The Effect of Temperature on Recovery of Buoyancy by *Microcystis*

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Colonies of *Microcystis* in Abbots Pool, Avon, UK, were found to regulate their buoyancy according to light (photon flux density). The autumnal decline of the population was associated with an increase in the proportion of colonies that were non-buoyant, and with declining temperatures in the pond. Non-buoyant colonies taken from the pond regained buoyancy in the dark rapidly at 20 °C but only slowly at 12 °C and below. A laboratory strain of *Microcystis* behaved in a similar manner. Comparisons of the behaviour of this organism placed at 8 °C and 20 °C were made; in high photon flux density buoyancy was lost at both temperatures due to accumulation of dense carbohydrate. When transferred to the dark cells at 20 °C became buoyant again as carbohydrate was utilized and more gas vesicles were made; at 8 °C much less carbohydrate was used and no increase in gas-vacuolation occurred. The failure to regain buoyancy in the dark at low temperatures accounts for the loss of buoyancy and sedimentation of the *Microcystis* in Abbots Pool.

INTRODUCTION

Microcystis is one of the most widespread and successful of the planktonic cyanobacteria and is well known for the formation of surface water-blooms (Reynolds & Walsby, 1975). It has been established that in temperate zones *Microcystis* populations reach a maximum in late summer and autumn before many of the colonies fall to the lake sediments where they overwinter in the vegetative state (e.g. Reynolds & Rogers, 1976; Fallon & Brock, 1981; Takamura *et al.*, 1984). The accumulation of colonies in sediments must result from increased excess density of the colonies, which are usually buoyant owing to the presence of gas vesicles in their cells.

A colony in the plankton must remain non-buoyant for sufficiently long to reach the lake bottom if it is to be lost by sedimentation. The possible causes of autumnal buoyancy loss have been discussed previously (Reynolds *et al.*, 1981; Oliver *et al.*, 1985). Oliver *et al.* (1985) found that buoyant *Microcystis* colonies fell out of the water column in one of the Blelham Tarn Lund tubes because of precipitation with a heavy iron-containing colloid that formed when iron-rich water of the hypolimnion became oxygenated. However, this mechanism cannot apply universally because it depends on the presence of large quantities of iron (or possibly other elements such as manganese) and a suitably high pH to allow precipitation of the oxidized metal.

Cells in *Microcystis* cultures are able to regulate their buoyancy according to photon flux density (Thomas & Walsby, 1985). It was quantitatively shown that in bright light the density of cells increased sufficiently to cause loss of buoyancy, primarily because of carbohydrate accumulation. Kromkamp & Mur (1984) showed that in cultures kept on light/dark cycles there was a close correlation between changes in buoyant density of cells and their carbohydrate content. In more general terms, it has been held that buoyancy in cyanobacteria depends on an interaction of light and limiting nutrients that affects the relative rates of photosynthesis, growth and gas vesicle synthesis (Walsby, 1977; Klemer *et al.*, 1982). Temperature will also affect these processes, but no investigations have been made into its effects on buoyancy. Most laboratory experiments on buoyancy have been done at 20 °C or higher, while cyanobacteria in nature are frequently present at temperatures well below the optima for photosynthesis and growth (Konopka & Brock, 1978; Krüger & Eloff, 1978).

Sedimentation of *Microcystis* populations is associated with declining water temperature (see data of Renolds & Rogers, 1976; Rother & Fay, 1977; Takamura *et al.*, 1984), but temperature changes have not previously been shown to cause loss of buoyancy. In this paper direct evidence is presented for light-mediated regulation of buoyancy in a natural population of *Microcystis* and for the involvement of low temperature in the seasonal loss of buoyancy.

METHODS

Natural population. (i) *Site.* Abbots Pool is a small eutrophic pond near Bristol (grid reference ST 535734). It has been described in detail by Moss (1969); it measures 40 × 100 m and has a maximum depth of 4 m. A *Microcystis* bloom was present in the autumn of 1984.

(ii) *In situ incubation experiments.* Samples of *Microcystis* were concentrated by towing a plankton net (100 µm, mesh) and were incubated in 100 ml conical flasks stoppered with rubber bungs and clipped to wooden battens that were suspended at required depths on a rope anchored at one end and buoyed at the other. Dark bottles were flasks that had been painted black. Buoyancy of colonies was determined with minimum delay by counting floating and sinking colonies in a Lund counting cell. The Lund cell is a normal microscope slide, with the edges raised 0.5 mm on which a 22 × 55 mm coverslip can be placed; a sample of 0.62 ml volume is pipetted under the coverslip. The principle of operation is the same as the haemocytometer method of Walsby & Booker (1980).

(iii) *Sampling and counting.* Depth profiles were taken at a station at the deepest point of the pond, using a 200 ml water sampler. Samples were taken at 0.5 m intervals from the surface to 3.5 m. When required, sediment samples were obtained by disturbing the soft sediment with the water sampler before closing it. The temperature of samples was determined immediately using a mercury thermometer. Samples of known volume were concentrated to a measured volume by drawing off water with a 50 ml syringe, the nozzle end of which had been cut off and replaced with nylon mesh, pore size 15 µm, tightly held on with elastic bands. This allowed backwashing of the mesh. The buoyancy and numbers of colonies in the concentrated samples were then determined simultaneously using the Lund cell as described above.

(iv) *Traps.* Colonies sedimenting out of the water column were trapped in plastic buckets that were weighted on the bottom of the pond and attached to surface marker buoys.

(v) *Temperature experiments.* Colonies collected from the pond were incubated in dark McCartney bottles at various temperatures. Buoyancy of colonies was determined as described above.

Culture experiments. (i) *Culture.* *Microcystis* BC 84/1 was grown at 20 °C in BG11 medium; this is *not* the same strain as the *Microcystis* in Abbots Pool. Details of the culture and the methods below are given by Thomas & Walsby (1985).

(ii) *Buoyancy.* The percentage of cells floating was determined after centrifuging cell suspensions in horizontally placed Microslides (Thomas & Walsby, 1985). Microslides are capillary tubes with a rectangular cross section and flat side walls that allow undistorted microscopical observation.

(iii) *Gas vesicles and cell density.* Gas vesicle collapse pressure distributions were determined using a pressure nephelometer as described by Walsby (1980). The volume occupied by gas vesicle gas space in cell suspensions was measured with a capillary compression tube (Walsby, 1982). Cell densities were determined on continuous gradients of Percoll (Oliver *et al.*, 1981) using the modifications of Thomas & Walsby (1985).

(iv) *Cell constituents.* Dry weight was determined using Whatman GF/C filters and a Cahn electrobalance (four replicates). Carbohydrate was measured with the anthrone reagent (Herbert *et al.*, 1971). Protein was assayed using BCA Protein Assay (Pierce Chemical Co.) and bovine serum albumin standards in a colorimetric test for copper(I), which is generated when copper(II) reacts with protein under alkaline conditions.

(v) *Light/dark cycles.* Light was supplied to cultures on timed cycles from fluorescent tubes underneath the culture flasks. The mean photon flux density in cultures was determined by the method of Van Liere & Walsby (1982) using a Macam quantum sensor (sensitive to wavelengths between 400 and 700 nm).

RESULTS

Diel buoyancy changes in early autumn

During October 1984 it was found that the buoyancy of *Microcystis* colonies near the surface of Abbots Pool changed at different times of day. This was shown by the following experiments.

Concentrated samples of buoyant *Microcystis* colonies collected in a net tow were incubated in duplicate bottles at each of 0, 1 and 2 m depth from 10.45 h to 16.00 h on 9 October. The proportion of buoyant colonies was determined at 16.00 h and again on the following morning at 09.00 h after dark incubation overnight. The results (Table 1a) showed that in the surface samples many colonies lost their buoyancy during the day and most regained it at night; at 1 m

Table 1. *Proportion of Microcystis colonies floating after incubation in light or dark and at different depths*

(a) Concentrated samples of colonies were incubated in flasks in Abbots Pool at three depths for 5.25 h. The sample from the surface was further incubated in the dark in the laboratory for 17 h (9 October 1984). (b) Concentrated samples were incubated in light and dark bottles at the surface of Abbots Pool for 4.7 h (10 October 1984).

	Samples (and time)	Percentage of colonies floating	Number of colonies counted
(a)	Initial (10.45 h)	99	135
	Surface (16.00 h)	49	677
	1 m (16.00 h)	99	217
	2 m (16.00 h)	99	190
	Surface (09.00 h)	92	242
(b)	Initial (12.00 h)	98	317
	Light (16.40 h)	54	445
	Dark (16.40 h)	100	135

and 2 m buoyancy was not lost. It was subsequently found that only 3% of incident light penetrated to 1 m and 0.1% to 2 m.

The importance of light in buoyancy loss was conclusively established by comparing the buoyancy of colonies in light and dark bottles suspended at the surface of the pool on 10 October (Table 1b); as before, many colonies lost buoyancy in the light, but none did in the dark. Gas vesicle collapse pressure curves showed that the median critical pressure of gas vesicles in cells with no turgor pressure was 0.73 MPa and that the weakest gas vesicles collapsed at 0.55 MPa. The highest average turgor pressure reached in any of the experiments was 0.47 MPa, which was insufficient to cause collapse of any gas vesicles.

Buoyancy changes by colonies free in the pool could also be seen. Fig. 1 shows data from depth profiles taken at 10.45 h and 16.45 h on a bright day (16 October 1984). At each station between the surface and 2 m depth the proportion of colonies floating had declined during the day. A decrease in the number of colonies in samples near the surface had also occurred.

The autumn decline of Microcystis

The abundance of *Microcystis* in Abbots Pool, estimated by taking depth profiles with stations at 0.5 m intervals, is shown for the period from 9 October 1984 to 29 November 1984 in Fig. 2(a). The percentage of colonies floating is also shown (Fig. 2b); it will be noted that the decline of the population coincided with a decrease in the proportion of colonies that were buoyant.

Large numbers of colonies were found in buckets that had been left on the bottom of the pool from 7 to 29 November. This suggested that at least part of the population decline was due to colonies falling out of the water column onto the sediments. In order to test whether the colonies were sinking due to attachment of extraneous material to them (as found by Oliver *et al.*, 1985), colonies collected in a net tow were washed thoroughly on Kleenex tissue. No significant difference in the proportion of colonies floating was found following this treatment.

During the period mid-October to mid-November the proportion of colonies in the water column that were buoyant fell from over 90% to 35% (Fig. 2b). This coincided with a decrease in the water temperature from 12 °C to 8 °C (Fig. 2c), which suggested that declining temperature might in some way be affecting the buoyancy of colonies.

Effect of temperature on buoyancy changes

Of colonies collected in net tows on 15 November 49% were buoyant. An experiment was done to test whether the sinking colonies would regain their buoyancy when incubated in the dark and whether this response was temperature related. Sub-samples were placed in the dark at a range of temperatures and the buoyancy of colonies was determined after 17 and 40 h (Fig. 3).

Fig. 1. Percentage of colonies floating (○, ●) and number of colonies at different depths (□, ■) in Abbots Pool on 16 October 1984. Samples were collected at 10.45 h (○, □) and at 16.45 h (●, ■).

Fig. 2

Fig. 2. Change during October and November 1984 in Abbots Pool of: (a) number of colonies per ml of water (averaged from samples taken at 0.5 m intervals); (b) the proportion of colonies floating; (c) the average water temperature.

Fig. 3

Fig. 3. Proportion of colonies floating after incubation in the dark at a range of temperatures for 17 h (○) and 40 h (●). The colonies were collected from Abbots Pool; 49% were buoyant initially. The lowest line (□) shows the proportion of cells of *Microcystis* BC 84/1 floating after 24 h dark incubation (the 95% confidence limits were within 4.6% of the mean for each value). Initially cells, grown at a photonflux density of $53 \mu\text{mol m}^{-2} \text{s}^{-1}$ for 16 h, were 10% floating.

Recovery of buoyancy was much less at lower temperatures; for example, after 40 h at 30°C 84% of colonies that were initially non-buoyant had recovered buoyancy, while at 7°C only 10% had recovered buoyancy.

In the next experiment samples in which 66% of colonies were buoyant were incubated at 7.5°C for 26 h at photon flux densities of 10 and $40 \mu\text{mol m}^{-2} \text{s}^{-1}$. Each sample was then divided, one half being incubated at 7.5°C and the other at 20°C for a further 92 h in the dark. The proportion of colonies floating at various times is shown in Fig. 4. Most colonies lost buoyancy at both photon flux densities. Little or no recovery of buoyancy occurred at the lower

Fig. 4. Changes in the proportion of colonies floating after a light (open bar) and a dark (shaded bar) period, and the effect of temperature on these changes. Samples were exposed to a photon flux density of $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ (●), or $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ (○); —, incubation at 7.5°C , ---, incubation at 20°C . Colonies were collected from Abbots Pool and incubated in the laboratory.

temperature; however, substantial recovery occurred at the higher temperature. There was a pronounced lag before the colonies that had been at the higher photon flux density recovered buoyancy.

Behaviour of colonies in the winter

Investigation of the colonies collected in the bucket traps showed that over 50% of them were buoyant and these seemed to be trapped on the bottom of the pool by debris in the sediment. In a sediment sample taken on 15 March 1985 many buoyant colonies were found; the exact proportion that were floating was not determined as non-buoyant colonies could not be reliably counted amongst the surrounding sediment particles.

Throughout the winter a small population of *Microcystis* was consistently found in the water, averaging about $0.5 \text{ colonies ml}^{-1}$.

Buoyancy changes in laboratory culture

Since the *Microcystis* in Abbots Pool was mixed with other algae and zooplankton, detailed measurements to determine the causes of buoyancy change could not be undertaken (unlike the relatively pure population in Blenheim Tarn investigated by Oliver *et al.*, 1985). Further studies were therefore undertaken using a laboratory strain of *Microcystis*, BC 84/1. The relationship between temperature and buoyancy recovery in the dark of cells that had been exposed to high photon flux density is plotted in Fig. 3. Like the *Microcystis* in Abbots Pool, the cells in this culture also regained buoyancy more rapidly at high temperatures. Cells of this strain of *Microcystis* grown at low photon flux densities lost buoyancy at high photon flux density due to an increase in cell density that resulted mainly from carbohydrate accumulation. The following experiment was therefore designed to test the theory that a failure to metabolize carbohydrate at low temperature prevented cells from regaining buoyancy. Cells grown at a photon flux density of about $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ were exposed to $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 8°C and at 20°C for 18 h. They were then kept at these temperatures in the dark for a further 22 h. Determinations of buoyancy, density after gas vesicle collapse, dry weight, and major cell constituents (protein, carbohydrate and gas vesicle space) were made (Table 2). On exposure to bright light the two cultures behaved in a similar manner; nearly all cells lost buoyancy and the density of cells increased by about 10 kg m^{-3} , from the original value of 1034 kg m^{-3} . The dry weight and carbohydrate content both increased significantly, but protein did not change greatly. During the period in the dark, however, the cultures behaved differently from one another. The buoyancy of cells at 20°C recovered to 95% floating, while at 8°C it increased to only 16% floating. The large change in buoyancy at 20°C was associated with a 64% decrease in carbohydrate, a 9% increase in gas vesicle gas space and a 17% increase in protein. The magnitude of all these changes was much smaller in the 8°C culture; most notably carbohydrate had decreased by only 11%.

Table 2. *Changes in major cell constituents contributing to cell density after a light and then a dark period at two temperatures*

(a) The proportion of cells floating, cell density and amounts of major constituents in a culture of *Microcystis* BC 84/1 were determined after growth at a mean photon flux density of $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 20°C (initial conditions), after exposure of the culture to $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 8°C for 18 h ($t = 18$ h), and then after dark incubation at 8°C for a further 22 h ($t = 40$ h). (b) As (a), but culture kept at 20°C throughout.

	Initial values ($t = 0$ h)	Values after incubation			
		$t = 18$ h	% change from $t = 0$ h	$t = 40$ h	% change from $t = 18$ h
(a) Incubated at 8°C					
Cells floating (%)	85	8		16	
Density (kg m^{-3})	1034	1043		1042	
<i>Amounts per ml culture:</i>					
Dry weight (μg)	179	214	(+20**)	209	(-2 NS)
Protein (μg)	105	101	(-4 NS)	105	(+4 NS)
Carbohydrate (μg)	15	35	(+133**)	31	(-11**)
Gas vesicle space (nl)	ND	44	—	45	(+2 NS)
(b) Incubated at 20°C					
Cells floating (%)	85	10		95	
Density (kg m^{-3})	1034	1044		1034	
<i>Amounts per ml culture:</i>					
Dry weight (μg)	179	223	(+25**)	195	(-12**)
Protein (μg)	105	99	(-6*)	111	(+17**)
Carbohydrate (μg)	15	39	(+160**)	14	(-64**)
Gas vesicle space (nl)	ND	46	—	49	(+9**)

ND, Not determined.

* t -test indicates values significantly different at 5% level; ** significant at 1% level; NS, not significant at 5% level.

Buoyancy changes of cells in four cultures maintained on light/dark cycles are shown in Fig. 5. The cultures were kept at temperatures of 8°C and 20°C and photon flux densities of $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ for five complete cycles. At 20°C cells at both photon flux densities showed diel regulation of buoyancy, losing buoyancy in the light and recovering it in the dark. However, at 8°C recovery in the dark was insufficient to make up for buoyancy lost in the light, causing progressive net loss of buoyancy in each cycle. This buoyancy loss was shown to be reversible by shifting part of the 8°C cultures back to 20°C after a light period. After 16 h in the dark at 20°C both had increased from around 20% cells floating to 50% floating; controls at 8°C had shown very small recoveries of buoyancy (Fig. 5).

Growth rates for the four cultures on alternating light/dark (8/16 h) regimes were determined from cell counts. Doubling times were: 3.5 d at $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 20°C ; 211 d at $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 8°C ; 5.4 d at $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 20°C ; 28 d at $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ and 8°C . The slow growth rates reflect the failure to produce new cell protein at the lower temperature.

DISCUSSION

The loss of *Microcystis* from the plankton to the sediment in Abbots Pool resulted from loss of buoyancy of colonies. The cause of this can be summarized in the following way. In early autumn colonies exhibited diel regulation of buoyancy in response to light. At high photon flux densities near the surface during the day, colonies lost buoyancy; in the dark, in deeper water and at night, buoyancy was regained. The mechanism of light-mediated buoyancy loss is likely to have been accumulation of dense carbohydrate as found in the laboratory strain of *Microcystis* by Thomas & Walsby (1985). Some other cyanobacteria are able to lose buoyancy by collapsing their gas

Fig. 5. Changes in the proportion of cells floating in cultures of *Microcystis* BC 84/1. (a) On cycles of 8 h light, $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ (open bar), 16 h dark (shaded bar) at 20°C ($\circ$) and 8°C ($\bullet$); (b) as (a) except using a photon flux density of $20 \mu\text{mol m}^{-2} \text{s}^{-1}$. Samples were transferred from 8°C to 20°C on day 7 ($\square$).

vesicles under increased turgor pressure (Oliver & Walsby, 1984). This mechanism could not have operated in the *Microcystis* from Abbots Pool, however, because its gas vesicles are too strong to be destroyed in this way.

As autumn progressed the water temperature in Abbots Pool decreased and the population of *Microcystis* colonies gradually lost its buoyancy. It appeared that the reason for this was that colonies near the water surface continued to lose their buoyancy in response to light intensity during the day but they failed to recover their buoyancy subsequently in the dark. This failure could be attributed to the temperature falling below a certain critical value, around 10°C , at which buoyancy could not be regained sufficiently rapidly in the dark to prevent colonies reaching the sediment.

Once on the sediment, colonies became entrapped there and although they gradually recovered buoyancy through depletion of carbohydrate they were not able to float free. This accounted for the presence of buoyant colonies in the sediment during winter. The colonies that were found in small numbers in the plankton during the winter may have been a residual population that had not sedimented out, or they may have been released from the sediment by disturbance; individual colonies left in suspension may have continued to lose buoyancy on exposure to light and to sink to the sediment during the winter. In the spring, as the water temperature increased, colonies released from the sediment would be able to remain buoyant again, and to grow if other factors permitted.

Experiments with *Microcystis* BC 84/1, which was shown to behave in a similar manner with respect to temperature, indicated that the magnitude of buoyancy loss in the light was not greatly different at 8°C and 20°C . However, at 8°C the rates of protein and gas vesicle synthesis in the dark were greatly reduced, as was the rate at which dense carbohydrate was lost, compared with the activity at 20°C (Table 2). There was thus a crucial difference in the relative rates of carbohydrate production in the light and its utilization in the dark between the two temperatures. The resultant buildup of carbohydrate in cells at low temperatures prevented them from regaining buoyancy.

Our results suggest that the ability of *Microcystis* cells to remain buoyant is linked to their ability to grow by producing protein at the expense of carbohydrate, and that these abilities are therefore similarly sensitive to the effects of temperature. The significance of this is that in a

lake the organism will fall out of suspension at temperatures where growth cannot occur; overwintering takes place in the sediments.

The results here can be related to other investigations into the effects of light and temperature on other cyanobacteria. Foy (1983) found that carbohydrate changed in a similar manner in two species of *Oscillatoria* grown on 3 h light/21 h dark cycles. At lower temperatures 'dark reactions' in cells were depressed so that the dark period was not long enough for cells to metabolize the store of carbohydrate laid down in the light. Below a critical temperature (11 to 14 °C) this resulted in a greater decline in growth rate than predicted by the expected Q_{10} of 2 for light-saturated photosynthesis. Kruger & Eloff (1978) also found a sharp decline in growth rate below about 15 °C in four strains of *Microcystis*, though the exact critical temperature varied between strains. Okino (1973) commented that in several Japanese lakes *Microcystis* did not appear until the water temperature had reached 20 °C, and again there were indications of strain-specific temperatures. The presence of *Microcystis* in a lake will be determined not only by its ability to grow but also by its ability to remain in suspension.

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