fig. 3). The afternoon depression in CO_2 fixation could not be linked to a decrease in the inorganic carbon pool, since no significant changes in this pool occurred on a diurnal basis. Furthermore, an inorganic carbon limitation was not demonstrated by bioassays (*13*). The total inorganic carbon was 15 to 20 mg liter^{-1} in both lakes.

Sequential optimization of CO_2 and N_2 fixation, which can be maintained for several months in natural *Anabaena* populations, has a number of advantages. By separating the optimal rates of two light-driven processes in time, competition for photogenerated reductant between both processes is minimized. A higher proportion of photogenerated reductant is allocated to CO_2 fixation during morning than afternoon hours. As CO_2 fixation shows signs of afternoon depression, NA plays an increasingly important role as a recipient for photogenerated reductant. In effect, during the afternoons N_2 fixation acts as a reductant sink, accepting increasing shares of photoreductant as CO_2 fixation becomes less able to utilize photosynthetically active radiation. The afternoon decrease in CO_2 fixation reduces O_2 evolution, which aids in minimizing further O_2 inhibition of N_2 fixation. Finally, adequate supplies of newly fixed carbon skeletons are assured in order to accept fixed nitrogen.

Earlier work has shown that, per unit biomass, a diatom community that does not fix N_2 had patterns and efficiencies of CO_2 fixation similar to *Anabaena*, being similarly inhibited during the afternoon (*14*). The ability of *Anabaena* to divert photoreductant to N_2 fixation pathways during this inhibited period therefore represents much more efficient use of the total daily energy input. Furthermore, 20 to 30 percent of the daily *Anabaena* N_2 fixation occurs in the dark, relying on endogenous carbon pools accumulated during photosynthetic periods.

Under thermally stratified conditions, surface lake waters often become depleted of combined nitrogen, forcing phytoplankton that do not fix N_2 to greater depths where the supplies of combined nitrogen are ample but photosynthetically available radiation is not. The ability of *Anabaena* to successfully dominate surface waters stems from its capacity to utilize N_2, to deal with high light intensity and O_2 concentrations, and to maximize the efficiency of utilization of the total daily available radiant energy.

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Nanosecond X-ray Diffraction from Biological Samples with a Laser-Produced Plasma Source

Abstract. *By using 4.45-angstrom radiation generated by Cl^{+15} ions in a laser plasma and nanosecond exposures, low-angle x-ray diffraction patterns were obtained from dried rat spinal nerves and a powder of cholesterol. Three to four 400-picosecond, 45-joule pulses were required for the exposure. This new technique should have wide application in structural kinetic studies.*

X-ray diffraction has provided important and often unique information about the structure of biological macromolecules such as proteins and nucleic acids and of macromolecular assemblies such as membranes and muscles. In one area x-ray diffraction has unfulfilled potential of great significance: real-time kinetic studies of structures and macromolecules responding to well-defined stimuli. With conventional sources such as x-ray tubes exposure times are too long for kinetic studies. The use of powerful synchrotron x-ray sources has reduced the required exposure times significantly, and with the completion of the National Synchrotron Light Source at the Brookhaven National Laboratory exposure times as short as a few milliseconds may be possible in x-ray diffraction work (*1*).

In this report we present x-ray diffraction patterns of biological structures obtained with a laser plasma x-ray source. The exposure time for these patterns was of the order of 1 nsec. We believe that the laser plasma x-ray source can be developed into an effective tool for structural kinetic studies with high time resolution. The important characteristics of the source that make this application possible are its highly localized distribution in space and time combined with high spectral brightness (*2*).

If a high-power laser pulse is focused on a solid, an electron avalanche is driven by the optical field turning the solid into an ionized gas. If the laser pulse is of nanosecond duration or less, most of the energy absorbed by the plasma cannot be transported away from the focal region. As a result, very high particle temperatures are produced in a small region, and the plasma exhibits a high degree of ionization. At focused intensities of 10^{14} W/cm^2 or greater, there is significant pro-

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