

## Photoinhibition and the availability of inorganic carbon restrict photosynthesis by surface blooms of cyanobacteria

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### Abstract

When buoyant cyanobacteria form a surface bloom, they may obtain inorganic carbon from the water and the atmosphere. In this study, artificial blooms of cyanobacteria were exposed to different concentrations of CO<sub>2</sub> in the atmosphere and HCO<sub>3</sub><sup>-</sup> in the water. The conditions and photosynthetic activity within the bloom were measured with microelectrodes sensing O<sub>2</sub>, pH, and light. Net rates of photosynthesis increased with the concentration of CO<sub>2</sub> in the atmosphere (air = 350 ppm). However, even under 3,500 ppm CO<sub>2</sub>, reducing the concentration of inorganic carbon in the water reduced rates of photosynthesis. Thus, both air and water acted as sources of inorganic carbon. Bloom formation may give cyanobacteria an advantage in competition for light and inorganic carbon with other groups of phytoplankton; by placing a dense biomass close to the water surface they are able to intercept a large amount of the flux of light and inorganic carbon. The high surface pH produced in the bloom will promote influx of CO<sub>2</sub> from the atmosphere by "chemical enhancement." The obvious advantage of being close to the water surface may be offset by the risk of photoinhibition at high photon irradiance. The dense packing of colonies in the bloom furthermore caused a high local demand for inorganic carbon and consequently extreme carbon depletion. This combination of high photon irradiance and carbon limitation proved to be especially detrimental in blooms. When the availability of inorganic carbon was restricted, nonphotochemical quenching of fluorescence increased, showing some removal of excess excitation energy, but not to the extent that photodamage was prevented. Depending on the outcome of several interacting processes, surface bloom formation may be advantageous or deleterious for the cyanobacteria involved. This also depends on the environmental conditions to which cells were acclimated prior to bloom formation. Cyanobacteria have a better chance of withstanding the conditions in surface blooms if the cells were already acclimated to high average irradiance prior to floating to the surface.

Many cyanobacteria possess gas vacuoles that are composed of hollow, proteinaceous gas vesicles filled with air (*see* Walsby 1994 for a review). The low-density gas vacuoles provide the cells with buoyancy that permit larger colonial cyanobacteria to migrate upward rapidly when the water column is stable. In this way they can concentrate their biomass in the illuminated layer near the surface, increase their daily rates of photosynthesis, and gain competitive advantage over nonbuoyant phytoplankton (Humphries and Lyne 1988; Ibelings et al. 1991). If mixing is very low (or absent), the populations may rise to the surface and form a surface bloom, particularly after a period of low average photon irradiance (Walsby et al. 1992).

Bloom formation results in an abrupt change in conditions

for the cyanobacteria, resulting in temperature and desiccation stress (Ibelings and de Winder 1994; Ibelings 1996). Stress factors have been defined by Grime (1979) as external constraints on the growth rate of organisms. Most notably, however, the cells at the surface of the bloom become exposed to full sunlight that will cause photoinhibition, a reduction in the quantum yield of photosynthesis resulting from (prolonged) exposure to higher photon irradiances than those to which the cells are acclimated (Powles 1984). Irradiance stress results from absorption of photons in excess of those used for photosynthesis. Abeliovich and Shilo (1972) and Eloff et al. (1976) observed photooxidative death of cyanobacteria in surface waterblooms. Cells, however, have evolved many defensive mechanisms, which operate on different time scales, in order to prevent photodamage (*see* Baker and Bowyer 1994). Nevertheless, damage may occur if photoprotection is incomplete. Under photoinhibitory conditions excess excitation energy may be dissipated as heat, either in the antenna or the reaction center of PS2 (Krause and Weis 1991). Increased thermal energy dissipation in PS2 manifests itself as an increase in the nonphotochemical quenching of fluorescence. Fluorescence accounts for a small proportion of the light absorbed—it competes weakly with photochemistry, thermal dissipation of energy, and transfer to nonfluorescent pigments (review by Krause and Weis 1991). Quenching analysis of fluorescence (*see Materials and methods*) is a fast, noninvasive technique that permits the analysis of photoinhibition *in situ*. Demmig-Adams

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(1990) and Demmig-Adams and Adams (1992) proposed a relationship between nonphotochemical quenching and the formation of the carotenoid zeaxanthin in the xanthophyll cycle. Although cyanobacteria lack a xanthophyll cycle, they can produce zeaxanthin more slowly and do so especially when grown under high average irradiance (Ibelings et al. 1994).

The supply of inorganic carbon for photosynthesis will also be altered in a surface bloom. Inorganic carbon is present in water as dissolved  $\text{CO}_2$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_3^{2-}$ , which are linked by equilibria controlled largely by pH. When the rate of photosynthesis is greater than the combined rate of resupply of  $\text{CO}_2$  from the atmosphere and regions of higher inorganic carbon in the water, such as the hypolimnion, deviation of inorganic carbon from the air-equilibrium will inevitably occur. At a constant alkalinity, this causes the pH to increase, shifting the equilibria away from  $\text{CO}_2$  and toward  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ . The concentration of  $\text{CO}_2$  can become depleted to values close to zero, and under extreme conditions the concentration of  $\text{HCO}_3^-$  can also become markedly reduced (Talling 1976; Maberly 1996). Many phytoplankton species, including cyanobacteria, possess a carbon-concentrating mechanism (CCM) that elevates the concentration of  $\text{CO}_2$  around the photosynthetic carboxylation enzyme (Rubisco), thus reducing photorespiration (see below; Badger et al. 1994). Use of  $\text{HCO}_3^-$  as an additional source of inorganic carbon is one means by which a CCM may operate; cyanobacteria appear to be particularly efficient in this respect (Talling 1976).

Oxygen uptake in blooms in the light can occur as a result of at least three processes: dark respiration, photorespiration, and the Mehler reaction, the direct reduction of  $\text{O}_2$  by PS1, which can drive photophosphorylation (Kana 1992). In cyanobacteria, dark respiration is suppressed in the light because respiratory and photosynthetic electron transport interact closely (Scherer 1990). Photorespiration, the oxygenase activity of Rubisco, depends on the relative concentrations of oxygen and inorganic carbon, whereby a high  $\text{O}_2:\text{CO}_2$  ratio stimulates photorespiration. Photorespiration is enhanced by high irradiance; the energy dissipation that is involved helps to protect the photosynthetic apparatus against overexcitation. Previous microelectrode studies by Ibelings and Mur (1992) and by Ibelings and de Winder (1994) have shown supersaturating oxygen concentrations in surface blooms. In combination with the high surface photon irradiance the conditions suit strong photorespiration, despite the often efficient CCM of cyanobacteria (e.g. Glud et al. 1992).

In a surface bloom, the high cell density will amplify biologically mediated changes in the concentration of inorganic carbon and the supply of inorganic carbon from the bulk water will be restricted by diffusion. Nevertheless, it has been suggested that cyanobacterial blooms may benefit from being close to the air-water interface and hence to a source of resupply of  $\text{CO}_2$  (Paerl and Ustach 1982). The potential advantage of an increased availability of inorganic carbon may be offset by the risk of photooxidation by prolonged exposure to high photon irradiance. In this study we investigated the relative importance of air and water as sources of inorganic carbon for photosynthesis by blooms of cyano-

bacteria. We also studied the interaction between carbon limitation and high photon irradiance in producing photoinhibition in surface blooms.

## Materials and methods

*Description of sampling site*—Cyanobacteria were collected during June–September 1994 from Esthwaite Water in the English Lake District (54°22'N, 2°29'W). Esthwaite Water is a eutrophic lake of 1 km<sup>2</sup>, which typically supports summer phytoplankton maxima of ~90  $\mu\text{g Chl } a \text{ liter}^{-1}$  (Talling 1993), and in recent years has been dominated by cyanobacteria. The alkalinity is relatively low (~0.4 meq liter<sup>-1</sup>; Carrick and Sutcliffe 1982), so the water is poorly buffered against pH changes resulting from the photosynthetic activity of the relatively large phytoplankton population. As a result, pH values can exceed 10 in the summer (Talling 1976), thereby reducing the concentration of total inorganic carbon from ~0.4 to 0.25 mM, the concentration of  $\text{HCO}_3^-$  to <0.14 mM, and the concentration of  $\text{CO}_2$  virtually to zero (Maberly 1996). Photon irradiance at the water surface will reach 2,000  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  on a cloudless day in midsummer, but becomes attenuated rapidly with depth.

*Surface bloom formation*—Cyanobacteria were collected in the early morning with a 55- $\mu\text{m}$  mesh-size plankton net and left overnight to float up in buckets at 20°C under low irradiance. The next morning the floating cyanobacteria were carefully layered on top of lake water (treated as described below) and contained in black perspex cylinders. The cylinders had a depth of 0.6 m, a diameter of 0.06 m, and were closed with a lid that left a sealed headspace of ~250 ml. The lid allowed access for the fiberoptic arm of the light source and for the  $\text{O}_2$  and pH microsensors used; these microsensors were lowered into the bloom at an angle of 90° to the bloom surface. Blooms were left to stabilize for 90 min in the dark at 20°C, a temperature that was maintained throughout the experiments.

*Experimental conditions*—Blooms were illuminated for 2 h before the start of the measurements with a Schott KL-1500 light source equipped with a flexible fiberoptic arm and an infrared filter to minimize heat input. The fiberoptic arm of the Schott lamp was fitted through a hole in the lids that sealed off the bloom incubators and illuminated the blooms at an angle of 90°. This produced 200  $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$  (400–700 nm) at the bloom surface, measured with a Macam  $2\pi$  quantum sensor (QD 101) connected to a Keithly picoammeter. Air was continuously passed through the headspace at 200 ml min<sup>-1</sup>. Four different concentrations of  $\text{CO}_2$  were used:  $\text{CO}_2$ -free air (0 ppm, produced by passing air through tightly packed soda-lime, i.e. a mixture of NaOH, CaO, and CaOH), normal air (350 ppm), air with double the normal amount of  $\text{CO}_2$  (700 ppm), and air with 10 times the normal amount of  $\text{CO}_2$  (3,500 ppm). For each set of experiments two blooms were used. One bloom received 350 ppm  $\text{CO}_2$  and subsequently 3,500 ppm  $\text{CO}_2$ ; the other bloom received 700 ppm  $\text{CO}_2$  and subsequently 0 ppm  $\text{CO}_2$ . During the first incubations half of the surface area of the bloom,

to be used in the second treatment, was covered by black netting that reduced the photon irradiance to  $20 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ . The 2-h incubation period allowed steady-state oxygen conditions to develop in the blooms. The short period allowed for acclimation to the conditions in the surface bloom intended to reproduce conditions in the field, where surface blooms can develop in a short period owing to the high flotation velocity of the colonies that make up the bloom.

Measurements were made in filtered water from Esthwaite, which has a natural alkalinity of  $\sim 0.4 \text{ meq liter}^{-1}$ . An alkalinity of  $0.7 \text{ meq liter}^{-1}$  was produced by adding NaOH to raise the pH to 9.5. A reduced alkalinity of  $0.2 \text{ meq liter}^{-1}$  was produced by adding a calculated amount of HCl, removing the inorganic carbon by bubbling with  $\text{CO}_2$ -free air, and adjusting the pH to 9.5 with carbon-free NaOH.

*Measurement of light*—The light field in the blooms was determined with scalar fiberoptic microprobes since these provide the most appropriate measure of light availability in a scattering environment (Kühl and Jørgensen 1994). The probes were connected to a photodiode and a Keithly picoammeter. The bead sensor had a diameter of  $\sim 0.1 \text{ mm}$  and was constructed and calibrated following Lassen et al. (1992). Each sensor was checked under the microscope by shining light through the other end of the fiber, and checking carefully for a uniform light emission by the bead sensor. Measurements were made every  $0.2 \text{ mm}$  by connecting the probe to an Uhl micromanipulator, controlled by a personal computer running designated software, and connected via a TiePie engineering interface (Leeuwarden, The Netherlands). The light sensors were pushed up through a hollow stainless steel rod, fixed in the middle of the bloom incubator, parallel to the incoming light (i.e. perpendicular to the bloom). Light profiles were measured in triplicate.

*Measurement of oxygen and photosynthesis*—Concentrations of oxygen and rates of photosynthesis in the blooms were measured every  $0.1 \text{ mm}$  using oxygen microelectrodes constructed to the specifications of Revsbech and Ward (1983). The electrodes were calibrated at  $0\% \text{ O}_2$  in a saturated  $\text{Na}_2\text{SO}_3$  solution and in water saturated with air. Rates of photosynthesis were measured using the light-dark shift technique (Revsbech et al. 1981) and calculated as the slope of the decrease in oxygen concentration in the first 2 s after darkening. The signal from the microelectrodes was recorded by a Keithly picoammeter connected to a personal computer. Oxygen profiles were measured in quadruplicate, rates of photosynthesis in triplicate. Positions in the bloom were chosen at random.

The diffusivity within the bloom was determined following Revsbech (1989). The diffusion flux  $J$  of a solute is given by Fick's first law of diffusion,

$$J = -\phi D_a \delta C / \delta x, \quad (1)$$

where  $\phi$  is the porosity,  $D_a$  the apparent diffusion coefficient, and  $C$  is the concentration at depth  $x$ . The essential parameters for calculation of diffusion fluxes between bloom and atmosphere are thus  $\phi$  and  $D_a$ , more precisely the porosity multiplied with the diffusion coefficient. Bloom material was

killed with  $5 \text{ mM HgCl}_2$ , and  $0.75 \text{ ml}$  of the cyanobacterial concentrate (resulting in a  $1.5\text{-mm-thick}$  bloom) was placed on top of a  $1.5\text{-mm-thick}$  layer of  $1\%$  agar, supported by a thin silicone membrane, in a special diffusion chamber (Revsbech 1989). The lower half of the chamber was flushed with oxygen and the chamber was left for  $2 \text{ h}$  at  $20^\circ\text{C}$  in a water-saturated atmosphere. The diffusion profile through the bloom and the agar was measured in quadruplicate with oxygen microelectrodes.

*Measurement of pH*—Borosilicate glass capillaries (GC 200-10) were pulled in two stages to yield a capillary with a tip of  $1\text{--}2 \mu\text{m}$ . The tip was silanized by submersion in a fresh solution of  $0.2\%$  (vol/vol) dimethyldichlorosilane in benzene (Fluka Chemicals). The electrodes were baked in an oven for  $90 \text{ min}$  at  $90^\circ\text{C}$ , and a small amount of Fluka hydrogen ionophore I-cocktail B (dynamic range pH of  $5.5\text{--}12$ ) was drawn into the tip. The remainder of the electrode was then filled with  $1 \text{ M KCl}$ , checked for the absence of air bubbles, and left to stabilize for a minimum of  $3 \text{ h}$ . An Ag/AgCl wire was inserted into the electrode, which was connected to the mV channel of a Radiometer PHM64 pH meter. A separate reference electrode (Ag/AgCl wire embedded in soda glass) was placed in the bloom at a short distance from the pH microelectrode. The electrodes were calibrated in lake water in which the pH was varied by adding small amounts of HCl or NaOH. The pH of these samples was measured with a calibrated combination pH electrode (Radiometer GK 2401C). The average of the calibration immediately before and after a series of measurements was used—the difference between readings before and after was  $1\text{--}2\%$ . pH profiles were also measured in triplicate at  $0.1\text{-mm}$  intervals. Readings stabilized within  $20 \text{ s}$ . Noise was minimized by enclosing the blooms within a Faraday-cage.

*Inorganic carbon*—Alkalinity was measured by Gran titration (Mackereth et al. 1989), and this was combined with temperature, a calculated ionic strength of  $1.35 \text{ M}$ , and the pH measurements to calculate carbon speciation as outlined in detail in Maberly (1996). At  $20^\circ\text{C}$ , the values for the adjusted carbonate dissociation constants were  $6.37$  for  $\text{p}K'_1$ ,  $10.32$  for  $\text{p}K'_2$ , and  $14.14$  for  $\text{p}K'_w$ .

*pH drift experiments*—A simple pH-drift experiment was performed to determine the maximum pH that the cyanobacteria could produce by removal of inorganic carbon during photosynthesis. Cyanobacteria in Esthwaite lake water were placed in stoppered bottles and kept in the light at  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  at  $20^\circ\text{C}$  for  $24 \text{ h}$ . At the end of this incubation, the pH was measured with a combination electrode and alkalinity was determined by Gran titration.

*Species composition*—Samples from the blooms were fixed with Lugol's iodine. The relative abundances of the several cyanobacterial genera present in the bloom were counted using a microscope.

*Fluorescence measurements*—A Hansatech MFMS fluorometer (excitation wavelength of  $585 \text{ nm}$ ) was used to study the effect of the availability of carbon in blooms on photo-

inhibition and recovery from photoinhibition. Experiments were performed at three concentrations of CO<sub>2</sub> in the headspace (0, 350, 3,500 ppm) and two alkalinities in the water (0.7, 0.2 meq liter<sup>-1</sup>). The bloom was given time to acclimate to the CO<sub>2</sub> concentrations during 90 min under a low photon irradiance of 30 μmol m<sup>-2</sup> s<sup>-1</sup>. A light shock of 1,000 μmol photons m<sup>-2</sup> s<sup>-1</sup> at 60 min in duration was applied using a Schott KL-1500 light source. Recovery from photoinhibition was followed during 90 min under 30 μmol photons m<sup>-2</sup> s<sup>-1</sup>.

Quenching analysis of fluorescence throughout the 4-h time course largely followed the protocol and terminology outlined by van Kooten and Snel (1990). Fluorescence can be used as a monitor of photosynthetic activity. Photosynthesis and other processes, mainly thermal energy dissipation, compete with fluorescence for excitation energy. Hence, photosynthesis induces photochemical quenching of fluorescence and thermal energy dissipation of nonphotochemical quenching. The minimum fluorescence yield,  $F_0$ , was determined on dark-acclimated samples (all photosynthetic reaction centers open,  $Q_A$  pool fully oxidized). The maximum fluorescence yield of dark-acclimated cells,  $F_m$ , and the maximum fluorescence yield of light-acclimated cells,  $F'_m$ , were determined with all photosynthetic reaction centers closed ( $Q_A$  fully reduced) by applying a saturating light pulse of 12,000 μmol photons m<sup>-2</sup> s<sup>-1</sup> at 0.9 s in duration. The difference between  $F_m$  and  $F_0$  (the variable fluorescence  $F_v$ ) describes the redox state of  $Q_A$ .

A single saturating pulse did not yield  $F_m$ , however, as was found before for other cyanobacteria by Romero et al. (1992) and Crotty et al. (1994). A saturating pulse in the presence of a low photon irradiance of 30 μmol m<sup>-2</sup> s<sup>-1</sup> did yield  $F_m$ , as was checked by using DCMU at a final concentration of 10 μM. DCMU blocks the electron transfer from  $Q_A$  to  $Q_B$  and leads to a complete reduction of all  $Q_A$  (Krause and Weis 1991). During incubation in the light, saturating pulses (increasing steady-state fluorescence  $F$  to  $F'_m$ ) were applied every 2 min by the Hansatech PLS1 light source. The minimum fluorescence yield in the light-acclimated state,  $F'_0$ , was determined by briefly switching off the actinic light source and administering a pulse of far-red light of 720 nm. From this analysis the following variables were calculated (see Hofstra et al. 1994):

$$\Phi_{po} = F_v/F_m, \quad (2)$$

the photochemical yield of open PS2 reaction centers, which is closely related to the maximum quantum yield of photosynthesis and is a good quantitative indicator of photoinhibition (Krause and Weis 1991);

$$\Phi_p = (F'_m - F)/F'_m, \quad (3)$$

the photochemical efficiency of PS2 per absorbed photon, or photon yield;

$$q_p = (F'_m - F)/(F'_m - F'_0), \quad (4)$$

the coefficient of photochemical quenching; and

$$q_N = 1 - [(F'_m - F'_0)/(F_m - F_0)], \quad (5)$$

the coefficient of nonphotochemical quenching.

## Results

Microelectrode studies of blooms receiving variable amounts of CO<sub>2</sub> were performed on five occasions. We first discuss general aspects of the blooms used in this study and then present a typical set of data from 13 July 1994 for lake water with an alkalinity of 0.7 meq liter<sup>-1</sup> (0.4 meq liter<sup>-1</sup> before pH adjustment to 9.5) and 7 July 1994, when the alkalinity of the lake water was reduced to 0.2 meq liter<sup>-1</sup>. Finally, the results are described from experiments on the occurrence of photoinhibition and recovery made between 12 and 14 September 1994.

*General description of the blooms*—Blooms that formed in the cylindrical bloom incubators varied in thickness from 1 to 1.5 cm. Although the blooms had a homogeneous appearance, microelectrode measurements showed that small-scale horizontal patchiness did occur. There was also a vertical gradient—the Chl *a* content decreased from ~70 mg liter<sup>-1</sup> in the top layer of the bloom to ~30 mg liter<sup>-1</sup> in the deepest layers. In the blooms, larger colonies were abundant in the top layer, while smaller colonies dominated the deeper layers. During the process of bloom formation, larger colonies arrive first at the surface because their higher flotation velocity. In the beginning of July 1994, *Anabaena flos-aquae* made up ~60% based on numbers in Esthwaite Water, with 30% being *Aphanizomenon flos-aquae* and 10% *Gomphosphaeria naegeliana*. The experiments on photoinhibition were conducted later in the year, in September. The species composition of Esthwaite Water had changed and consisted of roughly equal numbers of *Anabaena flos-aquae*, *Aphanizomenon flos-aquae*, *G. naegeliana*, and *Oscillatoria* spp. (two species).

*Depth profiles*—Light attenuation: Figure 1 shows profiles of photon irradiance measured on 7 and 13 July 1994. Log transformation of the profiles did not always result in straight lines, which was expected given the nonuniform distribution of Chl *a* with depth (the average  $r^2$  for all profiles on these 2 d was 0.86; min. of 0.8, max. of 0.98). Despite the relatively insensitive photodiode used in the setup, the profiles gave a reliable estimate of the depth in the bloom where irradiance was no longer sufficient to support net photosynthesis (corresponding roughly to 1–10% of the surface irradiance); this depth was found between 2 and 5 mm. The response of photosynthesis to photon irradiance by the algae in Esthwaite ( $P$  vs.  $I$  curve) showed that, the photon irradiance at the onset of light saturation,  $I_k$ , varied between 150 and 200 μmol m<sup>-2</sup> s<sup>-1</sup> (data not shown). Thus, photosynthesis by cells at the surface, receiving 200 μmol m<sup>-2</sup> s<sup>-1</sup>, should be just light-saturated but not photoinhibited, whereas 20 μmol m<sup>-2</sup> s<sup>-1</sup> at 10% of the surface irradiance will be strongly light-limiting. The rapid extinction of photon irradiance emphasizes the high density of cyanobacterial biomass in a bloom.

*Oxygen concentration*: When CO<sub>2</sub> was excluded from the headspace, the concentration of O<sub>2</sub> in the bloom at an alkalinity of 0.2 meq liter<sup>-1</sup> was less than air equilibrium throughout the depth profile (Fig. 2), suggesting that, overall,

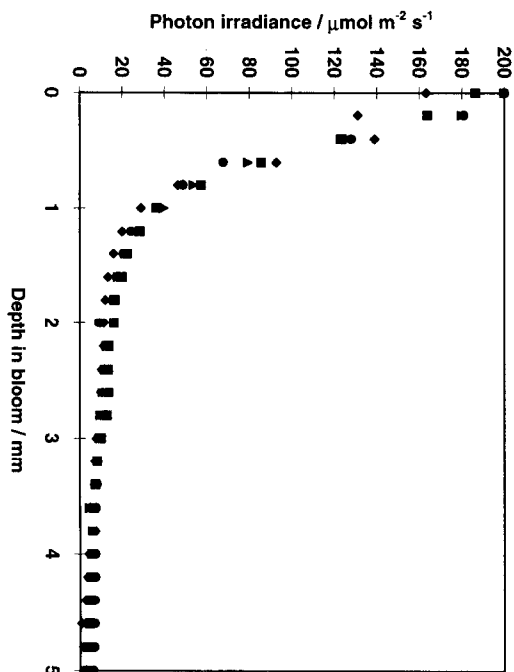


Fig. 1. Extinction of scalar photon irradiance in the two surface blooms used on 7 July 1994 and the two blooms used on 13 July 1994.

rates of  $O_2$  consumption were greater than rates of gross  $O_2$  production. At the higher alkalinity of  $0.7 \text{ meq liter}^{-1}$ , concentrations of  $O_2$  exceeded air equilibrium down to a depth of 2 mm (Fig. 2), indicating an increase in rates of net photosynthesis compared to the lower alkalinity. There was a consistent trend for the maximal concentration of  $O_2$  to increase with the concentration of  $CO_2$  in the headspace and, at a given headspace concentration of  $CO_2$ , for maximal oxygen concentrations to be greater at the higher alkalinity. At both alkalinities, the depth of maximal  $O_2$  concentration occurred nearer to the surface as the concentration of  $CO_2$  in the headspace increased. Experiments at the two alkalinities were performed 6 d apart, and so differences in bloom characteristics cannot be discounted, although the similarities in the light profiles suggest they are not major. The different depth of maximal  $O_2$  concentration at the two alkalinities is probably a real effect—it was substantially greater at the higher alkalinity when the headspace concentration of  $CO_2$  was 700 ppm or less, but similar for both alkalinities at 3,500 ppm. The  $O_2$ -profile data suggest that oxygen production by the bloom resulted from uptake of inorganic carbon from both the headspace and the water.

Rates of gross photosynthesis: At an alkalinity of  $0.7 \text{ meq liter}^{-1}$ , the profile of gross rates of photosynthesis under air ( $350 \text{ ppm } CO_2$ ) resembles profiles published earlier by Ibelings and Mur (1992). Two distinct peaks were found: one in the top layer of the bloom, and a second subsurface peak at a depth of  $\sim 1.4 \text{ mm}$  (Fig. 3). The removal of  $CO_2$  from the headspace resulted in a reduction of the near-surface peak, but left the subsurface peak largely unaffected. At an alka-

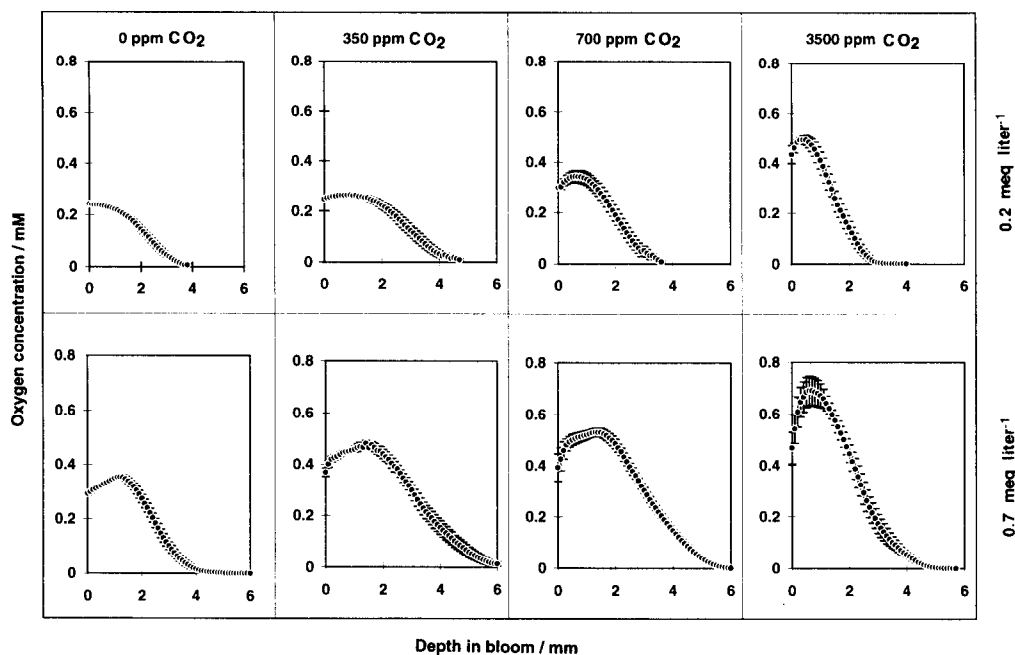


Fig. 2. Oxygen concentration profiles in cyanobacterial surface waterblooms under variable  $CO_2$  concentrations—0 ppm; air (350 ppm); twice the amount of  $CO_2$  in air (700 ppm); and  $10\times$  the amount of  $CO_2$  in air (3,500 ppm) at an alkalinity of  $0.2 \text{ meq liter}^{-1}$  (top) and  $0.7 \text{ meq liter}^{-1}$  (bottom). The error bars (where visible) denote the standard error of the mean. Air equilibrium is  $0.28 \text{ mM } O_2$ .

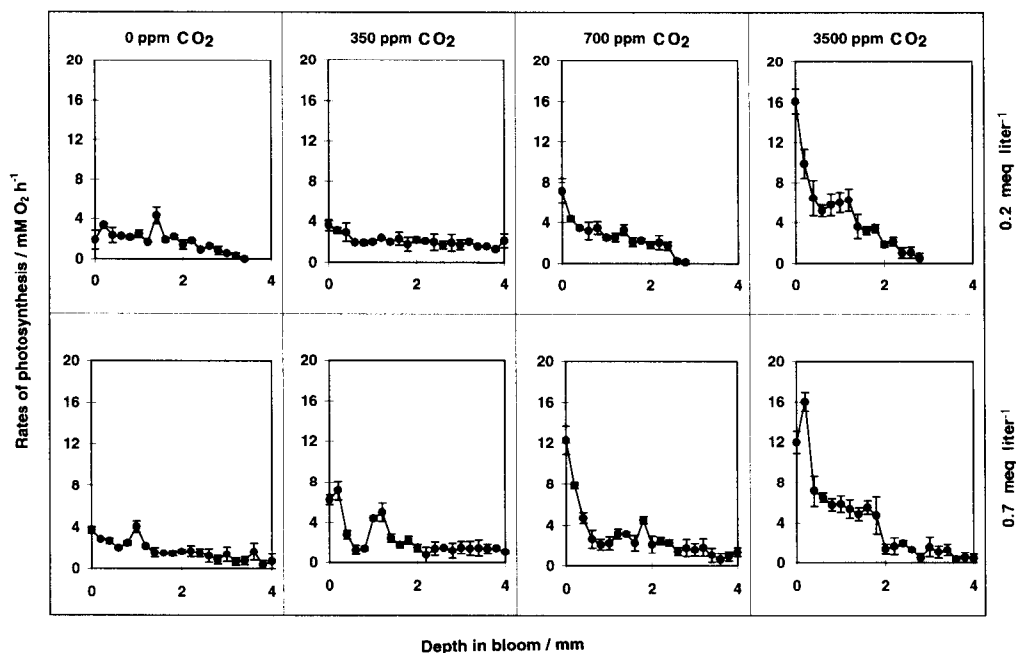


Fig. 3. Gross rates of photosynthetic  $O_2$  production, corresponding to the oxygen profiles shown in Fig. 2 at an alkalinity of  $0.2 \text{ meq liter}^{-1}$  (top) and  $0.7 \text{ meq liter}^{-1}$  (bottom) under variable  $CO_2$  concentrations in the headspace of the bloom—0 ppm; air (350 ppm); twice the amount of  $CO_2$  in air (700 ppm); and  $10\times$  the amount of  $CO_2$  in air (3,500 ppm). The error bars denote the standard error of the mean.

linity of  $0.2 \text{ meq liter}^{-1}$ , rates of gross photosynthesis changed less with depth than they did at the higher alkalinity. Although a midbloom peak in rate of gross photosynthesis was present under a headspace concentration of 0 ppm, it was absent at the higher headspace concentrations. Rates of gross photosynthesis were generally lower at the lower alkalinity, but were similar at the surface at the highest headspace  $CO_2$  concentration. Rates of gross photosynthesis near the surface increased with increasing concentration of  $CO_2$  in the headspace at both alkalinities (Fig. 3).

Areal integration of rates of photosynthesis through depth showed that gross photosynthesis increased significantly with the headspace  $CO_2$  concentration (Table 1). Areal gross photosynthesis under a headspace of 3,500 ppm was about twice that under 350 ppm.

pH: The absence of  $CO_2$  in the headspace at the higher alkalinity resulted in a constant pH of  $\sim 11$  from the bloom surface to a depth of up to 2 mm. With a rise in the availability of  $CO_2$  in the headspace, the pH in the near surface layer dropped. This was particularly noticeable under 700 or 3,500 ppm  $CO_2$ , where pH decreased more than one unit at the surface (Fig. 4). Below 2-mm depth, there was a sharp fall in pH wherein the pH dropped nearly four units to values close to 7. The depth where the pH dropped corresponded roughly with the bottom of the euphotic layer. Below the bloom the pH quickly reached 9.5, the pH of water that was used to fill the cylinders (not shown).

At reduced alkalinity the pH of the blooms did not exceed 10. In the deeper layers, however, pH dropped to lower values than before, probably because of reduced buffering ca-

Table 1. Areal concentrations of oxygen, integrated through depth, from the bloom surface to the anoxic layer (derived from data in Fig. 2), rates of total bloom gross photosynthesis (derived from data in Fig. 3), net photosynthesis derived from concentration gradients in Fig. 2, and calculated respiration (see text for details of calculation) at four concentrations of  $CO_2$  in the headspace and two alkalinities. Concentrations ( $\text{mmol } O_2 \text{ m}^{-2}$ ) and rates ( $\text{mmol } O_2 \text{ m}^{-2} \text{ h}^{-1}$ ) given as means with standard deviations in parentheses.

| Head-space<br>[ $CO_2$ ]/<br>ppm | Oxygen                   |                          | Gross photosynthesis     |                          | Net photosynthesis       |                          | Respiration              |                          |
|----------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                                  | 0.2 meq<br>liter $^{-1}$ | 0.7 meq<br>liter $^{-1}$ | 0.2 meq<br>liter $^{-1}$ | 0.7 meq<br>liter $^{-1}$ | 0.2 meq<br>liter $^{-1}$ | 0.7 meq<br>liter $^{-1}$ | 0.2 meq<br>liter $^{-1}$ | 0.7 meq<br>liter $^{-1}$ |
| 0                                | 0.6(0.01)                | 0.9(0.05)                | 6.3(0.5)                 | 7.2(0.9)                 | 0.6(0.1)                 | 1.5(0.1)                 | 5.7                      | 5.7                      |
| 350                              | 0.8(0.04)                | 1.7(0.08)                | 8.0(0.4)                 | 9.9(0.8)                 | 0.9(0.2)                 | 2.2(0.9)                 | 7.1                      | 7.7                      |
| 700                              | 0.8(0.04)                | 1.7(0.05)                | 8.8(0.1)                 | 12.5(0.9)                | 1.7(0.9)                 | 3.0(1.4)                 | 7.1                      | 9.5                      |
| 3,500                            | 0.8(0.05)                | 1.7(0.04)                | 14.7(1.1)                | 17.1(0.7)                | 3.2(2.2)                 | 6.2(2.7)                 | 11.5                     | 10.9                     |

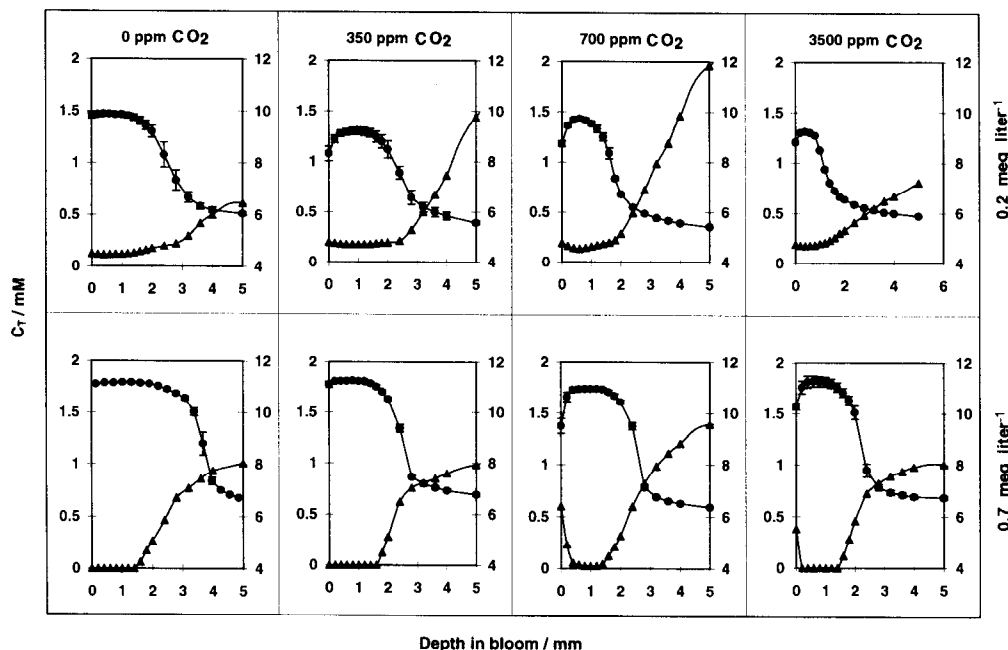


Fig. 4. pH profiles (●; right y-axis) of waterblooms under variable concentrations of  $\text{CO}_2$  in the headspace (0, 350, 700, and 3,500 ppm), whose oxygen concentration profiles and rates of photosynthesis are shown in Figs. 2 and 3 at an alkalinity of  $0.2 \text{ meq liter}^{-1}$  (top) and  $0.7 \text{ meq liter}^{-1}$  (bottom). The error bars (where visible) denote the standard error of the mean. Also shown are the concentrations of total inorganic carbon in the surface waterblooms (▲; left y-axis).  $C_7$  was calculated from temperature, alkalinity, and the pH profiles shown in the same figure.

capacity, reaching values between 5 and 6 (Fig. 4). In the bloom incubated under 3,500 ppm  $\text{CO}_2$ , water of low pH flooded the bloom and led to its demise; thus, the measurements of pH could not be finished in triplicate.

In general, the pH reflects the balance between inorganic carbon uptake during photosynthesis, which causes the pH to increase, and production of  $\text{CO}_2$  in respiration, which causes the pH to decrease, and is modified by fluxes of inorganic carbon down concentration gradients established by the biological activity. Thus, in the surface layers of the bloom where photosynthesis predominates, pH was high but decreased with depth as respiration became increasingly important.

**Inorganic carbon:** The maximum pH values measured in the blooms were greater than could be accounted for if alkalinity remained constant and all the carbon species were converted to hydroxide ions (note that alkalinity was not actually measured in the profiles; this sets limits on calculations of the concentration of inorganic carbon, which at a given pH will increase with alkalinity). Assuming a constant alkalinity of  $0.7 \text{ meq liter}^{-1}$ , the calculated maximum pH possible is 11.00, which is less than the maximum pH of 11.28 measured under 3,500 ppm at a depth of 0.6 mm. Two explanations for this discrepancy are possible. First, alkalinity may have increased within the bloom (e.g. as a result of nitrate assimilation). There is some evidence for this, as alkalinity increased from 0.6 to  $0.7 \text{ meq liter}^{-1}$  during pH drift experiments in small stoppered bottles. Triplicate pH-drift experiments confirmed that pH values of  $\sim 10.9$  could be

generated. The second explanation may be that the pH microelectrodes did not respond linearly at pH values  $> 11$ , which exceeded the upper pH used in calibration (pH 10–10.5).

Despite these problems, the main point is clear; that is, the concentration of  $C_7$  in the upper layer of the blooms was extremely low. At the higher alkalinity the concentration of  $C_7$  approached zero under a headspace of 0 or 350 ppm  $\text{CO}_2$  (Fig. 4). Under 700 or 3,500 ppm  $\text{CO}_2$ ,  $C_7$  increased toward the surface of the bloom, but quickly fell to zero below 0.2 mm. Below a depth of 1.5 mm,  $C_7$  concentrations in the bloom increased. There was a similar pattern at the lower alkalinity (Fig. 4), but here concentrations of  $C_7$  did not fall to such low values.

**Estimates of oxygen flux—Diffusivity:** The slope of the oxygen profiles in the bloom layer in the diffusion chamber was not significantly different from the slope in the agar layer. The identical values of  $\Phi D$ , in blooms and water show that diffusivity of gases in cyanobacterial surface blooms is neither enhanced nor restricted when compared to water. A diffusion coefficient for oxygen of  $1.7 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$  at  $20^\circ\text{C}$  was used.

**Rates of oxygen exchange:** The  $\text{O}_2$  content of a bloom results from net photosynthesis and is dependent on the balance between rates of photosynthetic oxygen evolution, respiratory oxygen uptake, and rates of exchange with the atmosphere. The oxygen concentrations in the blooms integrated through depth, from the surface to the anoxic lay-

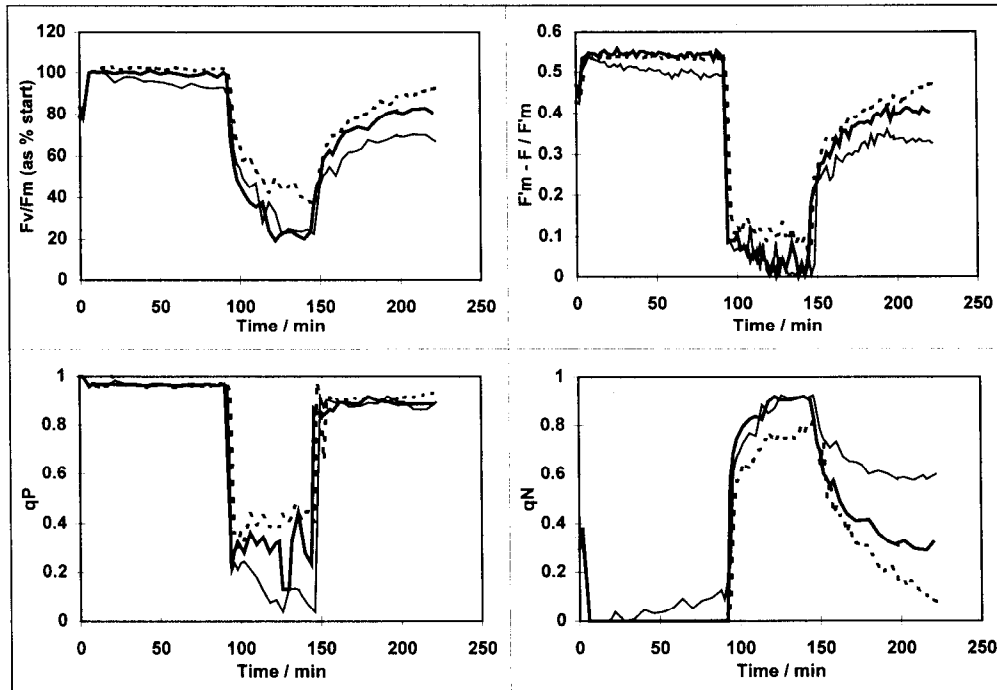


Fig. 5. Changes in the ratio of the photochemical yield of open PS2 reaction centers,  $F_v/F_m$ , as well as the photochemical efficiency of PS2 per absorbed photon  $(F'_m - F)/F'_m$ ; the coefficient of photochemical quenching  $q_p$  and the coefficient of nonphotochemical quenching  $q_N$ , during acclimation to various  $\text{CO}_2$  concentrations in the headspace (0 ppm, —; 350 ppm, —; 3,500 ppm, ·····). Photon irradiance was varied in time as follows:  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the first 90 min of the incubation, followed by 45 min exposure to a photoinhibiting photon irradiance of  $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  (between 90 and 135 min), and again a low photon irradiance of  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ , allowing recovery from photoinhibition during the final 90 min. Measurements were made at an alkalinity of  $0.2 \text{ meq liter}^{-1}$ .

er, increased with the presence of  $\text{CO}_2$  in the headspace or an increase in alkalinity (Table 1). In the upper layers of the bloom (where cyanobacteria are photosynthetically active) an increase in oxygen concentrations integrated through depth, with an increase in  $\text{CO}_2$ , was found: the increased availability of  $\text{CO}_2$  stimulated photosynthesis and suppressed photorespiration. In the deeper, photosynthetically inactive layers, however, the drop toward anoxia was steeper at the higher  $\text{CO}_2$  concentrations (see Fig. 2), hence the  $\text{O}_2$  content integrated through depth did not increase further.

The gradients of oxygen concentration from the midbloom maximum to surface and to depth characterize the net rates of oxygen production. The net rates of exchange with the headspace were calculated by linear regression from the gradient of  $\text{O}_2$  concentration in the surface  $0.3 \text{ mm}$  ( $0.2 \text{ mm}$  at  $3,500 \text{ ppm}$ ) by using Fick's first law of diffusion. The net rates of exchange at depth were calculated in a similar way over the  $1 \text{ mm}$  depth range where the  $\text{O}_2$  gradient was steepest. The middepths ranged between  $1.5$  and  $2.5 \text{ mm}$  over the eight treatments, which was broadly similar to the euphotic depth. The sum of the calculated rates of flux to the headspace and to depth were used to calculate the net areal rate of photosynthesis. Areal rates of respiration were calculated as the difference between the calculated net rate and the integrated measured gross rate.

The effect of the different concentrations of  $\text{CO}_2$  in the

headspace on the estimated areal net photosynthesis was much more marked than was the effect on the areal gross photosynthesis. Increasing the concentration of  $\text{CO}_2$  in the headspace from 0 to  $3,500 \text{ ppm}$  increased rates of net photosynthesis 5.3 and 4.1 times at alkalinities of  $0.2$  and  $0.7 \text{ meq liter}^{-1}$ , respectively (Table 1). The areal gross photosynthesis at a given headspace concentration of  $\text{CO}_2$ , increased between 1.1 and 1.4 times with alkalinity, but net photosynthesis increased much more at 1.7–2.5 times. The difference between net photosynthesis and gross photosynthesis is attributed to respiration, including photorespiration. This increased with the headspace concentration of  $\text{CO}_2$  but was not affected by alkalinity (Table 1). Areal respiration was between 64 and 90% of areal gross photosynthesis. The percentage decreased with increasing headspace  $\text{CO}_2$  concentration and alkalinity. Rates of photosynthesis measured in this study are comparable to values published by Ibelings and de Winder (1994) for a *Microcystis* bloom under air. By using a 1.75 times higher incident irradiance they measured a 1.45 times higher integrated rate of photosynthesis in the bloom.

*Photoinhibition and recovery*— $F_v/F_m$ : Figure 5 shows the pattern of  $F_v/F_m$  during the photoinhibition experiments in blooms with an alkalinity of  $0.2 \text{ meq liter}^{-1}$ . During acclimation to the various  $\text{CO}_2$  concentrations at low photon ir-

radiance,  $F_v/F_m$  decreased slightly under 0 ppm CO<sub>2</sub>, but not under 350 or 3,500 ppm CO<sub>2</sub>. A decrease in  $F_v/F_m$  during acclimation to low CO<sub>2</sub> at moderate photon irradiance was also observed in the green alga *Chlamydomonas reinhardtii* by Falk and Palmqvist (1992). The photon irradiance shock of 1,000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  under 0 or 350 ppm CO<sub>2</sub> induced a fast quenching of  $F_v/F_m$  to a level of 20% of the value at the start. The reduction of  $F_v/F_m$  under 3,500 ppm was less pronounced. Recovery from high photon irradiance at 30  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  improved with an increase in the availability of CO<sub>2</sub> in the headspace. The described phenomena can be seen even more prominently in the ratio of  $(F'_m - F)/F'_m$ , which is a measure of the photon yield of PS2 (Fig. 5). Similar observations were made in blooms incubated at an alkalinity of 0.7 meq liter<sup>-1</sup>, although the decrease in  $F_v/F_m$  was less prominent, especially under 0 ppm CO<sub>2</sub> (data not shown).

**Quenching analysis:** At an alkalinity of 0.2 meq liter<sup>-1</sup> photochemical quenching during the light shock almost dropped to zero under 0 ppm, while it remained at values close to 0.3 under 350 ppm and 0.4 under 3,500 ppm CO<sub>2</sub> (Fig. 5). Nonphotochemical quenching rapidly increased during the light shock, but the rise in  $q_N$  leveled off at a lower value under 3,500 ppm CO<sub>2</sub>. In the recovery phase,  $q_N$  remained at a high level under 0 ppm especially (Fig. 5). The slow recovery of  $F_v/F_m$  under 0 ppm, however, does suggest that there was still an excess of excitation energy during the light shock, despite the increase in thermal energy dissipation.

## Discussion

**Limitation and supply of inorganic carbon to surface blooms**—The high biomass of cyanobacteria within the bloom produced a high photosynthetic demand for inorganic carbon that outstripped the rate of supply. As a result, almost all of the available inorganic carbon had been removed from the photosynthetically active part of the bloom in all treatments (Fig. 4). Consequently, rates of inorganic carbon resupply had a major effect in controlling the areal rates of net photosynthesis in the bloom. Present-day concentrations of CO<sub>2</sub> in the atmosphere were insufficient to satisfy the photosynthetic demand of the blooms. Net photosynthesis increased around fourfold and threefold at alkalinities of 0.2 and 0.7 meq liter<sup>-1</sup>, respectively, when the headspace concentration of CO<sub>2</sub> was increased 10-fold (Table 1).

At atmospheric concentrations of CO<sub>2</sub>, the net rate of production at the higher alkalinity was about twofold greater than at the lower alkalinity. At steady state this difference must be caused by a different rate of supply of inorganic carbon. The gradients of inorganic carbon concentration below the bloom were similar for the two alkalinities (Fig. 4), and so the greater net flux at the higher alkalinity is presumably the result of more rapid uptake from the headspace. This difference is consistent with the greater pH generated at the surface at the higher alkalinity (Fig. 4), which will increase the rate of influx of CO<sub>2</sub> by "chemical enhancement," the reaction of CO<sub>2</sub> with water to form HCO<sub>3</sub><sup>-</sup>, and co-diffusion of HCO<sub>3</sub><sup>-</sup> ions across the subsurface boundary

layer (Portielje and Lijklema 1995; Maberly 1996; Waninkhof and Knox 1996).

The O<sub>2</sub> profiles, integrated oxygen content, profiles of gross photosynthesis, and calculated net photosynthesis for the blooms are all consistent with productivity being controlled by the rate of supply of inorganic carbon from the headspace above and the water below. The profiles of photosynthesis (Fig. 3) under 350 ppm CO<sub>2</sub> show two peaks, one near the surface and a second in deeper layers of the bloom. Ibelings and Mur (1992), who found a similar pattern, suggested that the lower peak was the result of separation by size of the cyanobacterial colonies in the bloom and differences in the degree of acclimation to low average irradiance by the size groups. The disappearance of the near-surface maximum under 0 ppm CO<sub>2</sub> suggests that a more likely explanation is that the twin peaks are a response to the dual availability of inorganic carbon, diffusing in from the air above and the water below.

The calculated rates of respiration plus photorespiration ( $R$ ) were a substantial part of the rates of gross photosynthesis ( $P$ ) (Table 1). From photosynthesis vs. irradiance curves (not shown), the standard photon irradiance, 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , was close to  $I_K$ , and so the Mehler reaction is unlikely to have been the major cause of the low  $P:R$  ratios (Kana 1992). A more likely cause of the low ratios is enhanced photorespiration promoted by supersaturating oxygen concentrations and very low carbon concentrations. Glud et al. (1992) observed that photorespiration in biofilms of *Oscillatoria* under high ratios of O<sub>2</sub>:CO<sub>2</sub> was much less than that in biofilms of diatoms. They suggested that the efficient CCM of cyanobacteria was responsible for the lower rates of photorespiration. Although a CCM is strongly indicated indirectly by the very low concentrations of inorganic carbon achieved within the bloom, it was evidently insufficient to prevent  $P:R$  ratios close to 1 under extreme carbon limitation.

**Interaction of light stress with carbon limitation**—Previous studies have shown that blooms of cyanobacteria are prone to photoinhibition (Ibelings and Mur 1992; Ibelings and de Winder 1994) and that recovery can be slow. Reuter and Müller (1993) reviewed acclimation of the photosynthetic apparatus of cyanobacteria to photon irradiance and CO<sub>2</sub>. When the concentration of CO<sub>2</sub> is low, the cellular carotenoid content, especially zeaxanthin, is considerably enhanced. It is well known that additional stress factors that limit the rate of photosynthesis enhance photoinhibition (Demmig-Adams and Adams 1992; Osmond 1994). An increased zeaxanthin content would offer partial protection against the larger excess of excitation under carbon-limiting conditions. The presence of zeaxanthin correlates with non-photochemical quenching of fluorescence ( $q_N$ ). Nonphotochemical quenching is the result of energy-dissipating mechanisms other than photochemistry. It provides the photosystem with a photoprotective mechanism when photosynthesis is restricted, as in this study by the limited availability of CO<sub>2</sub>. Paerl et al. (1985) found that enhanced carotenoid content, resulting from prolonged exposure to high average irradiance, can offer photoprotection in cyanobacterial surface blooms.

Recovery from light shock was lowest (Fig. 5) when carbon was restricted in air and water. Enhanced  $q_N$  under 0 ppm  $\text{CO}_2$  (Fig. 5) could not prevent all photodamage. Falk and Palmquist (1992) stated that even with a fully operational CCM, cells have a relative increase in excitation pressure under low  $\text{CO}_2$ . The short time allowed for acclimation to low inorganic carbon concentrations in our study (90 min) may have augmented the harmful combination of low  $\text{CO}_2$  and high photon irradiance since a fully induced CCM may not have been operational (Badger 1987). On the other hand, cyanobacteria from Esthwaite Water used in the experiments were already acclimated to a low availability of carbon; the pH varied between 9 and 9.8 during the period of study, and the concentration of  $\text{CO}_2$  was (much) less than air equilibrium. The stronger photoinhibition under low  $\text{CO}_2$  found in our study contrasts with the findings of Knoppova et al. (1993) for *Scenedesmus quadricauda*. These cells increased  $q_N$  sufficiently when carbon limited to make them less sensitive to photoinhibitory damage than found here for cyanobacteria.

*The consequences of bloom formation*—When cyanobacteria form surface blooms they alter the supply of essential resources for photosynthesis. Thus, buoyancy can increase the availability of light but may also expose cells to sufficiently high light to cause photoinhibition, particularly if the cells aggregate at the surface. Similarly, although a surface aggregation may place cells close to resupply of  $\text{CO}_2$  from the atmosphere, by concentrating a large number of cells within a small volume of water, carbon depletion may be accentuated. The supply of light and inorganic carbon interact and low carbon availability can intensify photoinhibition. Within the bloom itself there will be steep crossed gradients for these photosynthesis resources, producing very different environments for photosynthesis over short vertical distances.

In the system we used there was sufficient biomass to remove almost all the light within the bloom. As a result, the lower layers of the bloom were unable to photosynthesise and there was a net respiration rate. This produced relatively high concentrations of  $\text{CO}_2$  at the base of the bloom that in part fueled the flux of inorganic carbon into the bloom from below. While very thick surface blooms can occur in some lakes (see Zohary and Robarts 1990), in Esthwaite Water the blooms tend to be less dense, ephemeral, and often occur during periods when the pH of the water is already high and concentrations of inorganic carbon are already depleted (Maberly 1996). Under these conditions, the supply of inorganic carbon from below the bloom will be very much reduced and the rates of production controlled to a larger extent by resupply from the atmosphere.

Paerl and Ustach (1982) and Paerl (1983) emphasised that the atmosphere might be an important source of inorganic carbon for blooms of cyanobacteria. Our work supports this idea. Although Paerl and Ustach (1982) showed that *Anabaena oscillarioides* had a higher affinity for  $\text{CO}_2$  than for  $\text{HCO}_3^-$  and appeared not to use  $\text{CO}_3^{2-}$ , the concentrations of  $\text{CO}_2$  within the blooms in our study were very low and  $\text{HCO}_3^-$  is likely to have been the only available source. Cyanobacteria are known to be able to use this carbon source

effectively (Talling 1976; Paerl and Ustach 1982; Badger et al. 1994). In the top 0.1 mm, where the pH was reduced, it is possible that  $\text{CO}_2$  might have been available as a source of inorganic carbon, even though the computed equilibrium concentrations will have been low. The production of a high pH close to the air–water interface will chemically enhance the influx of  $\text{CO}_2$  by promoting co-diffusion of  $\text{HCO}_3^-$ . For example, for a subsurface boundary layer of variable depth, the rate of influx is 6–10 times greater at pH 10.5 than at pH 8.5 for a given alkalinity (Portielje and Lijklema 1995; Maberly 1996).

*Timescales of bloom formation and acclimation*—Cyanobacteria, like other organisms, have evolved many mechanisms to ameliorate stress imposed by the environment. For example, photoacclimation to the prevailing light climate involves a number of physiological processes (Falkowski and LaRoche 1991). These are set in motion on varying timescales, although typically on the order of the generation time of an organism (Post et al. 1985). The timescale involved in surface-bloom formation, however, often is much shorter than the timescale involved in photoacclimation; that is, cyanobacteria will not have time to invoke all defensive mechanisms. This results from the high flotation velocity of colonial cyanobacteria. Measurements have shown that cyanobacteria such as *Microcystis* or *Gomphosphaeria* can travel several meters per hour (see Reynolds 1987; Walsby et al. 1992), and thus surface blooms form rapidly when mixing subsides owing to the high flotation velocity of the colonies that make up the bloom. Most cyanobacteria in lakes are positively buoyant at all times (Ibelings et al. 1991), so blooms can potentially form at any time of day. Persistent surface blooms will form especially when mechanisms that normally lead to a loss of buoyancy in the light fail to operate as a result of an enhanced gas vacuole content (as in Walsby et al. 1992), photoinhibition (Ibelings et al. 1991), carbon limitation, or because a blanket of buoyant, inactive colonies in deeper layers of the bloom keeps colonies at the top of the bloom in position (Walsby 1994).

Because there is little time to acclimate to the conditions in a surface waterbloom, the condition of the algae prior to bloom formation is crucial for the initial survival of the cells in the waterbloom. If cells prior to bloom formation are acclimated to low average irradiance they will be unable to invoke photoprotective mechanisms on a sufficiently short timescale, and may suffer photodamage. Our study intended to investigate the response of cyanobacteria in the first few hours after bloom formation (we did not allow a long incubation time). Hence, our study did not investigate the long-term acclimation to the conditions in the surface blooms that will occur if cells survive the crucial initial stages after bloom formation.

*Conclusions*—Formation of blooms may give cyanobacteria an advantage in competition for light and inorganic carbon with other groups of phytoplankton. By placing a dense biomass close to the water surface they are able to intercept a large amount of the flux of light and inorganic carbon from above. The obvious advantage of this may be offset by the risk of photoinhibition when the photon irra-

diance at the surface is high. A combination of high photon irradiance and carbon limitation proved to be especially deleterious in blooms.

Cyanobacteria dominate many productive lakes in the summer. Although their successful competition for inorganic carbon is unlikely to be the only reason for this, it appears to play a certain role (Shapiro 1973, 1990). Our finding that increasing the CO<sub>2</sub> content in the headspace over a bloom stimulates productivity of the cyanobacteria may be ecologically relevant in the light of rising CO<sub>2</sub> levels in the atmosphere. It seems possible that cyanobacteria will benefit from the change in atmospheric conditions.

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