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Variations in the buoyancy response of *Microcystis aeruginosa* to nitrogen, phosphorus and light

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Microcystis aeruginosa displays a range of variability in buoyancy in response to light which is dependent upon the previous nutrient or light history of the cell. The short-term buoyancy response to light is nested into a longer term response, over a period of days, as cyanobacteria respond to their nutrient and light climate, which may be manifested in their gas vesicle volume, photosynthetic rate and carbohydrate metabolism. Microcystis aeruginosa cultures were subjected to a range of nitrogen (N), phosphorus (P) or light pre-treatments before exposure to saturating irradiance, and these results are used to illustrate that populations can display a range of buoyancy responses dependent upon subtle changes in nutrient and light conditions. N-limited (0 μM) cells suffered a dilution in gas vesicle volume and increased carbohydrate content, which resulted in a loss of buoyancy. Cells in 10 μM N increased their gas vesicle content during light exposure; however, this was insufficient to maintain buoyancy in the majority of cells as carbohydrate increased. Cells in 100 μM N increased their gas vesicle volume, metabolized carbohydrate more efficiently than the N-limited treatments and retained positive buoyancy. During light exposure, there was minimal change in buoyancy in 0 μM P pre-treated cells, although there was a dilution in gas vesicle volume and an increase in carbohydrate. In 0.5 μM P pre-treated cells, the proportion of individuals floating did not change, although gas vesicle volume and carbohydrate increased. There was a significant increase in gas vesicle volume in 10 μM P pre-treated cells during light exposure, and some buoyancy loss due to carbohydrate accumulation. Cells grown in the range of light intensities tested all increased their gas vesicle volume during the 24 h light treatment. The actual rate of gas vesicle production was equivalent for all three light pre-treatments; however, the growth-dependent dilution rate was determined by the previous light history, which resulted in different relative gas vesicle volume per cell. There was minimal increase in carbohydrate per cell during the light period, yet all treatments displayed the classical buoyancy loss response.

INTRODUCTION

Microcystis aeruginosa Kütz. emend. Elenkin is a noxious, bloom-forming cyanobacterium which is frequently associated with thermally stratified water bodies (Ganf, 1974; Robarts and Zohary, 1984). The ability of *M. aeruginosa* to exploit thermally stratified conditions can be attributed to gas vesicles, which provide buoyancy, reduce sedimentation losses (Reynolds and Walsby, 1975) and maintain colonies in a favourable light climate during periods of low turbulence (Humphries and Lyne, 1988).

Cyanobacterial buoyancy can be altered in response to light and nutrients. Turgor pressure generated by osmotically active photosynthates, or potassium ions, is strong enough to collapse gas vesicles in some species (Allison and Walsby, 1981; Oliver and Walsby, 1984). However, cell density is altered most rapidly by the accumulation or loss of dense polysaccharides during photosynthesis and respiration (Kromkamp and Mur, 1984; Utkilen, *et al.*, 1985; Kromkamp and Walsby, 1990). Buoyancy is also regulated via the molecular control of gas vesicle production relative to growth (Oliver, 1994; Walsby, 1994).

It has been proposed that buoyancy regulation may be a mechanism used by cyanobacteria to achieve vertical migration and overcome the vertical separation of light and nutrients (Ganf and Oliver, 1982). This hypothesis

developed because surface waters in thermally stratified lakes and reservoirs often become deficient in bioavailable nutrients, but hypolimnetic waters become enriched as nutrients flux from lake sediments.

In environments where the surface waters are nutrient deplete, the ability to migrate vertically and scavenge for nutrients is clearly advantageous. However, in environments where nutrients are in excess, persistent buoyancy may offer a more favourable strategy to maximize light capture and allow rapid flotation upon the onset of thermal stratification (Brookes *et al.*, 1999). If this line of argument is pursued, it follows that abundant nutrients should promote buoyancy, but nutrient limitation should act to decrease it in order for colonies to migrate and scavenge for nutrients. In general, this is what is observed. When nutrients are abundant, buoyancy is promoted; however, buoyancy decreases when the major nutrients, nitrogen (N) and phosphorus (P), are limiting (Oliver, 1994).

For example, N limitation generates a reduction in gas vesicle volume (Klemer, 1978; Klemer *et al.*, 1982). Coupled with less efficient carbohydrate (CHO) metabolism (Herzig and Falkowski, 1989; Turpin, 1991), N limitation leads to a reduction in cell buoyancy (Klemer *et al.*, 1982; van Rijn and Shilo, 1983; Spencer and King, 1985, 1989). However, this buoyancy loss is reversible as the addition of ammonia to N-limited cells enables a rapid recovery of buoyancy (van Rijn and Shilo, 1983; Konopka *et al.*, 1993).

Buoyancy loss/vertical migration has been observed in naturally occurring and cultured populations of *M. aeruginosa* (Ganf, 1974; Ganf and Oliver, 1982; Walsby and McAllister, 1987; Kromkamp *et al.*, 1988; Ibelings *et al.*, 1991; Visser *et al.*, 1996). Buoyancy loss in response to light is also well documented for *Anabaena flos-aquae* (Walsby and Booker, 1980; Oliver and Walsby, 1984; Spencer and King, 1985, 1989; Kinsman *et al.*, 1991), *Aphanizomenon* (Kromkamp *et al.*, 1986; Konopka *et al.*, 1987a; Konopka, 1989; Klemer *et al.*, 1995), *Anabaena circinalis* (Brookes *et al.*, 1999) and *Oscillatoria* spp. (Konopka, 1982; van Rijn and Shilo, 1983; Walsby *et al.*, 1983; Utkilen *et al.*, 1985).

However, even in populations where buoyancy loss is observed, often a significant proportion of the population retains buoyancy. Persistently buoyant populations have sufficient gas vesicles to maintain buoyancy upon the accumulation of dense polysaccharide and this has been observed in several *Anabaena* species. For example, persistently buoyant *Anabaena lemmermannii* and *Anabaena minutissima* filaments were observed in Lake Windermere (Walsby *et al.*, 1991) and Lake Rotongaio, respectively (Walsby *et al.*, 1987, 1989). It is interesting to note that the majority (88.9%) of *A. minutissima* suspended in bottles at the water surface retained buoyancy following 9 h of daylight; however, at the end of the light period on the second day of incubation, only 44.9% of filaments remained buoyant.

Persistent buoyancy is often associated with nutrient enrichment. Brookes *et al.* found that nutrient-enriched *Anabaena circinalis* displayed significantly attenuated buoyancy loss relative to the treatment with no nutrients added (Brookes *et al.*, 1999). Similarly, *Aphanizomenon* with nutrients added maintained buoyancy after a 5 h light incubation (Klemer *et al.*, 1995).

However, the buoyancy characteristics of cyanobacterial populations need not necessarily fall neatly into the categories of buoyancy regulating or persistently buoyant, but rather they may exhibit a range of buoyancy responses depending upon their previous nutrient or light history. The short-term buoyancy response to light is nested into a longer term response, over a period of days, as cyanobacteria respond to their nutrient and light climate, which may be manifested in their gas vesicle volume, photosynthetic rate and CHO metabolism. Furthermore, the buoyancy strategy adopted is a response to variations in the patterns of thermal stratification which optimizes the probability for a population to capture resources that are in limited supply. As water columns may stratify in numerous ways, it is suggested that cyanobacteria may respond differentially depending upon the vertical structure of the water column and the resource which is in most demand.

Although studies on buoyancy regulation exist for a number of species, Oliver states that there is a 'need for comprehensive studies on buoyancy regulation in response to changes in major nutrients (viz. carbon, N and P) and light availability. No such studies are available' (Oliver, 1994). Additionally, the role of both light and nutrients in buoyancy regulation is not always appreciated. For example, Bormans *et al.* suggested '…changes in the vertical distribution of phytoplankton suggest a response either to the dynamics of the surface mixed layer or to lateral advection. In natural systems, algal buoyancy appears to be dependent much more on light than on nutrients, this being consistent with the CHO ballast mechanism' (Bormans *et al.*, 1999).

The aim of this study was to test the hypothesis that the buoyancy status of *M. aeruginosa* cells was dependent upon their previous light and nutrient history. This paper accepts the view that many of the physiological characteristics of *M. aeruginosa* and other cyanobacteria may be an adaptation to transient availability of nutrients in pulse-fed systems (Bhaya *et al.*, 2000). To simulate this phenomenon, a pre-treatment was provided to mimic nutrient-limited cells. The range of buoyancy responses was examined by pre-treating cultured *M. aeruginosa* in a range of N concentrations, P concentrations or light

intensities before a 12 h dark period, followed by a 24 h incubation in 150 μmol m^{-2} s^{-1}, which is a saturating light intensity for photosynthesis and growth (Olesen and Ganf, 1986). The relative gas vesicle volume (RGV), CHO concentration and buoyancy status were measured prior to and following high-light exposure.

METHOD

Gas vesicle volume and buoyancy were examined in *M. aeruginosa* strain MASH 01, obtained from CSIRO Marine Laboratories, Hobart, Tasmania, grown in ASM-1 media (Gorham *et al.*, 1964) at a range of light intensities, and of N and P concentrations. All cultures were grown at 25°C. Cultures were exposed to the relevant pre-treatment before the treatment of 24 h exposure to high light. All experiments were performed in triplicate.

Nitrogen-limitation pre-treatment

Log-phase *M. aeruginosa* cultures were deprived of N for 2 days, to deplete intracellular stores of N, prior to inoculation into 0, 10 or 100 μM nitrate ($n = 3$) in otherwise normal ASM-1. Cultures were grown at 100 μmol m^{-2} s^{-1} on a 12 h:12 h light:dark cycle for 6 days to induce relative N limitation prior to experimentation.

Phosphorus-limitation pre-treatment

Log-phase *M. aeruginosa* cultures were deprived of phosphate for 8 days, to deplete intracellular polyphosphate stores, prior to inoculation into 0, 0.5 or 10 μM phosphate ($n = 3$) in otherwise normal ASM-1 media. Cultures were grown at 100 μmol m^{-2} s^{-1} on a 12 h:12 h light:dark cycle to promote relative phosphate limitation prior to experimentation.

Light-limitation pre-treatment

Log-phase *M. aeruginosa* cultures were inoculated into complete ASM-1 media and incubated at three irradiances, 10, 50 and 100 μmol m^{-2} s^{-1} (12 h:12 h light:dark cycle), and grown for 6 days prior to experimentation.

Treatment and quantification of parameters

Following the pre-treatment, cultures were incubated in darkness for 12 h before exposure to 150 μmol m^{-2} s^{-1} for 24 h. The RGV required to maintain buoyancy was estimated by measuring the RGV and the percentage of cells floating after a 12 h dark pre-treatment and following 24 h exposure to 150 μmol m^{-2} s^{-1}.

At each sampling time, subsamples of the cultures were subjected to incremental pressure increases (0, 100, 250, 300, 350, 400, 500, 600, 700 kPa) to progressively collapse gas vesicles. Following each pressure increase, the RGV was measured using the flow cytometer and the percentage of floating and sinking cells estimated using a haemocytometer and Zeiss microscope (Walsby and Booker, 1980). Samples were collected and frozen for CHO analysis on each sampling occasion. Carbohydrate content per cell was determined using the phenol method of Herbert *et al.* (Herbert *et al.*, 1971) following sonication for 1 min to disrupt cells.

Measurement of gas vesicle volume

Mean RGV was estimated as the difference in side scatter of cells with intact gas vesicles and the side scatter with all gas vesicles collapsed by the application of 1000 kPa pressure. Side scatter was measured on a single-cell basis using a Becton Dickinson FACStrak flow cytometer with an argon laser (488 nm) as the excitation light source (Brookes *et al.*, 2000). Side scatter sensitivity was adjusted to ensure that cells with intact gas vesicles appeared on scale. The flow cytometer was calibrated using fluorescein isothiocyanate-labelled beads and the instrument settings saved to allow comparisons between measurements made on different days. The units of RGV are relative side scatter units (RSU). Growth rate was determined by microscopic enumeration of cells using a haemocytometer and Zeiss microscope.

RESULTS

Buoyancy response of N-limited *M. aeruginosa* to light exposure

At atmospheric pressure, following the dark pre-treatment, the maximum recorded side scatter per cell was lowest in cells grown in 0 μM and highest in 10 and 100 μM nitrate (Table I). There was a decrease in the relative side scatter per cell with sequential increases in pressure, and the RGVs were calculated to be 1166, 1526 and 1478 RSU in the 0, 10 and 100 μM treatments, respectively. The minimum mean side scatter increased for each treatment following light exposure (Table I), which is consistent with an increase in cell size (Li, 1994) due to cellular CHO accumulation. The N-limited (0 μM) cells suffered a dilution in gas vesicle volume and increased CHO content, which resulted in a loss of buoyancy (Table IVA). Exposure to light resulted in an accumulation of CHO, which in the 0 μM treatment increased from 9 to 24 pg $cell^{-1}$ and decreased the proportion of floating cells from 63 to 29%.

Cells in the 10 μM treatment increased their gas vesicle content during the extended light exposure; however, this was insufficient to maintain buoyancy in the majority of cells as CHO increased (Table I). Cells in the 100 μM treatment increased their gas vesicle volume, metabolized CHO more efficiently than the N-limited treatments

Table I: The relative side scatter per cell measured before (max SSC) and after the collapse of all gas vesicles (min SSC), the relative gas vesicle volume (RGV), the proportion of cells floating, CHO content and the RGV required to maintain 50% buoyancy in M. aeruginosa cells grown in 0, 10 or 100 μM nitrate

Parameter	Light exposure	Nitrate concentration (μM)		
		0	10	100
Max SSC	Dark	1375	1735	1734
(GV intact)		(11.5)	(27.6)	(49.4)
(relative units – RSU)	24 h light	1311	1839	2036
		(43.9)	(66.3)	(124.5)
Min SSC (RSU)	Dark	209	209	256
		(9.0)	(10.0)	(64.2)
(GV collapsed)	24 h light	273	247	453
		(19.1)	(9.9)	(86.2)
RGV	Dark	1166	1526	1478
(RSU)	24 h light	1038	1592	1583
% cells floating	Dark	63	99	100
		(32.7)	(1.5)	(0.6)
	24 h light	29	34.6	93
		(28.7)	(9.6)	(4.0)
RGV required to maintain	Dark	1052	1052	1005
50% buoyancy	24 h light	>max recorded RGV	>max recorded RGV	1499
Growth rate over 24 h light		0.06	0.31	0.32
(day^{-1})		(0.01)	(0.02)	(0.05)
Carbohydrate	Dark	9.00	4.96	6.88
(pg $cell^{-1}$)		(0.3)	(0.46)	(0.54)
	24 h light	24	27.3	19.6
		(7.3)	(5.6)	(5.4)

Parameters were recorded prior to and following 24 h exposure to 150 μmol m^{-2} s^{-1}. The SD is in parentheses.

(increase in CHO: 12.7 pg CHO $cell^{-1}$ in 100 μM N, 15 pg CHO $cell^{-1}$ for 0 μM and 22.3 pg CHO $cell^{-1}$ for 10 μM; Table IVA) and retained positive buoyancy even though they required a much greater gas vesicle volume to do so (+494 RSU for 50% buoyancy).

Microcystis aeruginosa cells grown in 100 μM nitrate had more gas vesicles than the other treatments, and although the CHO content increased from 6.88 to 19.6 pg $cell^{-1}$, only 7% of cells lost buoyancy (Table I). The volume of gas vesicles required to maintain buoyancy increased as CHO content increased. Following the application of 400 kPa pressure, gas vesicle volume decreased (Figure 1) and 63% of cells in the 100 μM treatment maintained buoyancy, whereas the 0 and 10 μM treatments had 0 and 8% of cells floating (Figure 2). The critical gas vesicle volume was determined as the RGV required to maintain buoyancy in 50% of cells. Prior to light exposure, a mean gas vesicle volume of 1052 RSU supported buoyancy in 50% of cells, but following high light, a gas vesicle volume of 1499 RSU was required for 50% buoyancy (Figures 1 and 2). However, this gas vesicle volume was only realized in cells grown in the 100 μM treatment.

Buoyancy response of P-limited *M. aeruginosa* to light exposure

The initial mean gas vesicle volume was greatest in cells grown in media with no added phosphate (1426 RSU) and lowest in the 10 μM treatment (1201 RSU); however, this increased to 1722 following 24 h of light exposure (Table II). Cells grown in the 0.5 μM treatment had a gas vesicle

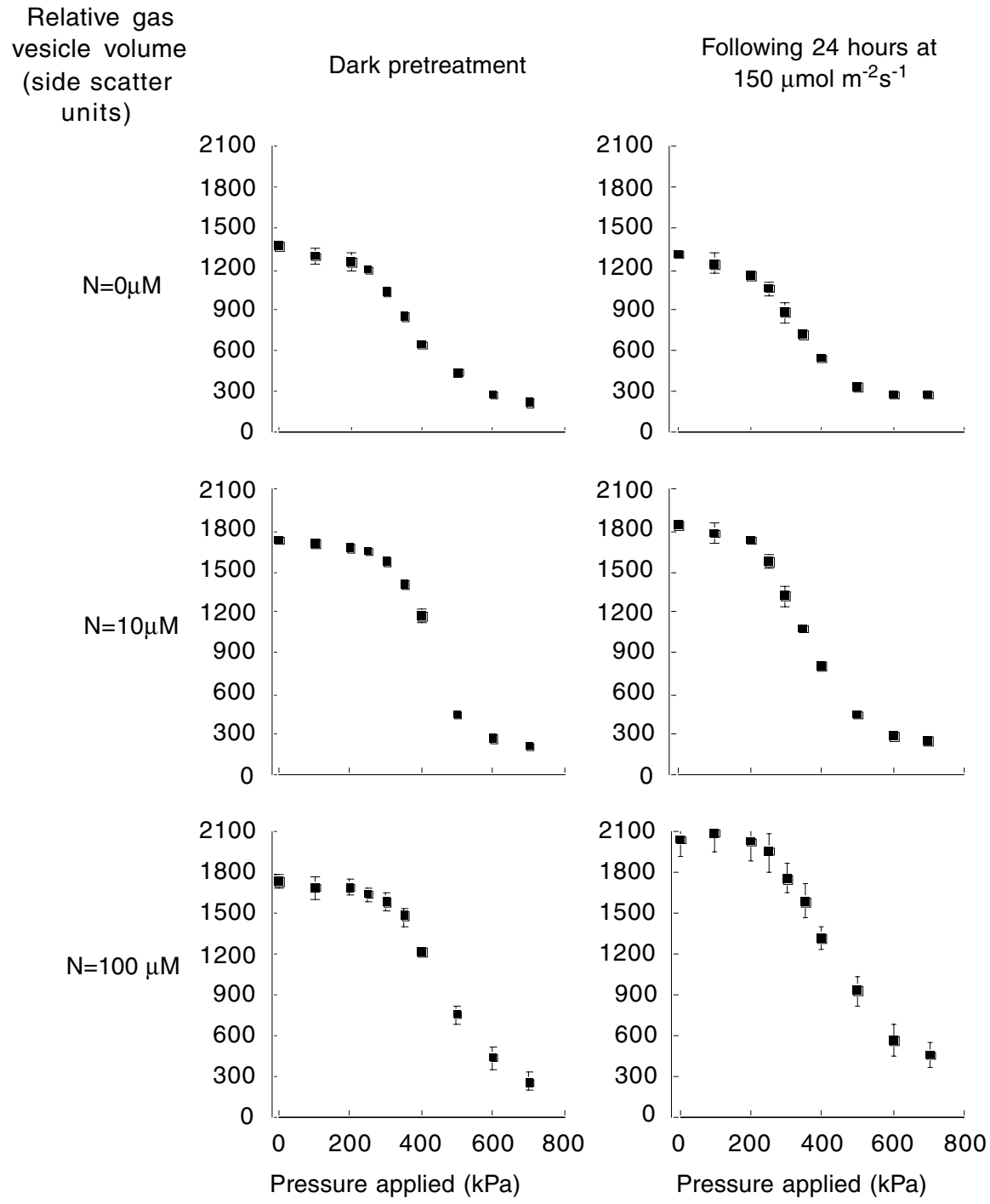

Fig. 1. Relative gas vesicle volume of *M. aeruginosa* cells grown in three nitrate concentrations and subjected to incremental increases in pressure, before and after light exposure. Error bars represent 1 SD.

volume of 1450 RSU, which remained about the same after 24 h of light exposure.

Initially, the gas vesicle volume per cell was less in the 10 μM treatment than in the 0 and 0.5 μM treatments; however, the pressure required to render 50% of cells in the 10 μM treatment non-buoyant was 375 kPa, but only 117 and 283 kPa in the 0 and 0.5 μM treatments, respectively (Table II). This was attributable to the greater

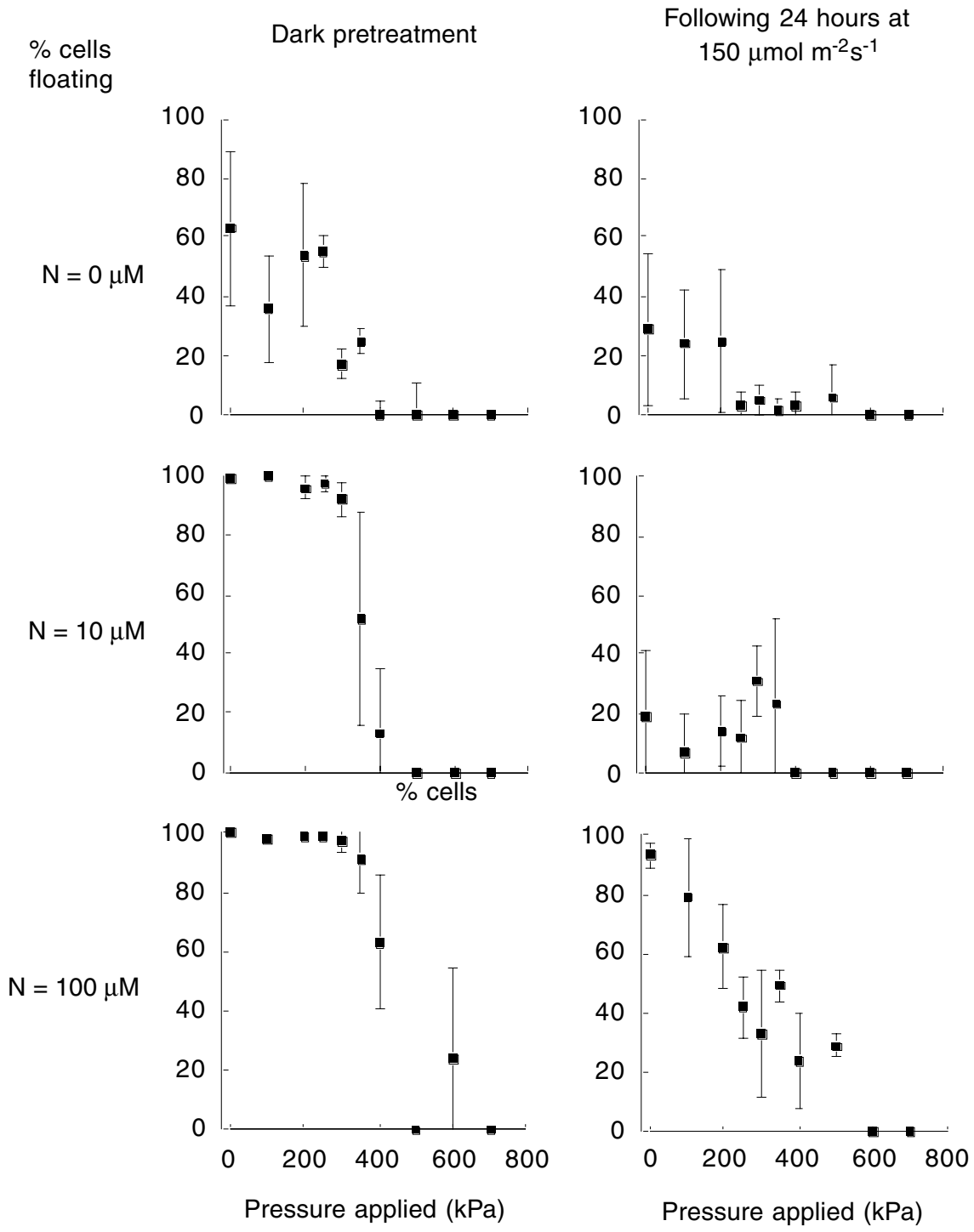

Fig. 2. The percentage of *M. aeruginosa* cells that are floating following incremental increases in pressure. The cells were grown in three different nitrate concentrations in otherwise normal ASM-1 media, and subjected to a dark pre-treatment and a light treatment. Error bars represent 1 SD ($n = 3$).

CHO content in the phosphate-limited cultures (Table II), which contributed greater turgor pressure. Following 24 h of light exposure, the CHO content per cell increased in all treatments; however, the increased CHO content did not change the percentage of cells floating in the 0 and 0.5 μM treatments, whereas 32% of cells lost buoyancy in the 10 μM treatment. This may indicate that some other dense cellular constituent, such as

Table II: The relative side scatter per cell measured before (max SSC) and after the collapse of all gas vesicles (min SSC), the relative gas vesicle volume (RGV), the proportion of cells floating, CHO content and the RGV required to maintain 50% buoyancy in cells of M. aeruginosa cells grown in 0, 0.5 or 10 μM phosphate

Parameter	Light exposure	Phosphate concentration (μM)		
		0	0.5	10
Max SSC	Dark	1710	1695	1503
(GV intact)		(104.3)	(79.8)	(113.3)
(relative units – RSU)	24 h light	1673	1694	2075
		(118.9)	(67.3)	(111.6)
Min SSC	Dark	284	245	302
(GV collapsed)		(7.8)	(27.6)	(10.4)
(RSU)	24 h light	297	223	353
		(8.4)	(12.8)	(11.5)
RGV (RSU)	Dark	1426	1450	1201
	24 h light	1376	1471	1722
% cells floating	Dark	83	96	100
		(8.3)	(5.3)	(0)
	24 h light	79	95	68
		(13.7)	(4.0)	(16.6)
RGV required to maintain	Dark	1267	1306	892
50% buoyancy	24 h light	1278	1352	1665
Growth rate over 24 h light		0.06	0.18	0.47
(day^{-1})		(0.06)	(0.07)	(0.05)
Carbohydrate	Dark	26.4	20.7	7.3
($pg\ cell^{-1}$)		(2.1)	(3.4)	(3.1)
	24 h light	37.5	27.3	22.7
		(1.6)	(2.2)	(4.3)

Parameters were recorded prior to and following 24 h exposure to 150 μmol m^{-2} s^{-1}. The SD is in parentheses.

polyphosphate, was contributing to buoyancy loss in addition to CHO.

The critical RGV required to maintain buoyancy in 50% of cells was calculated to be 1267 and 1306 RSU for the 0 and 0.5 μM treatments after the dark pre-treatments, and 1278 and 1352 RSU after the light treatment. The small difference before and after high light reflects the small change in gas vesicle volume and CHO during light exposure. In the 10 μM treatment, the critical gas vesicle volume required to maintain 50% buoyancy increased from 892 to 1665 RSU after the light treatment.

In summary, during high-light exposure, the 0 μM treatment showed a dilution in gas vesicle volume and an increase in CHO; however, there was minimal change in buoyancy (Table IVB). The 0.5 μM treatment increased gas vesicle volume and CHO; however, there was no change in the proportion of individuals floating even though the gas vesicle volume required to maintain buoyancy increased. There was a significant increase in gas vesicle volume in the 10 μM treatment during the high-light exposure; however, there was some loss of buoyancy due to CHO accumulation.

Buoyancy response of light-limited *M. aeruginosa* to light exposure

Microcystis aeruginosa cells grown at 10, 50 and 100 μmol m^{-2} s^{-1} had similar gas vesicle volumes at the commencement of the light exposure (Table III). However, after 24 h of light, the gas vesicle volume of cells increased in all treatments. The growth rate during the 24 h light

Table III: The relative side scatter per cell measured before (max SSC) and after the collapse of all gas vesicles (min SSC), the relative gas vesicle volume (RGV), the proportion of cells floating, CHO content and the RGV required to maintain 50% buoyancy in cells of M. aeruginosa cells grown in 100, 50 or 10 μmol m^{-2} s^{-1} irradiance

Parameter	Light exposure	Light (intensity μmol m^{-2} s^{-1})		
		10	50	100
Max SSC	Dark	1238	1242	1269
(GV intact)		(59)	(33)	(12)
(relative units – RSU)	24 h light	1671	1582	1426
		(70)	(30)	(180)
Min SSC	Dark	139	146	159
(GV collapsed)		(2)	(3)	(6)
(RSU)	24 h light	228	201	169
		(19)	(11)	(29)
RGV	Dark	1099	1096	1110
(RSU)	24 h light	1443	1381	1257
% cells floating	Dark	99	98	100
		(2)	(3)	(1)
	24 h light	23	24	52
		(17)	(20)	(2)
RGV required to maintain	Dark	561	764	861
50% buoyancy	24 h light	>max recorded RGV	>max recorded RGV	1216
Growth rate over 24 h light		0.21	0.43	0.75
(day^{-1})		(0.13)	(0.03)	(0.1)
Carbohydrate	Dark	14.8	7.4	4.5
(pg $cell^{-1}$)		(0.17)	(0.84)	(0.45)
	24 h light	15.5	6.6	4.5
		(0.17)	(0.34)	(0.81)

Parameters were recorded prior to and following 24 h exposure to 150 μmol m^{-2} s^{-1}. The SD is in parentheses.

treatment was dependent upon the light intensity at which the cells had previously been grown, with greater growth in cells adapted to higher light intensities (Table III). The actual rate of gas vesicle production is about the same for all three light pre-treatments; however, the dilution rate, due to growth, is dependent upon previous light history, which results in different RGV per cell.

There was no net accumulation of CHO per cell, which may be because the CHO was used to fuel the observed growth. However, ~76% of cells initially grown at 10 and 50 μmol m^{-2} s^{-1} lost buoyancy during exposure to 150 μmol m^{-2} s^{-1} for 24 h. Forty-eight per cent of cells lost buoyancy in the 100 μmol m^{-2} s^{-1} treatment. The small changes in CHO during light exposure did not forecast the large decrease in buoyancy that was observed.

Prior to light exposure, an RGV of 561 RSU was sufficient to maintain buoyancy in 50% of cells initially grown at 10 μmol m^{-2} s^{-1}, but a greater gas vesicle pool was required to maintain 50% buoyancy in the 50 and 100 μmol m^{-2} s^{-1} treatments: 764 and 861 RSU, respectively. This suggests that the density of cellular constituents was greatest in cells in the 100 μmol m^{-2} s^{-1} treatment. The gas vesicle volume required to maintain buoyancy in 50% of cells increased from 861 to 1216 RSU; however, soluble CHO content per cell did not change. Cells in this treatment had the lowest soluble CHO content and therefore it is probable that some other cellular constituent, such as

Table IV: Growth rates and the relative change in cell features in response to 24 h of 150 μmol m^{-2} s^{-1} light

(A) Different nitrate concentrations

Nitrogen (μM)	Growth rate (day^{-1})	RGV (RSU)	CHO (pg $cell^{-1}$)	RGV 50%F	%F
0	0.06	–128	+15	>max recorded	–34
10	0.31	+66	+22.3	>max recorded	–80
100	0.32	+105	+12.7	+494	–7

(B) Different phosphate concentrations

Phosphorus (μM)	Growth rate (day^{-1})	RGV (RSU)	CHO (pg $cell^{-1}$)	RGV 50%F	%F
0	0.06	–50	+11.1	+11	–4
0.5	0.18	+21	+6.6	+46	–1
10	0.47	+521	+15.4	+773	–32

(C) Different light intensities

Light (μmol m^{-2} s^{-1})	Growth rate (day^{-1})	RGV (RSU)	CHO (pg $cell^{-1}$)	RGV 50%F	%F
10	0.21	+344	+0.7	>max recorded	–77
50	0.43	+285	–0.8	>max recorded	–76
100	0.75	+147	0.0	+355	–48

Data calculated from the mean values given in Tables I–III. RGV, relative gas vesicle volume; CHO, carbohydrate; RGV 50%F, the relative gas vesicle volume required to maintain 50% of cells floating; %F, the percentage of cells floating.

insoluble starch, was more abundant in these cells and contributing significantly to cell density.

DISCUSSION

Nitrogen and phosphorus

The limitation of N resulted in a decrease in the gas vesicle volume to the extent that it was unable to maintain buoyancy upon the accumulation of CHO. When N was not limiting (100 μM), persistent buoyancy was observed in 93% of cells. However, when the phosphate concentration was manipulated, the greatest buoyancy loss was observed in the highest phosphate treatment. The high P and N treatments were grown in similar media, but there was a difference in buoyancy in response to light. It is possible that cells in the high-phosphate treatment had accumulated excess amounts of polyphosphate, which has a density of 2170 kg m^{-3} (Jacobson and Halmann, 1982) and would contribute significantly to net cell density.

In the 0 μM nitrate and 0 μM phosphate treatments, the rate of gas vesicle production and assembly did not match the growth rate during the 24 h light exposure, and consequently there was a dilution of the gas vesicle volume per cell. However, a net increase in gas vesicle volume per cell was observed in all other treatments, suggesting that the rate of gas vesicle production exceeded the growth rate.

In phosphate-limited populations, growth, which is normally a sink for photosynthate, is reduced and CHO tends to accumulate rather than being metabolized (Konopka *et al.*, 1987b). Although this may be offset, to some extent, by a reduction in photosystem II efficiency relative to P-replete cells (Geider *et al.*, 1993), a reduction in gas vesicle volume would further act to decrease cell buoyancy. However, Konopka *et al.* found no difference in the gas vesicle volume of *Aphanizomenon* grown at different dilution rates in phosphate-limited continuous culture (Konopka *et al.*, 1987b).

When examining a population of *Aphanizomenon*

flos-aquae, Konopka found that phosphate affected the recovery of buoyancy of sinking filaments (Konopka, 1989). Phosphate-limited cells exposed to 100 μmol m^{-2} s^{-1} light lost buoyancy, but cells in 2 μM phosphate required 200 μmol m^{-2} s^{-1} in order to lose buoyancy. This demonstrates how the buoyancy mechanism works in favour of the cell, overcoming the vertical separation of light and nutrients. Buoyancy is promoted when a cell's nutrient requirements are fulfilled, which enables the cell to float to near the surface and satisfy its light requirements. However, if a cell is suffering nutrient stress, buoyancy is reduced and the cell is able to scavenge deeper into the water column for nutrients.

Carbohydrate changes rapidly in response to light and can alter buoyancy. Experiments detailed here suggest that other cell constituents (starch and polyphosphate), which change in response to previous light and nutrient conditions, may also affect cell buoyancy.

Light

Light limitation generally has the opposite effect to nutrient limitation. When light is in short supply, gas vesicles tend to accumulate (Reynolds and Walsby, 1975; Utkilen *et al.*, 1985; Kromkamp *et al.*, 1986; Konopka *et al.*, 1987b; Deacon and Walsby, 1990). Carbohydrate content is also low in light-limited cells, provided that nutrients are not limiting. Upon transfer to higher light, cells photosynthesize, accumulate carbon as polyglucose and, depending upon the gas vesicle volume, may induce sinking (Thomas and Walsby, 1985; Utkilen *et al.*, 1985; Kromkamp *et al.*, 1986, 1988; Konopka *et al.*, 1987a).

The RGV of *Microcystis* cells was negatively correlated with growth and decreased with increasing light pre-treatment. This is consistent with the study by Reynolds and Walsby, who documented an inverse correlation between growth rate and gas vesicle volume and observed the lowest gas vesicle volume in log-phase cultures (Reynolds and Walsby, 1975). Kromkamp *et al.* found no light-dependent regulation of gas vesicle content per unit protein and a decrease in gas vesicle volume per cell with increased growth in their strain of *Microcystis* (Kromkamp *et al.*, 1988). However, Kromkamp *et al.* found increasing gas vesicle volume with irradiance 40 h after a phosphate pulse to P-limited cultures, which was maximal at 50 μmol m^{-2} s^{-1} (Kromkamp *et al.*, 1989).

Deacon and Walsby showed that their strain of *M. aeruginosa* had the greatest gas vesicle volume at 30 μmol m^{-2} s^{-1}, which decreased at higher irradiances (Deacon and Walsby, 1990). Cells incubated in the dark following light exposure formed more gas vesicles if their previous irradiance was high and they had adequate CHO stores.

Cultures in the light experiment, documented here, had the highest growth rates and the greatest dilution of gas vesicles. However, less gas vesicles were required to maintain 50% buoyancy. This may be because the light-limited cells had less starch because of a high carbon demand to sustain growth. In soybean, less carbon is stored as starch when the demand for sucrose is high, and cyanobacteria may operate in a similar manner.

Variations in buoyancy in response to resource limitation

These experiments suggest that there are a range of buoyancy responses related to the previous light and nutrient histories and the subsequent exposure of these cells to a range of nutrient and light conditions which represent either optimal or suboptimal resource availability. The variations observed span the gradient from the classical buoyancy regulation response as CHO accumulates in the light and the gas vesicle volume cannot maintain lift and the cells sink, to the opposite extreme where the cells appear to maintain persistent buoyancy.

Do these variations in buoyancy responses correlate with changes in the vertical structure of a water body? Most temperate and Mediterranean water bodies show an increase in water column stability as solar input intensifies. This may lead to a deeper permanent thermocline overlaid by a diurnal surface mixed layer (Monismith *et al.*, 1990). Furthermore, there is a tendency for the available nutrient supply in the surface mixed layer to diminish as the available nutrients are incorporated into an existing population and non-buoyant cells sediment below the thermocline (Ganf and Oliver, 1982).

As thermal stratification first appears, it is likely to be accompanied by abundant nutrients and the resource most likely to be in short supply is light as the vertical extent of mixing may depress the average illuminance experienced by a cell circulating through a strong vertical light gradient. Nevertheless, persistent residence within the euphotic zone, or the formation of surface accumulations, may cause serious photo-oxidation and a significant decrease in photosynthetic efficiency. Our results (Table III) suggest that under low light and high nutrients, *Microcystis* cells exhibit the classical response, although the growth rate is suppressed compared with the rate under optimal light conditions. However, under optimal light, the CHO per cell neither accumulates nor reaches the concentrations found in the other light treatment. Consequently, only a fraction of the cells became negatively buoyant, perhaps as a result of the fast growth rate, or synchronized cell division, and a sampling time that obscured any change in CHO per cell. Although the RGV in all light treatments increased during the 24 h light period, this was insufficient to prevent the loss of buoyancy and may imply that either starch accumulation or protein synthesis contributed to cell density.

In many Australian water bodies, N is the resource most likely to limit growth as the season progresses, which may explain the absence of non-N-fixing species from rivers like the River Murray and the Darling River (Baker *et al.*, 2000). In a hypothetical scenario, if N-starved cells were present when thermal stratification was intense, and N remained a limiting resource, growth rates would be minimal, although cells would retain some buoyancy regulatory strategy via significant accumulation of CHO per cell, which would enable them to scavenge the water column for N. If the cells encountered N (in our experiments 10 μM), the growth rate would increase and the classical buoyancy regulation mechanism would be apparent. However, at higher nitrate concentration, the cells would tend to lose the ability to become negatively buoyant even though there are significant changes in the CHO per cell. This implies that the production of gas vesicles, when N, light and P are abundant, outstrips the density increases caused by CHO accumulation.

In a similar hypothetical scenario, if P-limited cells were present when P was the limiting resource, growth rates would be minimal, CHO concentrations per cell high, yet there would be little change in the frequency of sinking and floating cells as cells were transferred from the dark to the light, although there may be a slight fall in the RGV. If higher phosphate concentrations were encountered, the cells would become persistently buoyant, the growth rate would increase, but there would be little change in the RGV content, although a change in the CHO content per cell would be expected. Even higher phosphate concentrations are accompanied by an increased growth rate, a significant change in CHO per cell, which leads to an increase in the RGV, but 32% of cells lose buoyancy.

If available phosphate again becomes limiting, it would signify the end of the population as the growth rates fall to near zero, the RGV continued to fall and could not provide the lift for the increasing cell density as CHO accumulated.

These experiments were carried out under controlled laboratory conditions where resource availability was manipulated in order to induce a physiological response. The cyanobacteria have a capacity to store nutrients and consequently the observed buoyancy regulation will occur in response to the intracellular nutrient store, which is a function of previous nutrient history. Similarly, in field conditions, the cyanobacteria will respond to their previous nutrient environment and therefore the buoyancy response need not necessarily reflect the immediate nutrient status of the water column, but will be a manifestation of nutrient stores, nutrient uptake rates and resource availability.

Buoyancy significantly increases a cell's light-harvesting capacity. For example, Walsby *et al.* calculated that net photosynthesis of an *Aphanizomenon flos-aquae* population increased 3-fold by floating upwards into higher irradiance following deep mixing (Walsby *et al.*, 1997). Persistently buoyant populations will maximize light capture in stratified environments; however, they may suffer photoinhibition in the surface layer.

Nutrient-deplete cultures typically have reduced buoyancy and consequently are more likely to display vertical migration. The vertical migration need not necessarily be deep excursions into the water column, but migration will increase the nutrient pool, which can be scavenged for required elements. Indeed, the numerical models simulating vertical migration would suggest that migrations for nutrient-replete cultures are relatively shallow (Kromkamp and Walsby, 1990; Visser *et al.*, 1997; Wallace and Hamilton, 1999). Our results suggest that severely N-starved cells would sink until they encountered sufficient nutrients to assemble gas vesicles and recover buoyancy. Failing this, cells would tend to sediment and dominance of the phytoplankton community would shift towards species better adapted to the prevailing conditions.

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