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CYANOBACTERIAL BUOYANCY REGULATION: THE PARADOXICAL ROLES OF CARBON[1]

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ABSTRACT

In stratified lakes, dominance of the phytoplankton by cyanobacteria is largely the result of their buoyancy and depth regulation. Bloom-forming cyanobacteria regulate the gas vesicle and storage polymer contents of their cells in response to interactive environmental factors, especially light and nutrients. While research on the roles of nitrogen and phosphorus in cyanobacterial buoyancy regulation has reached a consensus, evaluations of the roles of carbon have remained open to dispute. We investigated the various effects of changes in carbon availability on cyanobacterial buoyancy with continuous cultures of Microcystis aeruginosa *Kuetz. emend. Elenkin (1924), a notorious bloom-former. Although CO_2 limitation of photosynthesis can promote buoyancy in the short term by preventing the collapse of turgor-sensitive gas vesicles and/or by limiting polysaccharide accumulation, we found that sustained carbon limitation restricts buoyancy regulation by limiting gas vesicle as well as polysaccharide synthesis. These results provide an explanation for the positive effects of bicarbonate enrichment on cyanobacterial nitrogen uptake and bloom formation in lake experiments and may help to explain the pattern of cyanobacterial dominance in phosphorus-enriched, low-carbon lakes.*

Key index words: blooms; Cyanobacteria; cyanobacterial buoyancy; dominance; gas vacuolation; gas vesicles; lake eutrophication; limiting nutrients; Microcystis aeruginosa

The importance of gas vacuoles to the success of nuisance-bloom-forming cyanobacteria has been recognized for decades (Fogg 1969). The advantages that cyanobacteria derive from buoyancy regulation effected by changes in the cellular content of the protein inclusions now known as gas vesicles include better access to light and atmospheric CO_2 at or near the surface as well as to nutrient-rich layers well below the surface of stratified water columns (Fogg and Walsby 1971).

Gas vesicles, of course, have a positive effect on buoyancy; also involved in cyanobacterial buoyancy regulation are storage polymers such as polysaccharide that act as ballast molecules with negative effects on buoyancy (Gibson 1978, Kromkamp 1987). The relative contributions of these different cell components to the buoyancy status of a cyanobacterium depend on interactive effects of environmental factors, especially light and nutrient availability. For thorough reviews of research on the role of light intensity in cyanobacterial buoyancy regulation as well as of molecular studies on gas vesicle proteins, see Oliver (1994) and Walsby (1994).

The association of cyanobacterial blooms with low ambient nutrient concentrations first appeared in print in Pearsall's (1932) classic study of English lakes, and the paradoxical nature of this association remained a topic of discussion three decades later (Hutchinson 1967, Fogg 1969). Over the next two decades, there were observations (Reynolds and Walsby 1975, Lewis 1983) that apparently explained the paradox by linking gas vesicle accumulation in cells with nutrient limitation as well as with light limitation of growth. However, those observations conflicted with experimental evidence indicating that cyanobacterial blooms could be induced *in situ* by additions of limiting N or P (Klemer 1973, 1976), and subsequent experiments with continuous cultures showed that the gas vesicle content of N-limited *Oscillatoria rubescens* varied directly with the availability of N (Klemer 1978, Klemer et al. 1982, see also review by Reynolds 1987). More recently, Klemer et al. (1995) described both short-term, negative effects (polyphosphate as ballast) and longer term, positive effects (gas vesicle synthesis) of P additions on the buoyancy of P-limited *Aphanizomenon schindleri*. To some extent, such differences in short-term and long-term effects of limiting nutrients may account for the different interpretations of their roles in cyanobacterial buoyancy regulation. Another confounding factor is the tendency of enrichment with one limiting nutrient (e.g. N) to induce limitation by another (e.g. P). In the case of non-N_2-fixing cyanobacteria, additions of either N or P may simply substitute one restriction on buoyancy for another (Klemer 1976, Klemer and Konopka 1989).

In the 1970s and early 1980s, experiments in several laboratories indicated that, unlike N and P, C

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mimicked light in promoting gas vesicle accumulation and cyanobacterial buoyancy by limiting photosynthesis and growth (Dinsdale and Walsby 1972, Booker and Walsby 1981, Klemer et al. 1982, Paerl and Ustach 1982). In articles based on those experiments, the authors interpreted the effects of dissolved inorganic carbon (DIC) on cyanobacterial buoyancy in terms of Walsby's hypotheses on gas vesicle regulation. That is, buoyancy changes were explained by growth-rate effects (dilution or concentration) on the relative cell volume occupied by gas vesicles and/or by photosynthesis-driven changes in turgor pressure on gas vesicles; Gibson's (1978) notions about polysaccharide ballast were, as yet, unappreciated.

In contrast to the consistent laboratory findings on this topic, *in situ* experiments on the role of C in buoyancy regulation by cyanobacteria produced what appeared to be contradictory evidence. Klemer et al. (1988) found that surface additions of bicarbonate to mesocosms containing natural (*Anabaena*) and induced (*Oscillatoria*) blooms in low-C lakes hastened their collapse, but they also found that bicarbonate enrichment induced more rapid and more intense surface blooms by subsurface (metalimnetic) populations of N- and P-enriched *Oscillatoria*. The negative effect of bicarbonate enrichment on the buoyancy of populations already at the surface might be viewed as consistent with nutrient-stimulated growth (gas vesicle dilution), photosynthesis-driven turgor increases leading to gas vesicle collapse or, possibly, polysaccharide accumulations in cells whose reserves of N or P were depleted in the presence of excess DIC. However, the more positive buoyancy responses by N- and P-enriched, metalimnetic populations in the presence of additional bicarbonate cannot be explained by nutrient-limited growth (gas vesicle concentration) or by reductions in turgor or polysaccharide ballast. Those responses were accompanied by increases in N uptake and, presumably, in gas vesicle synthesis.

In sum, the effects of carbon on cyanobacterial buoyancy have resisted easy identification and interpretation at times, especially in lake experiments. Much of the difficulty in discerning which of carbon's effects is operative resides in the variety of those effects; carbon availability governs not only the generation of osmotically active photosynthate and polysaccharide ballast, but also, as we demonstrate later, the production of gas vesicles.

MATERIALS AND METHODS

To investigate the different roles of carbon in cyanobacterial buoyancy regulation, we observed the effects of pulses of limiting nutrients on continuous cultures of a highly gas vacuolate strain of *Microcystis aeruginosa* supplied with different ratios of nitrogen and carbon and grown on a light–dark cycle in "cyclostats." The culture, *M. aeruginosa* AK1, was isolated by A. E. Konopka, Purdue University, from Lake Mendota, Wisconsin, and was supplied to us by Dr. Dorothy L. Parker, University of Wisconsin at Oshkosh. Our cyclostat systems consisted of 2-L reaction kettles with intermittent overflow siphons, magnetic stirrers, and Gilson peristaltic pumps. All cultures were grown in modifications of filter-sterilized Gorham's medium diluted 1:4 (Hughes et al. 1958). The principal modifications included several different NH_4-N and HCO_3-C concentrations, the use of NaCl to keep the [Na] constant, and the addition of the trace elements Mo, Mn, Zn, Co, and B at a 1:4 dilution of Gaffron's trace element concentrations (Hughes et al. 1958). All experiments were run on a 12:12 h LD cycle in an environmental chamber at 20° C. Irradiance from "warm-white" fluorescent lamps was measured at the surface of the growth vessels with a Biospherical Instruments QSL-100 4π light sensor.

In each experiment, the buoyancy status of *M. aeruginosa* was determined with live samples incubated in the dark in hemacytometer chambers. After 30 min, the number of buoyant cells (under the coverslip) was expressed as a percentage of the total number of cells counted ($n > 150$) with a microscope (Walsby and Booker 1980). Relative gas vacuolation (RGV) was determined nephelometrically with a Turner 111 fluorometer connected to a μamp meter and fitted with a green (Corning #58) excitation filter and neutral-density, emission filters. RGV represents the ratio of light scattered by gas vesicles (ΔT) to light scattered by cyanobacterial cells (T_c) after their gas vesicles have been collapsed by pressure (Walsby and Booker 1980). The RGV measurements in this study were also corrected for background light scattering (Klemer et al. 1982). Cell numbers and cell volumes were determined with a Coulter Counter Model Zb and a Coulter MCV accessory. Total polysaccharide (phenol method) and protein in samples collected on Whatman GF/F glass-fiber filters were determined by methods described in Herbert et al. (1971). An Orion 940 Ionalyzer and NH_3 (Orion) and CO_2 (HNU) electrodes were used to monitor culture-vessel concentrations of NH_4-N and DIC.

Specific experimental protocols. In preliminary experiments (Fig. 1), we ran N-limiting cyclostats at a dilution rate of 0.33 day^{-1} and with an incident irradiance (I_o) of 55 μmol photons$\cdot m^{-2}\cdot s^{-1}$. These cultures received the same limiting nutrient concentration (0.1 mM NH_4Cl) but different concentrations of bicarbonate (0.5 mM and 2.0 mM) in their input media. Once they had attained the oscillating steady state typical of cyclostat cultures, we pulsed them with 0.2 mM NH_4-N to simulate the effects a natural population might experience in sinking through the chemocline of a stratified lake.

In the experiments described in Figures 2 and 3, N- and C-limiting cyclostats were run with dilution rates of 0.16–0.18 day^{-1} and at an I_o of 110 μmol photons$\cdot m^{-2}\cdot s^{-1}$ In these experiments, the limiting nutrients were supplied in the input media at C:N (atomic) ratios that ranged from 20:1 to 0.625:1, and nutrient limitation was relieved by pulses of 0.4 mM NH_4Cl (Fig. 2a–c) or 4 mM $NaHCO_3$ (Fig. 2d), by an increase in dilution rate from 0.16 to 0.32 day^{-1} (Fig. 2e), or by increases in pH-stat-controlled aeration (Figs. 2f, 3). Increases in C supply by aeration were effected by decreases in the pH setting that triggered aeration.

RESULTS

Preliminary experiments with nitrogen-depleted cultures. Microcystis in a high-C, N-limiting (C:N = 20) cyclostat responded to an ammonium pulse with an increase in buoyancy (peak = 100%) that was sustained through three photoperiods (12:12 h LD) (Fig. 1a). In contrast, an ammonium pulse of the same magnitude to a low-C (C:N = 5) culture elicited a less positive (peak = 77%) and a shorter lived (ca. one photoperiod) buoyancy response (Fig. 1b). While in oscillating steady state prior to the N pulses, both cultures experienced N depletion below the limit of detection (<7 μg or 0.5 μmole $N\cdot L^{-1}$) of our am-

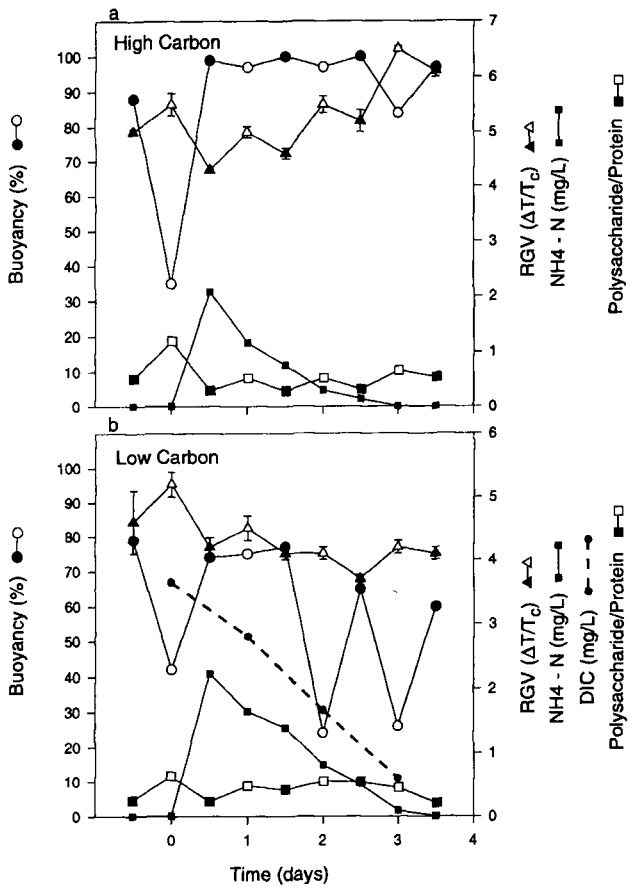

FIG. 1. Changes in *Microcystis aeruginosa* after a N pulse. a) Changes in cell buoyancy (buoyant cells as a percentage of the total cell count in a sample), RGV, polysaccharide : protein ratio, and [NH_4-N] after a N pulse at T = 0 to a cyclostat culture of *M. aeruginosa* receiving 2.0 mM $NaHCO_3$. Where closed symbols alternate with open symbols, the former indicate determinations at the beginning of a photoperiod and the latter those at the end of a photoperiod. RGV ($\Delta T/T_c$) determinations are presented with their 95% confidence limits. b) Changes in cell buoyancy, RGV, polysaccharide : protein ratio, [NH_4-N], and [DIC] after a N pulse at T = 0 to a culture receiving 0.5 mM $NaHCO_3$.

monia electrode, and, at time 0, the cultures did not differ greatly with respect to the buoyancy status or the relative polysaccharide or gas vesicle (RGV) contents of their cells. Within 1 day of the pulses, polysaccharide : protein ratios had fallen in both cultures. Within 3 days, RGV had fallen to 4.2 in the low-C culture but had risen to 6.5 in the high-C culture. After the pulses, the [DIC], measured at the end of each photoperiod, fell steadily in the low-C culture to less than 1 mg $C \cdot L^{-1}$ (<80 μM). In the high-C culture, [DIC] never fell below 1 mM.

C versus N effects on cyanobacterial buoyancy. To obtain a better understanding of the limiting effects of C on the buoyancy and buoyancy-related cell characteristics of *M. aeruginosa*, we ran a series of experiments with a broader range of C:N ratios in cyclostat input media, a higher irradiance (I_o = 110 vs. 55 μmol photons $\cdot m^{-2} \cdot s^{-1}$) and lower dilution rates (0.16–0.18 vs. 0.33 day^{-1}) than those used in the preliminary experiments. In comparison to the latter, the three N-pulse experiments of this series (Fig. 2a–c) produced differences with respect to initial states (e.g. buoyancy) but similarities with respect to long-term (>1 generation time) responses. With C:N ratios of 10 and 20 in the nutrient input, *Microcystis* experienced continuous depletion of N in growth vessel media, as had the cultures used in the preliminary experiments, but, unlike the latter, none of the cells in these cultures was buoyant prior to ammonium pulses. However, as in the preliminary experiment with the high-C culture (Fig. 1a), N enrichment produced not only rapid and persistent reductions in polysaccharide content but also increases in gas vesicle content and in the percentage of cells that were buoyant after a generation time (Fig. 2a, b). With a C:N ratio of 5 in the inflowing medium and under the new oscillating steady-state conditions, *Microcystis* experienced not the continuous N depletion of the low-C culture in the preliminary experiments (Fig. 1b) but C depletion (DIC < 10 μM) during the photoperiod and N depletion (NH_4-N < 0.5 μM) during the dark period (data not shown). With alternating C and N depletion prior to the N pulse, polysaccharide ballast was relatively low, gas vesicle content was relatively high, and a majority of the cells were buoyant, e.g. 97% at the beginning of a photoperiod (Fig. 2c). Nevertheless, after a generation time in N-enriched medium, cell polysaccharide, gas vesicle content, and buoyancy had decreased (Fig. 2c) as they had, along with [DIC], in the preliminary low-C experiment (Fig. 1b).

In C-limiting cyclostats (Fig. 2d–f), *Microcystis* responded to additional DIC supplied as bicarbonate (in a discrete pulse or at a higher dilution rate) or as CO_2 (in air) with increases in cell polysaccharide, gas vesicle content, and buoyancy over the course of a generation time or more. In general, gradual increases in [DIC], provided by an increase in dilution rate or by pH-stat-controlled aeration (Fig. 3), produced smaller initial increases in polysaccharide and transient decreases in buoyancy than 4-mM pulses of bicarbonate. Over the course of a generation time, the use of aeration to keep the pH from rising above 8.0 (Fig. 2f) also produced larger increases in gas vesicle content and buoyancy than either an increase in dilution rate (Fig. 2e) or a pulse of bicarbonate (Fig. 2d). When we increased the aeration time by resetting the pH stat to pH 7.0, cell gas vesicle content increased again, cell polysaccharide also increased, and buoyancy remained high, but when we provided continuous aeration during photoperiods and the pH approached 6.0, cell gas vesicle content and buoyancy decreased as polysaccharide content remained high (Fig. 3). We repeated this experiment three times, including once with lower trace metal concentrations (a 1:250 rather than a 1:4 dilution of Gaffron's concentrations), and observed the same pattern of responses each time.

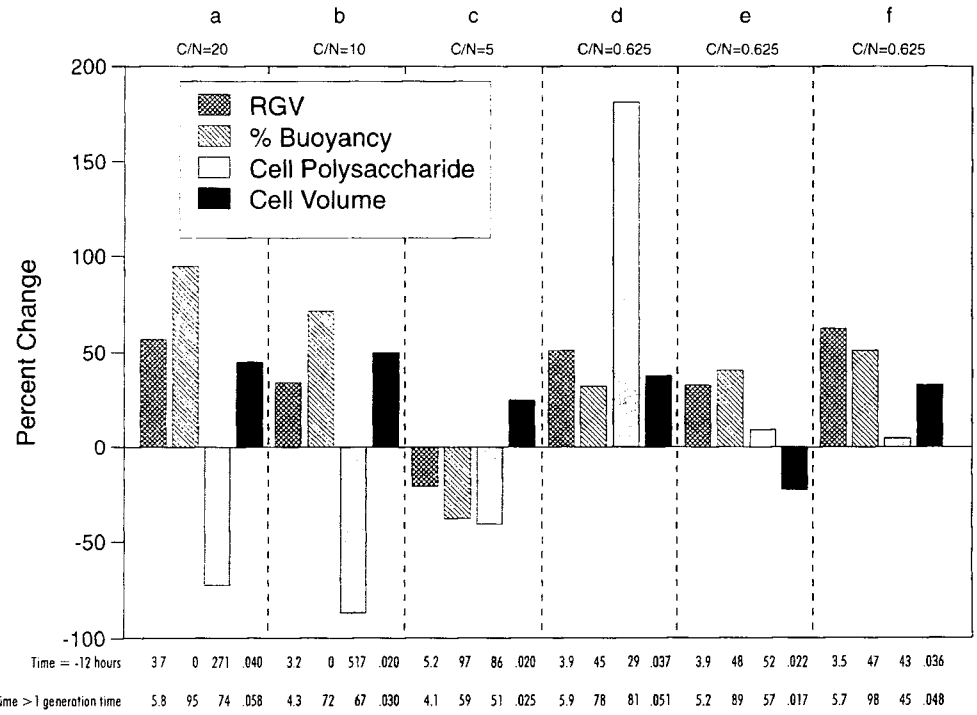

FIG. 2. Percentage of changes in RGV, cell buoyancy (as changes [final–initial] in the actual percentages of cells that were buoyant), cell polysaccharide, and cell volume after N pulses (a–c), a $NaHCO_3$ pulse (d), an increase in dilution rate (e), and pH-stat-controlled aeration at pH 8.0 (f). Prior to treatment, cyclostat cultures a and b were N-limited, d–f were C-limited, and c was alternately N- (AM) and C- (PM) depleted. The numbers directly under each bar represent the actual AM values (determined at the beginning of each photoperiod) for each parameter 12 h prior to and at least one generation time after each treatment. Here, RGV is expressed as $\Delta T/T_c$, cell buoyancy as the percentage of cells that were buoyant, cell polysaccharide as μg per μL of cell volume, and cell volume as μL per mL of culture.

DISCUSSION

Buoyancy responses to the relief of N limitation are both immediate and sustained because they involve short-term reductions in polysaccharide ballast and longer term increases in gas vesicle synthesis. Both effects were evident in the buoyancy responses by the high-C (C:N = 10–20) cultures. The response patterns involved in transitions from N limitation or from alternating N and C limitation to sustained C limitation may be more complicated, but despite differences in initial growth conditions and buoyancy states the two low-C (C:N = 5) cultures exhibited similar long-term responses to N pulses. After a generation time, the shift toward sustained C limitation had produced reductions in the relative gas vesicle content and buoyancy status of both cultures.

With sustained C limitation (Fig. 2d–f), the initial (T = −12 h) gas vesicle content of *Microcystis* was as low as it was with sustained N limitation (Fig. 2a, b) under the growth conditions (higher irradiance, lower dilution rates) of the second series of experiments, and because polysaccharide content was also low during C limitation the initial buoyancy of *Microcystis* was greater when C-limited than when N-limited (Fig. 2). However, under C limitation, less than half the *Microcystis* cells were buoyant (Fig. 2d–f), and as indicated by the small changes in buoyancy prior to aeration (Fig. 3) the range of daily buoyancy regulation was restricted. In a natural situation (e.g. a stratified lake), limited gas vesicle and polysaccharide production could mean limited vertical migration between adequate light above and adequate nutrients below. Thus, by limiting the synthesis of cell components that are important in cyanobacterial buoyancy regulation, DIC could limit the ability of bloom formers to succeed in stratified water columns.

Evidence that DIC is a factor that can limit buoyancy may seem paradoxical because it conflicts with published evidence that C limitation of photosynthesis has a positive effect on cyanobacterial buoyancy. However, the claim that CO_2 limitation of photosynthesis promotes buoyancy in cyanobacteria by limiting the turgor-effected collapse of gas vesicles holds only for cells with sufficient gas vesicles to be buoyant. Dinsdale and Walsby (1972) demonstrated that CO_2 limitation could prevent the loss of buoyancy in cells with enough turgor-sensitive gas vesicles to be buoyant. On the ballast side, C limitation not only limits the accumulation of polysaccharide but also promotes polysaccharide consumption. Hence, C limitation could produce as well as main-

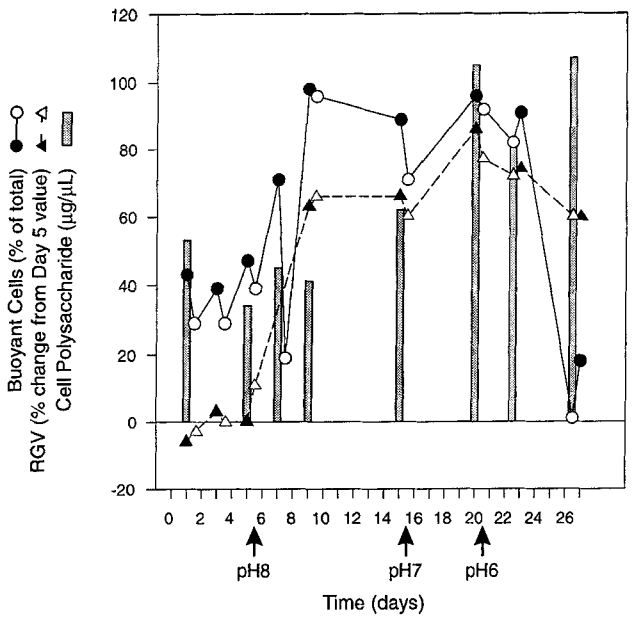

FIG. 3. Changes in cell buoyancy (buoyant cells as a percentage of total cells counted), RGV (expressed as a percentage of the mean value just prior to aeration), and mean polysaccharide content (the mean of pre- and post-photoperiod determinations on the days indicated) in response to pH-stat-controlled aeration of a cyclostat culture of *M. aeruginosa* that was C-limited prior to aeration. Cell buoyancy and RGV determinations are represented by closed symbols at the beginning of a photoperiod and by open symbols at the end of a photoperiod.

tain positive buoyancy in cells with sufficient gas vacuolation.

More importantly, the only evidence for gas vesicle–related increases in buoyancy during CO_2 limitation is from experiments with cyclostat cultures that underwent transitions from N to C limitation (Klemer et al. 1982). In those experiments, the *Oscillatoria* cultures that were subjected to CO_2 limitation were rich in polysaccharide as a result of prior N limitation and were receiving ca. 0.4 mM bicarbonate in the inflowing medium. Their gas vesicle increases were very likely the result of a gradual increase in N availability per cell as biomass fell after CO_2 was stripped from the air stream and the growth vessels; such an increase would have enabled those cells to synthesize the protein necessary for gas vesicle production at the expense of their polysaccharide ballast.

Although cyanobacterial blooms have long been associated with bicarbonate-rich waters (Naumann 1932, Pearsall 1932, Moyle 1945, Talling et al. 1973), such blooms have been linked also with the depletion of DIC. The ability of cyanobacteria to compete at low CO_2 concentrations has been construed as the means by which they attain dominance (King 1970, Shapiro 1973). In very low bicarbonate or mildly acidic waters, some cyanobacteria do flourish. At pH 6 or less, in waters at or near equilibrium with the atmosphere, the [DIC] is only about 10 μM (Stumm and Morgan 1981). However, the cyanobacteria that prevail under those conditions are usually benthic or meroplanktonic taxa and, in general, are species that do not synthesize gas vesicles or concentrate at the surface. Brock (1973) found cyanobacteria absent from benthic habitats where the pH was less than 4.0 but present in mats in the pH range between 4.0 and 5.0. On the basis of his benthic samples and a literature survey, he concluded that because cyanobacteria are "uncommon" even in mildly acidic waters (pH 5.0–6.0) mild acidification of lakes should control or eliminate cyanobacterial blooms.

In fact, the results of some lake experiments seem to support Brock's conclusion as to the fate of cyanobacteria in acidified lakes. Carl Watras (pers. commun.) found that N_2-fixing, filamentous cyanobacteria (*Anabaena* sp.) disappeared from the epilimnion as the pH of Little Rock Lake, Wisconsin, fell to 5.2. Also, during the acidification of Lake 302S in the Experimental Lakes Area (ELA) of northwestern Ontario, a littoral cyanobacterial association of *Anabaena* sp. and *Lyngbya* sp. was dominant until the pH fell below 5.0 and filamentous Chlorophyta became dominant under C-limiting conditions (Turner et al. 1995). However, when the pH fell below 5.0, a cyanobacterial succession from *Anabaena* and *Lyngbya* to *Merismopedia* to *Chroococcus* ensued in that lake. And during the experimental acidification of another ELA lake, Lake 223, Findlay and Kasian (1986) found that non–gas vacuolate cyanobacteria first appeared in 1978 when the average pH was ca. 5.9 and continued to increase through 1983 as the average pH fell to ca. 5.1 (David Findlay, pers. commun.). Thus, other cyanobacteria as well as certain eukaryotic algae seem to respond more positively to the combination of low pH and low DIC than gas vacuolate bloom formers. We realize that acidification has many environmental impacts including increased metal toxicities to which cyanobacteria are sensitive, especially below pH 6 (Gensemer and Kilham 1984). Our point here is that gas vacuolate cyanobacteria seem to be particularly sensitive to acidification. In our pH-stat-controlled aeration experiments, RGV and buoyancy fell before the pH reached 6.0, at 6.2, for example, and a large reduction in trace metal concentrations made no difference.

The reduction in RGV and loss of buoyancy that we observed repeatedly in pH-stated cultures of *Microcystis* as the pH approached 6.0 may reflect an impairment of gas vesicle synthesis that gas vacuolate cyanobacteria also experience as pH falls in lakes. Gas vesicles account for between 6 and 19% of cell protein in cyanobacteria (Oliver 1994), and some of the amino acids in gas vesicle protein (Walsby 1994) are among those that require anaplerotic bicarbonate fixation for the assimilation of their N (Turpin et al. 1991). The hypothesis (Klemer 1991, Klemer et al. 1995) that gas vesicle synthesis is selected against

under C limitation is being tested and the results of our initial experiments support it.

In enriched lakes that are low in DIC, N_2-fixing species seem to fare better than those gas vacuolate cyanobacteria that require fixed N (Klemer 1991). The former may have two advantages in low-C lakes with respect to other taxa, including those that are at least as efficient at DIC uptake under non-N-limiting conditions. Among gas vacuolate cyanobacteria, N_2-fixers can balance their assimilation of C and N more readily (Paerl and Kellar 1979). With access to atmospheric reservoirs of N as well as C, N_2-fixing cyanobacteria are able to assimilate N in the presence of bicarbonate as the result of photosynthesis-driven increases in pH that promote the invasion of atmospheric C (Schindler 1977). With C and N in balance (Fig. 2c), P-sufficient cyanobacteria can avoid both sustained nutrient limitation of gas vesicle synthesis and sustained polysaccharide ballast increases. Second, in P-enriched, low-carbon lakes, N limitation may favor N_2-fixers in the competition for DIC as well as for N (Klemer et al. 1995).

Even in lakes with high [DIC], C limitation may develop in intense cyanobacterial surface blooms and sustain their buoyancy, but, like the depletion of other nutrients, the depletion of DIC in the midst of a bloom is more accurately perceived as a consequence than as a cause of the bloom (Klemer 1991). The results of the laboratory experiments described here together with results from lake experiments (Klemer 1985, Klemer et al. 1988) indicate that the ability to redistribute (vertically) as well as to accumulate biomass that enables gas vacuolate cyanobacteria to dominate eutrophic lakes is a direct function of the availability of C as well as of N and P. In stratified eutrophic lakes, bloom populations develop initially in subsurface layers or in benthic habitats (Reynolds and Walsby 1975, Sirenko 1987) in which photosynthesis is much more likely to be limited by light than by nutrients. In such lakes, the intensity of cyanobacterial blooms often increases rather suddenly (due to vertical migration), not as gradually as one might expect if dominance at the surface were simply the result of relatively small differences in nutrient uptake kinetics. When nutrient supplies and water column stability permit effective buoyancy regulation as well as biomass generation, cyanobacteria create the conditions under which they dominate the phytoplankton in the process of forming their blooms.

In forming blooms, cyanobacteria alter conditions in surface layers in ways detrimental to other phytoplankton. Depletion of ambient C, N, P, or Fe (Murphy et al. 1976) and high pH (Shapiro 1990) may be among the conditions that favor cyanobacteria in surface blooms: however, they do not account for the development of the biomass or the buoyancy-regulating apparatus required for bloom formation. During a recent experiment, Joseph Shapiro (pers. commun.) observed that cyanobacterial blooms developed simultaneously in two basins of the same lake despite large differences in pH and [CO_2]. Consequently, he has concluded that high pH/low CO_2 conditions do not determine bloom initiation or magnitude, but he maintains that superior uptake kinetics for CO_2 account for cyanobacterial dominance of dense phytoplankton accumulations such as those generated during blooms.

We maintain that, in stratified eutrophic lakes, buoyancy regulation can confer an advantage with respect to access to light that allows highly gas vacuolate cyanobacteria to prevail over other species, even those with superior growth kinetics for a particular limiting nutrient (Klemer 1985). A clear-cut example of this advantage was described by Knoechel and Kalff (1975), who observed slower growing but rising cyanobacteria replacing faster growing but sinking diatoms. The paradox of nutrient depletion in the midst of cyanobacterial blooms that are associated with nutrient-rich waters is resolved for C as well as for other nutrients by focusing on where the nutrient requirements for bloom initiation are met. Even in the case of C, the requirements for midsummer blooms are met, initially, not at the surface of a stratified lake, but at depth, where nutrient conditions permit gas vesicle synthesis and the transport of storage forms of N and P toward the surface. Once at the surface, cyanobacteria may establish a monopoly on light as well as on other requirements for growth.

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