Gas vesicle collapse by turgor pressure and its role in buoyancy regulation by *Anabaena flos-aquae*

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Filaments of *Anabaena flos-aquae* lost over half of their gas vesicles when exposed to light of high irradiance ($> 115 \mu\text{mol m}^{-2} \text{s}^{-1}$) for long periods (up to 23 h). An investigation using different irradiances showed that the major loss of gas vesicles occurred after prolonged exposure to irradiances exceeding $80 \mu\text{mol m}^{-2} \text{s}^{-1}$. In a time-course experiment it was found that 56% of the gas vesicles were lost after 16 h exposure to an irradiance of $135 \mu\text{mol m}^{-2} \text{s}^{-1}$. Most of this loss could be accounted for by collapse under turgor pressure, which rose by 0.24 MPa to 0.54 MPa after 16 h. A further unexplained loss of 15% after 23 h exposure may indicate that prolonged high irradiance can cause weakening of gas vesicles in the cells. In this time-course experiment 98% of the filaments lost their buoyancy after 6 h exposure, before any gas vesicles were lost; this buoyancy change was quantitatively accounted for by the rise in cell carbohydrate. The relative importance of gas vesicle collapse and carbohydrate accumulation as mechanisms of buoyancy loss is discussed in relation to vertical movements of planktonic cyanobacteria in lakes.

Introduction

Planktonic cyanobacteria derive their buoyancy from gas vesicles. It was shown in *Anabaena flos-aquae* that the gas vesicle content was highest in cells cultured at low irradiance and that transfer to high irradiances could cause a rapid loss of buoyancy (Walsby, 1971). It was speculated that the buoyancy loss occurred either as a consequence of gas vesicle production not keeping pace with the accumulation of new cell material or through the collapse of gas vesicles under increasing cell turgor pressure (Walsby, 1970). Cells grown at high irradiances were shown to produce higher turgor pressures that would have caused some of the weaker gas vesicles to collapse (Walsby, 1971). Dinsdale & Walsby (1972) showed that an increase in irradiance caused a turgor rise of over 0.1 MPa in only 4 h and that this accounted for the collapse of the weaker gas vesicles and the loss of buoyancy by the filaments. The turgor rise was dependent on photosynthesis: about half the rise was generated by photosynthetic products (Grant & Walsby, 1977) and half by the light-stimulated uptake of potassium ions (Allison & Walsby, 1981).

The development of methods for measuring the

buoyant density of cells (Oliver *et al.*, 1981), gas vesicle volume (Walsby, 1982) and the ballast mass of the other principal cell components (i.e. their mass minus the mass of water they displaced) made it possible to determine the causes of buoyancy loss in different cyanobacteria (Oliver *et al.*, 1982; Oliver & Walsby 1984). The major cause of density increase in filaments of *Anabaena flos-aquae*, after a day's exposure to high irradiance ($120\text{--}150 \mu\text{mol m}^{-2} \text{s}^{-1}$), was the decrease in gas vesicle content, to half of the original value: increases in ballast contributed by protein and carbohydrate would not have been large enough by themselves to have counteracted the initial state of buoyancy (Oliver & Walsby, 1984).

The cause of buoyancy loss was different, however, in other cyanobacteria that possessed gas vesicles too strong to be collapsed by turgor pressure. In *Oscillatoria agardhii* gas vesicle production ceased on transfer to high irradiance; buoyancy was lost due to the accumulation of carbohydrate and, to a lesser extent, protein (Oliver *et al.*, 1982; Utkilen *et al.*, 1985). In *Microcystis* sp. buoyancy loss could be accounted for entirely by the increase in carbohydrate (Thomas & Walsby, 1985). Diel changes in carbohydrate content, which increased during the day and decreased at night, were found to be

positively correlated with changes in cell density and inversely correlated with the buoyancy state (Kromkamp & Mur, 1984; Thomas & Walsby, 1986; Konopka *et al.*, 1987a; Kromkamp *et al.*, 1988). These quantitative studies have shown that there are at least three physiological mechanisms – gas vesicle collapse, cessation of gas vesicle synthesis, and carbohydrate accumulation – which may contribute to the loss of buoyancy by cyanobacteria at high irradiance.

Studies on planktonic cyanobacteria in lakes have shown that buoyancy regulation in response to light explains how these organisms perform controlled vertical migrations (Walsby & Klemer, 1974; Konopka *et al.*, 1978; Walsby *et al.*, 1983). In some cases buoyancy loss at high irradiances near the water surface has been correlated with rise in turgor pressure (Walsby & Klemer, 1974; Reynolds, 1973, 1975; Konopka *et al.*, 1978; Konopka, 1982) though it was not shown that turgor rise was the cause of the buoyancy loss. In other instances buoyancy loss has been correlated with carbohydrate rise (e.g. van Rijn & Shilo, 1985) or shown to be caused by carbohydrate accumulation (Walsby *et al.*, 1989; B. W. Ibelings and others, unpublished). Detailed studies by Kromkamp *et al.* (1986) and Konopka *et al.* (1987b) on *Aphanizomenon flos-aquae* have also called into question the importance in buoyancy regulation of gas vesicle collapse by turgor pressure. They found that carbohydrate accumulation caused buoyancy loss after only 2 h at high irradiance whereas gas vesicle loss occurred only after 5 h.

These findings have prompted our reinvestigation of the mechanism of buoyancy loss by *Anabaena flos-aquae*, in which a number of uncertainties remained. First, the experiments of Oliver & Walsby (1984) showed a large loss of gas vesicles (up to 50%) after 24 h but no information was obtained on changes over shorter periods. Secondly, although substantial short-term turgor pressure rises had been demonstrated in this organism (Grant & Walsby, 1977; Allison & Walsby, 1981) they would not have caused as much as 50% of the gas vesicles to collapse. The largest turgor rise, observed by Dinsdale & Walsby (1971), was 0.17 MPa over 4 h at 10 klx (about $200 \mu\text{mol m}^{-2} \text{s}^{-1}$), which caused collapse of only 15% of the gas vesicles. Thirdly, no information was available on the short-term changes in carbohydrate.

To resolve these uncertainties we have performed detailed quantitative analyses of the major factors that affect buoyancy during the exposure of *Anabaena flos-aquae* to light of high irradiance. The study was facilitated by the development of a capillary compression tube in which replicate measurements of gas vesicle volume could be made at frequent intervals. The investigation has demonstrated the rapid accumulation of carbohydrate, which can cause buoyancy loss before

there is significant destruction of gas vesicles. It has also demonstrated turgor rises that were higher than previously recorded and do cause collapse of half the gas vesicles within a 16 h light period. These findings are discussed in relation to buoyancy regulation by water-bloom-forming cyanobacteria.

Methods

Cultures. *Anabaena flos-aquae* CCAP 1403/13F (Booker & Walsby, 1979), the same strain as used by Oliver & Walsby (1984) and in other investigations referred to above, was cultured in the medium described by Walsby & Booker (1980). Cultures were grown with aeration at 20 °C at a photosynthetically active photon irradiance (PAR) of $20 \mu\text{mol m}^{-2} \text{s}^{-1}$. Buoyant filaments that had floated were harvested from the surfaces of cultures left standing at $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ overnight.

Exposure to light. Shallow layers (<5 mm) of cultures in 2-litre capacity Erlenmeyer flasks were bubbled with moist air at 0.8 litres min^{-1} and illuminated from below with five fluorescent lamps (Thorn 30 W warm white). The PAR was determined by measurements with a Macam quantum sensor held at the illuminated bottom surface (I_0) and just above the culture (I_b) and the mean irradiance in the culture was calculated by integration, with the equation of van Liere & Walsby (1982).

Buoyancy state. Samples of the suspension were left in a haemocytometer chamber (depth 0.4 mm) for 15 min to allow the separation of floating filaments, under the coverslip, and sinking filaments, on the platform. Counts of the two classes were made by microscopy (Walsby & Booker, 1980).

Turgor pressure. Measurements were made by pressure nephelometer of the critical pressure distribution of gas vesicles in cells suspended in culture medium and in medium containing 0.5 M-sucrose (Walsby, 1971). The collapse of gas vesicles was determined from the change in turbidity measured by pressure nephelometer (Walsby, 1973). Readings were made on suspensions diluted to give an initial turbidity reading not exceeding 40 nephelometric units (NU). It was shown by the procedure of Deacon & Walsby (1990) that below 60 NU there was a linear relationship between turbidity and concentration of gas-vacuolate filaments of *A. flos-aquae*. The turgor pressure was calculated as $P = \bar{p}_c - \bar{p}_a$, where $\bar{p}_c$ and $\bar{p}_a$ are the median critical pressures determined in the sucrose solution and culture medium, respectively (Walsby, 1988). The standard deviation of the turgor pressure measurement was calculated as $(s_c^2 + s_a^2)$, where s_c and s_a are the standard deviations of $\bar{p}_c$ and $\bar{p}_a$ respectively.

Gas vesicle volume. The volume of gas vesicle gas space in a sample was determined from the contraction caused by application of pressure in a capillary compression tube (Walsby 1982) using the method of Oliver & Walsby (1988) with the following modifications. Triplicate measurements were made in quick succession using three compression tubes inserted into three pressure tubes housed in a common thermostatted water jacket. The time required for temperature equilibration was reduced to about 5 min by filling the pressure tubes with water to improve thermal contact with the compression tubes. The culture samples were enriched with 0.05 M-sucrose and degassed by evacuation to prevent subsequent bubble formation in the compression tubes; the addition of sucrose increased the external osmotic pressure, and consequently decreased the turgor pressure by more than one atmosphere, and so prevented gas vesicle collapse on releasing the vacuum.

Mass and ballast mass of cell constituents. Dry mass was determined by weighing the cyanobacteria collected on filters dried for 48 h at 80 °C (Oliver *et al.*, 1985). Protein was determined with the Coomassie blue G-250 reagent (Pierce) using bovine serum albumin as a standard. Carbohydrate was determined with the anthrone reagent (Herbert *et al.*, 1971) using D-glucose as a standard. The mass of carbohydrate was calculated as 162/180 of the glucose equivalent to allow for the gain in water on hydrolysis. Lipid was determined gravimetrically after extraction into methanol/chloroform/0.15 M-acetic acid (10:5:4, by vol.) and phase separation into chloroform (Thomas & Walsby, 1985; modified from Bligh & Dyer, 1959). All determinations were made in triplicate.

Results

Gas vesicle loss confirmed

We investigated buoyancy loss and changes in gas vesicle gas volume of *Anabaena flos-aquae* cultures exposed to high irradiances, in the range 115–142 $\mu\text{mol m}^{-2} \text{s}^{-1}$, in seven different experiments lasting between 18 and 23 h. In each case all of the filaments sank and a substantial decrease in gas vesicle volume was recorded; the average loss represented 51% of the initial value. In four of the experiments similar cultures were maintained at low irradiance; they all showed small gains in gas vesicle content, representing an 11% increase over the initial value. The results of these experiments confirmed the findings of Oliver & Walsby (1984) that in this strain of *A. flos-aquae* exposure to high irradiance (120–150 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for long duration (24 h) results in a substantial loss of gas vesicles (up to 49% of the volume initially present).

The irradiance required for loss of buoyancy and gas vesicles

A series of cultures were exposed to light of different photon irradiances for 22 h to define further the conditions required to cause loss of buoyancy and gas vesicles (Fig. 1). Half of the filaments lost buoyancy after 22 h at 12 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and there was total loss at irradiances exceeding 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$; this is in general agreement with the findings of Walsby & Booker (1980). In contrast there was no significant change in gas vesicle content at irradiances below 17 $\mu\text{mol m}^{-2} \text{s}^{-1}$; the greatest loss of gas vesicles, over 50%, required exposure to irradiances exceeding 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This experiment was repeated with substantially the same results: 50% loss of buoyancy after 22 h at 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and maximum gas vesicle loss at irradiances exceeding 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The results of these two experiments suggested that buoyancy loss by *Anabaena flos-aquae* did not always depend on loss of gas vesicles.

Fig. 1. The buoyancy state of filaments (○) and gas vesicle volume (●) in samples of a culture exposed to different photon irradiances for 22 h. The arrow marks the initial gas vesicle volume.

The proportion of gas vesicle loss caused by turgor rise

Several experiments were performed to investigate the time course of changes in buoyancy state, gas vesicle volume and turgor pressure. The results of one experiment are shown in Fig. 2. After transfer to high irradiance the turgor pressure increased steadily by 0.24 MPa (2.4 bar) to a maximum of 0.544 MPa after 16 h, and then declined slightly. The gas vesicle volume in the culture increased slightly over the first few hours and then began to decline; by 10 h it had fallen below its initial value and by 16 h $55.6 \pm 5.5\%$ of the initial gas vesicle volume had been lost. The following analysis demonstrates that, within the error of measurement, all of this loss could be accounted for by the turgor pressure rise. The gas vesicle critical-pressure distributions at the start of the experiment and after 16 h at high irradiance are plotted in Fig. 3. The value of $\bar{p}_c$ (with the filaments in 0.5 M-sucrose) rose from 0.540 ± 0.001 to 0.641 ± 0.003 MPa whereas the value of $\bar{p}_a$ (with filaments in culture medium) decreased from 0.236 ± 0.006 to 0.097 ± 0.007 MPa. From this it is calculated that the turgor pressure rose from 0.304 ± 0.006 to 0.544 ± 0.008 MPa. Measurements made on large-scale graphs of the initial p_c distribution (Fig. 3) indicated that a pressure of 0.544 MPa would have caused the collapse of $52.9 \pm 0.6\%$ of the gas vesicles present at the start of the experiment: this value is within the standard deviation of the percentage that had been collapsed, $55.6 \pm 5.5\%$, as determined by compression tube measurements. Much or all of the gas vesicle loss at 16 h can therefore be explained by the turgor pressure rise.

Also shown in Fig. 3 are derivative curves of the percentage of the original gas vesicles that survived application of different pressures in the culture after 16 h high irradiation. If the only change from the start of the experiment had been a uniform turgor pressure increase

Fig. 2. Changes in cultures transferred to low ($5 \mu\text{mol m}^{-2} \text{s}^{-1}$) or high ($135 \mu\text{mol m}^{-2} \text{s}^{-1}$) photosynthetically active radiation (PAR): (a) turgor pressure (∇ , $\blacktriangledown$) and gas vesicle content ($\diamond$, $\blacklozenge$) at low (filled symbols) and high (open symbols) PAR; (b, c) buoyancy state ($\triangle$) and net ballast masses ($\blacksquare$) and changes in ballast due to gas vesicles ($\circ$), protein ($\bullet$) and carbohydrate ($\square$) of filaments in cultures at (b) high and (c) low PAR. The arrow in (b) shows the time (2.9 h) at which 50% of the filaments had lost buoyancy and net ballast mass had risen to zero.

in all of the cells then the derivative p_c curve (for cells in 0.5 M-sucrose) would have been identical with the lower (below 0.45) part of the original p_c curve. The discrepancy is probably explained by variation in the critical pressure distribution and turgor pressure rise in different cells. It is otherwise noted that all of the criteria for gas vesicle collapse by turgor, specified by Dinsdale & Walsby (1972), are met: (a) the turgor pressure rise was sufficient to explain the collapse of the gas vesicles that were lost; (b) the collapse-pressure curve of gas vesicles

in turgid cells starts to fall abruptly from zero applied pressure, indicating that the weakest of the remaining gas vesicles were on the verge of collapsing under turgor pressure alone; and (c) the increase in the p_c of gas vesicles, in cells in 0.5 M-sucrose, indicates that the weaker gas vesicles had disappeared as a result of the turgor pressure rise. In the final 24 h sample, however, there was evidence that further gas vesicle collapse occurred which could not be explained by turgor rise (see below).

Fig. 3. Distributions of critical pressures (p_c) of gas vesicles in cells suspended in 0.5 M-sucrose ($\circ$, $\bullet$) and apparent critical pressures (p_a) of gas vesicles in cells suspended in culture medium ($\square$, $\blacksquare$), at the start of the experiment (open symbols) and after 16 h exposure to $135 \mu\text{mol m}^{-2} \text{s}^{-1}$ (filled symbols). The broken lines are the derivative curves for p_c and p_a of the 45% of the original gas vesicles remaining. Vertical arrow shows that a turgor pressure of 0.545 MPa (value at 16 h) would collapse 46% (horizontal arrow).

A balance sheet of ballast mass

The large-scale loss of gas vesicles measured in this experiment would undoubtedly have caused loss of buoyancy in the absence of any other changes in cell constituents. However, while it took 7 h before the gas vesicle content had decreased below its initial level, 50% of the filaments had already lost their buoyancy after only 2.9 h at high irradiance, and by 6 h less than 2% of the filaments remained buoyant (Fig. 2b). This buoyancy loss must have been caused by other changes in the cell constituents, as was demonstrated by analysing the changes in the principal classes of components.

Much of the dry mass of the *Anabaena flos-aquae* filaments could be accounted for by protein, carbohydrate and lipid. There was an increase in dry mass of 81% during the 16 h at high irradiance (Table 1). Protein accounted for 40–50% of the dry mass in the first 4.5 h of incubation but it then steadily decreased in both total amount and relative amount until it represented less than 25% after 24 h. Carbohydrate, in contrast, steadily increased, from 25% to over 65% of the total dry mass. Analysis of lipid in some samples indicated that it represented about 11% of the total mass.

From the mass of each component (M_c), the ballast mass (M_b) was calculated, using the equation $M_b = M_c (1 - \rho_w/\rho_c)$, where ρ_c is the density of the component and ρ_w is the density of water, which is 998.2 kg m^{-3} at 20°C . By taking the value of ρ_c as 1550 kg m^{-3} for carbohydrate, 1300 kg m^{-3} for protein, and 1050 kg m^{-3} for lipid (see Thomas & Walsby, 1985) the total ballast mass of these components was calculated (Table 1). Since these components accounted for much of the dry matter

present, the sum of their ballast masses would account for much of the ballast mass of the cell material. Set against this is the negative ballast provided by the gas vesicle gas space of density $\rho_g = 1.2 \text{ kg m}^{-3}$. With this value added in, the net ballast mass of the gas-vacuolate cells is calculated. (Irrespective of its volume, cell water makes no contribution to the ballast mass, though it does, of course, affect the final density of the cell.)

The net ballast mass of the gas-vacuolate cells showed a steady increase throughout the incubation at high irradiance and became positive after 2.95 h (Fig. 2b). This is very close to the time of 2.90 h at which 50% of the filaments had lost their buoyancy. The analysis of net ballast mass therefore provides a quantitative account of the buoyancy change. When the changes in ballast of the individual components are analysed it is seen that carbohydrate and protein initially made a similar contribution to the rise in ballast over the first 1.5 h, but thereafter protein decreased while carbohydrate continued to increase (Fig. 2b). In this experiment both the 50% loss of buoyancy at 3 h and the 99% loss of buoyancy at 6 h were dependent on the increase in carbohydrate ballast.

In the culture kept at $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ there was a slow increase in ballast and a small decline in buoyancy state, from 99% down to 70% filaments floating, over 24 h (Fig. 2c). Even at this relatively low photon irradiance there was sufficient carbohydrate accumulation to increase the density of the filaments.

Possible loss of gas vesicles unexplained by turgor rise

At the end of the time-course experiment in high irradiance there was a reduction in gas vesicle content to only $29.3 \pm 3.8\%$ of the volume present at the start (Fig. 2a). Measurements made on the initial p_c distribution (Fig. 3) indicate that to collapse 70.7% of the gas vesicles would have required a pressure of 0.580 MPa, substantially higher than the maximum value that the turgor pressure reached during exposure to high irradiance (0.544 MPa at 16 h); in fact the turgor pressure had actually decreased a little at 24 h. A statistical analysis in which 95% confidence limits are partitioned between estimates of the turgor rise and the loss of gas vesicles shows that the upper estimate of the turgor rise, 0.548 MPa, would account for loss of only 55% of the gas vesicles, whereas the minimum estimate of gas vesicle collapse is 68%, which would require a rise of 0.574 MPa. We conclude that either the further loss of gas vesicles between 16 and 24 h had had some other cause, or the turgor pressure had risen to a higher value and then dropped back again.

The possibility that there might have been fluctuations in turgor pressure after prolonged exposure to high

Table 1. *Balance sheet of changes in cell constituents and their ballast masses*
 Numbers are means of three measurements (with standard deviations shown in parenthesis).

Treatment	Time (h)	Amount per litre of culture				Ratio* P+C+L D	Ballast mass per litre of culture			
		Gas vesicle (µl)	Protein (mg)	Carbohydrate (mg)	Dry mass (mg)		Gas vesicle (mg)	Protein (mg)	Carbohydrate (mg)	G + P + C + L (mg)
Pre-treatment	-2	94.7 (2.8)	118.2 (5.9)	73.4 (1.1)	290.3 (17.5)	0.78	-94.4 (2.8)	27.5 (1.4)	27.6 (0.4)	-37.7
	0	94.7 (7.2)	115.0 (8.8)	83.6 (2.0)	352.8	0.77	-94.5 (7.2)	36.0 (2.1)	31.5 (0.8)	-25.3
High irradiance	1.5	102.7 (0.1)	175.9 (3.6)	111.2 (7.0)	369.2 (21.4)	0.87	-102.4 (0.1)	40.9 (0.8)	41.8 (2.6)	-18.0
	3	95.7 (3.1)	175.5 (10.9)	142.5 (11.0)	351.9 (2.6)	1.00	-95.4 (3.1)	40.8 (2.6)	53.6 (4.1)	0.7
	4.5	104.9 (11.3)	175.4 (9.0)	177.8 (9.4)	364.8 (7.4)	0.96	-104.5 (11.2)	40.8 (2.1)	66.9 (3.5)	4.8
	6	98.4 (4.9)	161.8 (7.8)	225.1 (13.8)	394.2 (14.4)	1.07	-98.1 (4.9)	37.6 (1.9)	84.7 (5.2)	25.9
	10	84.1 (3.7)	139.6 (5.9)	278.5 (17.9)	448.7 (25.7)	1.01	-83.9 (3.7)	32.4 (1.4)	104.8 (6.7)	55.1
	16	42.0 (4.3)	145.2 (5.0)	314.2 (8.5)	525.3 (5.6)	0.94	-41.9 (4.3)	33.7 (1.2)	118.2 (3.2)	111.7
	24	27.8 (2.2)	122.0 (2.5)	328.9 (6.1)	496.1	0.98	-27.7 (2.2)	28.4 (0.6)	123.7 (2.3)	126.1
Low irradiance	7	99.0 (3.7)	164.5 (3.7)	105.6 (8.0)	275.9 (45.2)	1.10	-98.7 (3.7)	38.2 (0.9)	39.7 (3.0)	-20.8
	15	95.5 (2.5)	171.2 (7.6)	112.7 (6.7)	308.9 (16.6)	1.03	-95.2 (2.5)	39.8 (1.8)	41.9 (2.5)	-13.6
	23	95.8 (11.1)	166.8 (5.4)	119.7 (1.4)	350.0	0.91	-95.5 (11.1)	38.7 (1.3)	45.0 (0.5)	-11.8

* The ratio of (P + C + L)/D includes 33.7 mg lipid in each row; the ratio gives the proportion of mass accounted for. C, carbohydrate; D, dry mass; G, gas vesicle; L, lipid; P, protein.

irradiance was investigated by making hourly measurements on another culture that had been incubated for 15 h at $126 \mu\text{mol m}^{-2} \text{s}^{-1}$. The turgor pressure rose from an initial value of 0.39 MPa to 0.53 MPa after 16 h and then remained within 0.013 MPa of this value over the next 6 h (see Fig. 4). There was, therefore, no evidence of large-scale rises in turgor pressure over this period.

Discussion

The results obtained here confirm the finding of Oliver & Walsby (1984) that cells of *Anabaena flos-aquae* can lose half of their gas vesicles when exposed for many hours to high irradiance, and they demonstrate that this loss can be quantitatively accounted for by the collapse of gas vesicles by rising turgor pressure. The buoyancy loss, however, can be brought about more rapidly (Fig. 2*a, b*), and perhaps also at lower irradiances (Fig. 1), by carbohydrate accumulation. These findings therefore raise questions about whether gas vesicle collapse by turgor rise has any importance in regulating buoyancy. The answers to these questions lie in an analysis of the changes in cell components.

For a cell to lose buoyancy by accumulating carbohydrate, the increase in carbohydrate ballast must exceed the cell's initial net buoyancy (negative net ballast). Reference to the last column in Table 1 shows that the initial net buoyancy was only 37.7 mg per litre of culture; the carbohydrate ballast increased by this amount within about 4.5 h at high irradiance. Obviously, the greater the initial net buoyancy the longer it would take for

counteraction by carbohydrate accumulation. In the extreme case it might be necessary for gas vesicles to collapse in order for buoyancy to be lost. This was evidently the case in the experiments of Oliver & Walsby (1984), in which the increase in carbohydrate ballast was insufficient to overcome the initial net buoyancy. In comparing the results of their analyses (Tables 1 and 2 in Oliver & Walsby, 1984) with those in Table 1 of this paper it is seen that their cultures had a slightly higher initial gas vesicle content (a ratio of gas vesicle ballast to dry mass of 0.35 compared with 0.32 here) and a lower initial carbohydrate content (0.20 of dry mass compared with 0.25 here). The major difference, however, was that the relative increase in carbohydrate was less in their cultures (2.5-fold over 24 h, compared with 4.5-fold here). The reason for this is uncertain, but we speculate that it might have been related to the rate of aeration, which supplied CO_2 to the cultures. In the complete absence of CO_2 there can be no carbohydrate synthesis and it has been demonstrated that there is no turgor rise or buoyancy loss (Walsby 1971; Dinsdale & Walsby, 1972). When CO_2 is not absent but is in limited supply, carbohydrate production might be affected more than turgor rise, as the K^+ -dependent mechanism of turgor increase would not be directly affected.

A number of other studies have also indicated that CO_2 limitation can cause a relative increase in buoyancy in both *A. flos-aquae* (Booker & Walsby, 1981; Spencer & King, 1985) and other gas-vacuolate cyanobacteria (Klemer *et al.*, 1982; Paerl & Ustach 1982). Spencer & King (1985) observed that at irradiances of $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ buoyancy increased when the CO_2

Fig. 4. The turgor pressures at different times after increasing the irradiance from 5 to $126 \mu\text{mol m}^{-2} \text{s}^{-1}$. Error bars are standard deviations calculated from three pairs of measurements ($p_c - p_a$).

concentration fell below $5 \mu\text{mol l}^{-1}$ in the culture. Klemer *et al.* (1982) found that there was an increase in pressure-sensitive turbidity, an indicator of relative gas vesicle content, in cultures of *Oscillatoria* deprived of CO_2 . Measurements are now needed of how gas vesicle volume and carbohydrate content change in response to changes in CO_2 .

The potential advantage of buoyancy-regulating mechanisms that destroy gas vesicles is that they act directly on the sole component that provides buoyancy. The turgor-collapse mechanism is, however, effective only on gas vesicles whose range of critical pressure overlaps with the range of turgor pressure that can be exerted by the cell. The time taken for this mechanism to provide reduction in buoyancy depends on both the turgor rise required and the rate of turgor rise. There has been little investigation of either of these factors. The turgor rise required depends on the difference between the initial turgor and the critical pressure of the weakest gas vesicles, both of which may vary (compare critical pressure distributions illustrated in Walsby 1971, 1980, and Walsby & Bleything, 1988). The rate of turgor rise is dependent on the irradiance and also on the external K^+ concentration (Allison & Walsby, 1981; Walsby 1980). Previous investigations have shown some relatively rapid rates of turgor increase, which gave gas vesicle collapse in as little as 30 min (Grant & Walsby, 1977). This prompted the suggestion that collapse by turgor rise might provide a more rapid response than carbohydrate accumulation (Walsby, 1987); in contrast, the results obtained here, and those obtained with *Aphanizomenon* by Kromkamp *et al.* (1986), show that under certain conditions carbohydrate accumulation can provide a more rapid response. Clearly, the effectiveness of the

different responses will be greatly affected by the initial physiological state of the cyanobacteria when subjected to increased irradiance. The overall buoyancy state is greatly affected by the availability of nutrients such as nitrogen (Klemer, 1978; Klemer *et al.*, 1982; Spencer & King, 1985) and phosphate (Konopka *et al.*, 1987a, b; Kromkamp *et al.*, 1989). Quantitative studies of the type described here are now required to elucidate the rates at which the two mechanisms operate under different conditions.

Although the rise in turgor pressure can account for much or all of the substantial gas vesicle loss that occurred in the first 16 h of incubation at high irradiance, there was an additional loss after 24 h incubation that could not be definitely attributed to turgor rise. The collapse of the gas vesicles might have been caused by some factor that caused them to become weaker; Buckland & Walsby (1971) showed that the critical pressures of isolated gas vesicles were decreased by exposure to proteolytic enzymes, detergents, extremes of pH and high concentrations of certain substances. They commented that if such weakening occurred inside cyanobacterial cells the gas vesicles would collapse under existing turgor pressures and this would provide an additional means of regulating buoyancy. Evidence for such destabilizing factors should now be sought in cells exposed for long times to light of high irradiances.

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