ON THE ANNUAL CYCLE OF THE BLUE-GREEN ALGA *MICROCYSTIS AERUGINOSA* KÜTZ. EMEND. ELENKIN

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A 4 year series of field, light-microscope and ultrastructural observations is presented to illustrate biological aspects of the annual cycle of natural *Microcystis* populations enclosed in Lund tubes. Nine morphological stati, all referable to *M. aeruginosa* f. *aeruginosa*, feature at various stages of the cycle. Summer bloom-forming populations originate from vegetative colonial stock that overwinters on the bottom sediment each year, but there is no mass transfer of these colonies to the water column: intensive growth from individual cells in the old colonies leads to the formation of new infective colonies, being stimulated when the bottom water approaches anoxia and light penetrates to the bottom sediments. Growth is slow but the developing populations sustain only minor losses through grazing and settling out, eventually becoming dominant over other species. Allelopathy possibly contributes to this effect. In post-maximal populations, several mechanisms can contribute to net buoyancy loss and a (usually) rapid recruitment of vegetative colonies to the sediments is observed.

Hypotheses are advanced to account for the observed behaviour, and some of these have been tested in the laboratory. The apparent physiological flexibility of *Microcystis* seems well suited to growth and survival in the microenvironments encountered in eutrophic lakes.

1. Introduction

One of the consequences of accelerating eutrophication in many temperate lakes and reservoirs is the mass development of planktonic blue-green algae, or cyanobacteria (Fogg 1969). Whereas increased exogenous nutrient loadings may be invoked to explain the maintenance of higher average standing crops (Vollenweider 1968; Dillon & Rigler 1974), they do not account for the shift in species dominance in favour of blue-green algae. Possible causal mechanisms, each arguably an indirect consequence of advancing trophic status, include: raised pH and the diminishing fraction of aqueous carbon dioxide available for photosynthesis (Shapiro 1973; Keenan 1973); the interaction between the attenuation of incident light and the physical stability of the water column (Kirk 1975; Reynolds & Walsby 1975; Foy *et al.* 1976); the ability to tolerate, if not flourish under, conditions of low oxygen tension and low redox potential (Gusev 1962; Sirenko *et al.* 1968; Eloff 1977), wherein weaker mineralization of organic matter (Topachevskiĭ *et al.* 1969), greater mobility of metals (especially iron: see Shapiro 1967)

or the availability of sulphur-containing compounds (see, for example, Volodin 1971, 1975), including hydrogen sulphide (Castenholz 1976, 1977; Oren & Padan 1978), may be important. Other selective mechanisms might include the production of iron-scavenging siderochromes (Simpson & Neilands 1976; Murphy *et al.* 1976) and differential effects of changes in the pressure of zooplankton grazing (Briand & McCaulay 1978; Nilssen 1978).

None of these explanations has yet been shown to be generally applicable although the competitive advantage to blue-green algae is undoubtedly enhanced by the low rates of loss through sinking, grazing, death and washout sustained by vegetative populations (Biermann 1976; Kalff & Knoechel 1978).

One of the most successful and cosmopolitan species is *Microcystis aeruginosa* Kütz. emend. Elenkin. Because its abundance sometimes poses serious economic and, occasionally, health problems (see, for example: Krüger & Eloff 1977; Collins 1978), practical methods for its control are actively sought. Laboratory cultures of *Microcystis* have been used for many years in the investigation of the alga's physiological characteristics and growth requirements (see, for example: Gerloff *et al.* 1952; Zehnder & Gorham 1960; Krüger & Eloff 1977, 1978) but these factors have not explained the ability of *Microcystis* to dominate over other species in the plankton of eutrophic lakes. We believe the experimental approach to be correct but there is still a need to relate laboratory studies to the behaviour of natural populations of *Microcystis*.

In this paper we describe morphological, cytological and physiological changes associated with the cycle of growth and perennation of *Microcystis* populations isolated within two experimental limnetic enclosures ('Lund tubes' A and B) installed in Blelham Tarn, Cumbria (National Grid Reference NY 365004). The Butylite walls enclose some 18500 m^3 of water (mean depth 11.4 m) and about 1630 m^2 of lake sediment (for further details see Lack & Lund (1974)). The present study was part of a wider investigation of losses sustained by the phytoplankton; complementary results are given in Reynolds & Wiseman (1981) and in Reynolds *et al.* (1981). Reference is also made to observations on natural *Microcystis* populations in Rostherne Mere, Cheshire (NGR SJ 742842), and to a series of laboratory experiments on *Microcystis* collected in the field or on isolates maintained in laboratory culture.

2. Methods

(*a*) *The maintenance of the experimental enclosures*

The observations to be described were made on natural populations isolated in enclosures A and B over three consecutive growing seasons (1977–1979); additional observations made on a population in enclosure B in 1976 are also presented. The experimental systems were isolated early in the year and kept closed to the tarn until the following winter; they were kept continuously closed during the 1978–1979 winter. To maintain the fertility of the enclosed water, inorganic nutrients were dissolved out from hessian sacks towed behind a boat rowed back-and-forth across the enclosure. Nutrients were added at weekly intervals to one (B, 1977) or both enclosures (1978, 1979) in quantities calculated to restore epilimnetic concentrations to arbitrary predetermined levels (300 μg of nitrogen per litre, 20 μg of soluble reactive phosphorus per litre, 1000 μg of soluble reactive silica per litre, and 100 μg of total iron per litre; for full details, see Reynolds & Butterwick (1979)). Enclosure B was fertilized only once in 1976, with a large concentration of phosphorus (*ca.* 60 μg of phosphorus per litre); enclosure A was left

unfertilized throughout 1977. The alkalinity of the water in enclosure A was artificially raised to a maximum of 1.08 milliequiv/l early in 1978 to improve its buffering capacity; enclosure B was similarly treated in 1979 (maximum alkalinity, 0.64 milliequiv/l).

(b) *Sampling and analytical procedures*

The standing populations were sampled at weekly (or more frequent) intervals with a 5 m length of flexible polyethylene hose (Lund & Talling 1957); vertical sample profiles were collected with a 1 m long modified Friedinger sampler (Irish 1980). In 1978 and 1979 only, these samples were integrated in the field to give five samples representing columns of 0–3, 3–5, 5–7, 7–9 and 9–11 m respectively. In July 1978, a series of close-interval sample profiles were collected simultaneously in Heaney's (1974) pneumatically operated sampler. The sediment–water interface was sampled by means of a Jenkin mud corer fitted with a Perspex cylinder having a vertical series of 2 mm holes drilled at 10 mm intervals and sealed externally by a length of transparent adhesive tape (Collins 1977); the semi-fluid surface layer was drawn off into a 50 ml syringe from the segmental area reached by the Luer needle (for further details of this method and the transformation of data to areal units, see Reynolds (1979)).

In the laboratory, chlorophyll was extracted from the residues of Whatman GF/C-filtered water samples in hot 90% (by volume) methanol; chlorophyll *a* concentration was calculated from spectrophotometric absorbance of the extracts at 665 nm, according to the equation of Talling & Driver (1963). *Microcystis* colonies were enumerated directly by the iodine sedimentation and inverted microscope technique of Lund *et al.* (1958). To obtain cell counts, colonial mucilage was hydrolysed in hot caustic soda or potash or discharged by ultrasonic vibration (*ca.* 12 μm, 20 kHz) transmitted in 150 W disintegrator (M.S.E.: Crawley, England); these methods and their statistical validity are described fully in an earlier paper (Reynolds & Jaworski 1978). Ultrasonic disintegration was frequently necessary to ensure adequate separation of cells from the thick peripheral mucilage investment of *Microcystis* colonies harvested from the plankton of the enclosures. Throughout the study, care was taken to avoid damage to cells through excessive exposure and no treatment exceeded 1 min (see Reynolds & Jaworski 1978). In spite of recent criticisms of the use of sonication (Humphries & Widjaja 1979), reasonable agreement ($\pm 10\%$) with other methods of enumeration was obtained (see figure 1). However, estimates of cell concentration in enclosure B during 1979 calculated from the regression equation of Reynolds & Jaworski (1978) were approximately double those estimated in sonicated samples.

Maximum external dimensions of cells and colonies were measured directly in live material by means of a calibrated micrometer eyepiece; quoted cell volumes were calculated from the geometrical equation for a sphere. Individual colony volumes (inclusive of mucilage) were approximated from geometrical equations for spheres, ellipsoids or cylinders as appropriate; more complex structures were visualized as composites of simpler shapes, and their volumes were approximated by summation of the component volumes. Quoted dry masses were measured in colonial suspensions concentrated in a phytoplankton net; portions were oven-dried at 105 °C to constant mass; a further portion was diluted for cell or colony counts as outlined above.

Two methods were used to determine the density of colonies. In the first, concentrated algal suspensions were weighed in a relative density bottle; the suspension was diluted for counting and calculation of the total colony volume (Walsby & Xypolyta 1977). More routinely, the

second method was followed, in which the density of individual colonies was calculated by solution of the Stokes equation (see Walsby & Reynolds 1980). Observed velocities of individual quasi-spherical colonies (viewed directly through a laterally mounted microscope: Reynolds 1972) of measured diameter (to the nearest 5 μm) and the density of the suspending water were interpolated. Form resistance was ignored throughout, as was the effect of Reynolds stress on the largest colonies: the particle Reynolds number of colonies 500 μm in diameter and having a density of 10^3 kg m^{-3} moving through lake water whose viscosity is 10^{-3} kg m^{-1} s^{-1} is 0.5; even in this extreme case the error of the Stokes equation is less than 10% (McNown & Malaika 1950).

Simultaneous equations are developed in §4*e* to evaluate the contribution of cells and mucilage to the density of the colonies. These assume that

$$x\rho_x' + y\rho_y' = \rho_c', \tag{1}$$

where ρ_c', ρ_x' and ρ_y' are respectively the densities of the colony, the mucilage and the cell, and y is the fraction of the colony volume occupied by cells. The fractional volume of the mucilage (x) is equal to $1-y$.

(*c*) *Electron microscopy*

Fixation was based on the method of Wildman *et al.* (1975). Samples were fixed at 4 °C for 2 h in a mixture of equal volumes of osmium tetroxide solution (20 g/l) and glutaraldehyde solution (25 g/l) in 0.1 M phosphate buffer (pH 7.0). After dehydration in a graded ethanol series the material was embedded in Spurr's (1969) resin, polymerized at 70 °C for > 12 h, sectioned on an OMU 2 microtome (Reichert) and stained with uranyl acetate solution (10 g/l) (Watson 1958) and lead citrate (Reynolds 1963). Sections were examined at 80 kV in a Siemens Elmiskop-1 (Siemens) electron microscope.

(*d*) *Fluorescence microscopy*

Chlorophyll fluorescence was used in assessing the physiological condition of benthic *Microcystis* colonies (cf.: Goryunova 1956; Sirenko 1972). Colonies were observed under a Leitz Epifluorescence Microscope (Leitz: Wetzlar), by means of a BG12 glass filter to provide suitable light for excitation of chlorophyll autofluorescence (see Jones 1979).

(*e*) *Sediment traps*

Two designs of sediment trap were employed during the study: one was a lidded Perspex cylinder according to Tauber's (1974) design; the other was a polystyrene screw-cap jar, into which a 60 mm length of plastic tubing was inserted through the cap. The performance characteristics and the methods for calculating their areal catches are described in Reynolds (1979). Traps were placed in both experimental enclosures. The jar traps were normally deployed in pairs on simple platforms constructed of folded aluminium sheeting, secured to a vertical buoyed rope so that they operated approximately 0.25 m above the bottom. Except during the early months of 1977, the aperture of one of the traps pointed upwards, and that of the other downwards. Both traps faced downwards in April and May 1977. Tauber traps were operated in enclosure B in 1976, when they were deployed in pairs (attached to Dexion framing) at depths of approximately 1, 3, 5 and 8 m below the surface. Tauber traps were also operated in both enclosures throughout the 1977, 1978 and 1979 seasons (see also

Reynolds & Wiseman 1981). Formaldehyde (effective concentration *ca.* 40 g/l) was used as a fixative in all the traps. Trap catches were enumerated by the iodine sedimentation and inverted microscope method, and expressed in terms of number of colonies recovered per unit area per unit time.

(*f*) *Bioassays*

The method employed was essentially that of Lund *et al.* (1971), and developed for *Microcystis* by Box (1977). The water tested was collected from a depth of 2–3 m in the 1 m Friedinger bottle (Irish 1980). The water was vacuum-filtered through pre-washed Whatman glass filters (GF/C) on the day of collection and volumes were apportioned between quartz flasks capped with quartz crucibles. Nutrient solutions (a.r. grade) were added, either singly or in combination, in quantities calculated to increase their effective concentrations in the test solutions by 14.1 μg of phosphorus, 59 μg of nitrogen or 340 μg of iron per litre. Iron was always added after chelation with an equimolar quantity of sodium ethylene diamine tetracetate (EDTA–Na). In some of the experiments EDTA–Na was added alone. Experiments are described in which salts of other metals (copper, manganese and zinc) were chelated and added to assay media in concentrations to give 340 μg of metal per litre.

The test organisms used were *Microcystis* L155 (originally isolated from Esthwaite Water in 1968) and L305 (isolated from Blelham enclosure B in 1977). Non-axenic cultures of both were maintained in ASM-1 medium (Gorham *et al.* 1964), modified by G. H. M. Jaworski (see Reynolds & Jaworski 1978), and were illuminated continuously from below (1 klx or 12.6 $\mu E\ m^{-2}\ s^{-1}$, at the base of the flasks) in a Droop cabinet (Droop 1969), thermostatically controlled at 23 ± 1 °C.

Only exponentially growing colonial subcultures were used for the tests: algae were washed on 0.45 μm Millipore membrane filters to which gentle suction was applied and subsequently resuspended into *ca.* 50 ml of filtered test medium by slow magnetic stirring (Box 1977). Fixed volumes of this suspension were inoculated into each of the test flasks, which were then incubated for 14 days under light and temperature conditions identical to those under which the stock cultures were maintained (above). Each treatment was duplicated. Samples of inocula and the cultures were fixed in iodine, and cell concentrations† were enumerated after either alkaline hydrolysis or ultrasonication. Results for growth rates are expressed as number of divisions per day or per week. Strain L155 was used continuously as test organism in 1977 and again between June and November 1978. Strain L305 was used between July and September 1977, during May and June 1978 and throughout 1979. Both strains were used simultaneously between July and September 1977. The results obtained with either strain were qualitatively identical though L155 gave consistently larger yields and average growth rates.

(*g*) *Experimental methods*

Several simple experiments were carried out during the course of this study that were designed to illuminate specific aspects of the ecology of *Microcystis*. These are not described separately here since the methods employed are briefly outlined at the appropriate point in the text. The carbohydrate analyses of *Microcystis* (see §10) were kindly undertaken by Dr C. E. Gibson at the laboratories of the Freshwater Biological Investigation Unit, Antrim, following the anthrone colorimetric method of Herbert *et al.* (1971; see also Gibson 1978).

† The cell or colony concentration is the number of cells or colonies per unit volume. The areal cell or colony concentration is the number of cells or colonies per unit area.

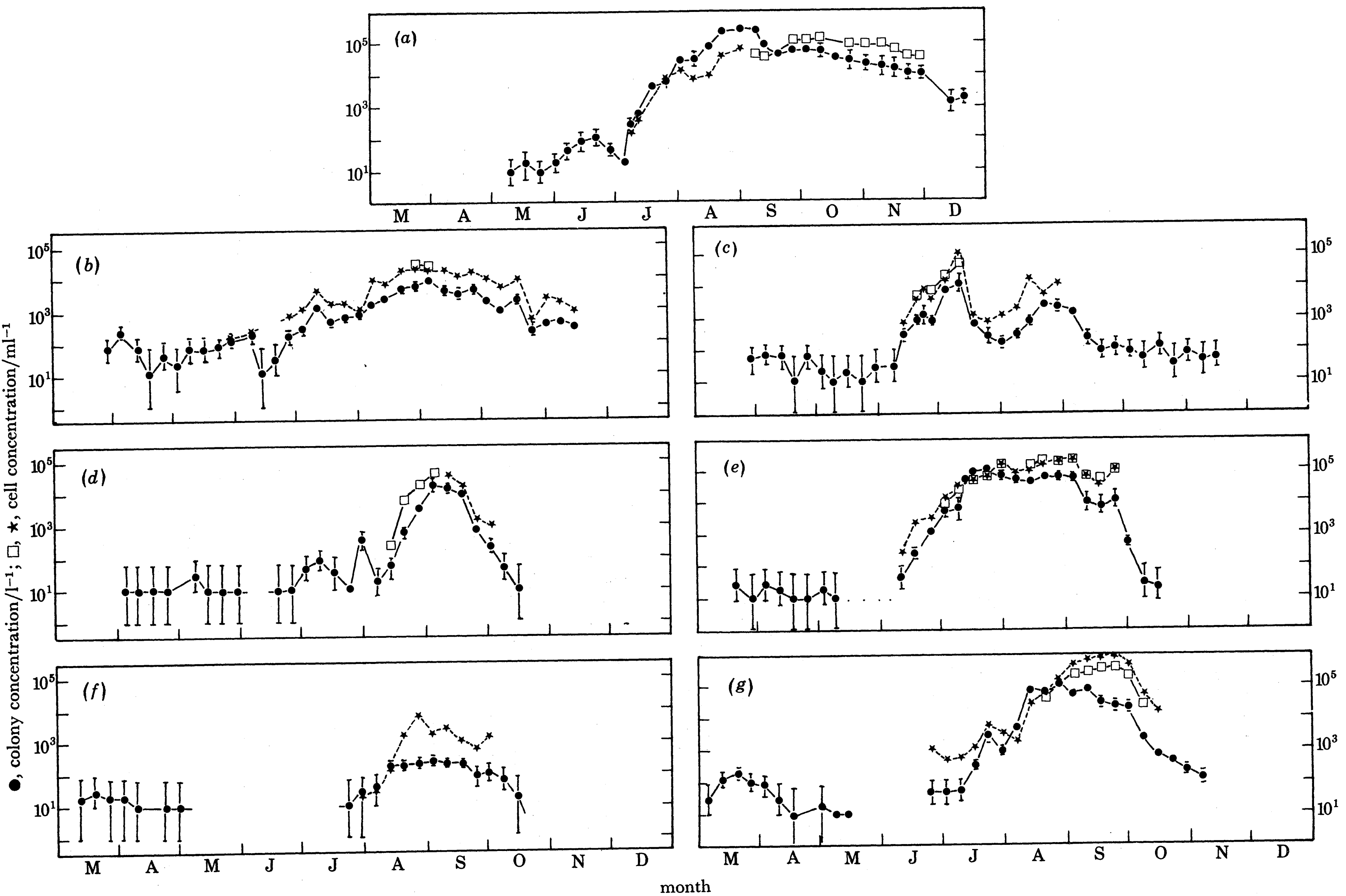


FIGURE 1. Semilogarithmic plots of the seasonal development of seven *Microcystis* populations in the experimental enclosures (Lund tubes A, B): (*a*) B, 1976; (*b*) A, 1977; (*c*) B, 1977; (*d*) A, 1978; (*e*) B, 1978; (*f*) A, 1979; (*g*) B, 1979. Populations are expressed as number of colonies (± 95% confidence limits) per litre (●) or as number of cells per millilitre, either measured directly after ultrasonication of colonies (□) or calculated from the regression solution based on colony size (★) (Reynolds & Jaworski 1978).

3. General features of the *Microcystis* populations

The seasonal development of the *Microcystis* populations in the upper 5 m of the experimental enclosures is represented in figure 1. Relatively small concentrations of colonies were sometimes maintained in suspension during the spring but these decreased after the onset of thermal stratification (see figure 2). In each of the years considered, *Microcystis* increase was restricted to the late spring or summer months, although the precise timing, the rate and the

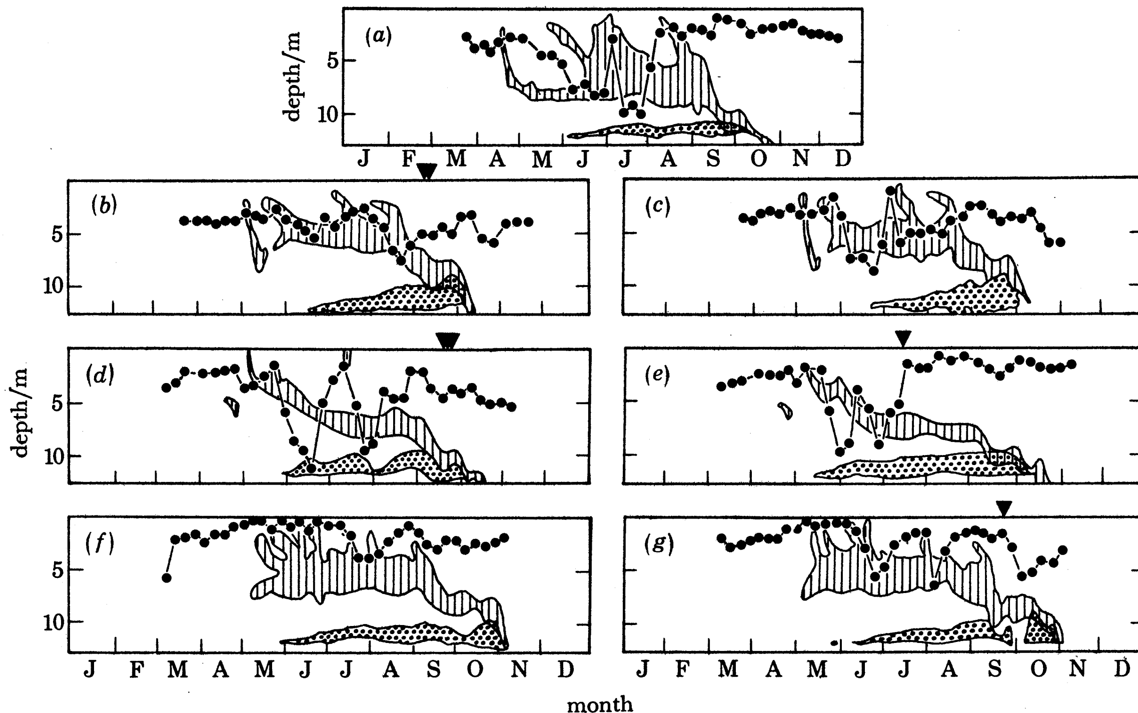


Figure 2. Seasonal changes in the physiological and chemical characteristics of the water column in the experimental enclosures during the study period: (*a*) B, 1976; (*b*) A, 1977; (*c*) B, 1977; (*d*) A, 1978; (*e*) B, 1978; (*f*) A, 1979; (*g*) B, 1979. Vertical hatching denotes the extent of the metalimnion (defined here as the part of the water column in which the temperature gradient exceeds 1 °C m^{-1}); stippling represents the extent of 'anoxic' water (oxygen concentration < 1 % of saturation at atmospheric); ● shows depth of Secchi disc extinction; ▼ indicates known occasions on which a substantial surface bloom was formed.

size of the eventual standing crop varied conspicuously from year to year. The onset of increase was typically abrupt, a feature common to *Microcystis* populations in Rostherne Mere (see Belcher & Storey 1968).

Many of the populations persisted until the end of summer but colony concentrations were abruptly reduced, by factors of between 10 and 100, during October or November. Decline could not be attributed to dilution of the epilimnetic population in the increased autumnal circulation. A contemporaneous removal, either through death and decomposition or by sinking out, is implied (cf. Reynolds & Rogers 1976). Known incidences of surface bloom formation (marked on figure 2), with or without subsequent lysis, were rare and cannot be invoked to explain the disappearance of the populations. On the other hand, rapid autumnal recruitment to the bottom deposits, similar to that reported in the literature (see, for example:

Vladimirova 1968; Topachevskiĭ *et al.* 1969; Reynolds & Rogers 1976), has been observed in the experimental enclosures (Reynolds & Wiseman 1981; see also § 10).

Where comparative data are available (figure 1), changes in colony and cell concentration in these populations were not in constant proportion: mean cell:colony ratio varied over one or two orders of magnitude during the course of a single growing season. The full range of the ratio during the study was 130 to 28200 cells per colony.

4. Morphological characteristics of *Microcystis*

(*a*) *Colony morphology*

The variability in size, shape and cell arrangement of *Microcystis* is well known and for a long time resulted in a confused and cumbersome taxonomy. Wesenberg-Lund (1904) was one of the first investigators to recognize that hitherto separate species of the genus *Polycystis* Kützing (*P. flos-aquae*, *P. aeruginosa*), previously ascribed to different genera (*Anacystis* Meneghin, *Clathrocystis* Henfrey, respectively), were ontogenetically continuous. Komárek's (1958) rationalization of the nomenclature reduced the genus (which he referred to *Diplocystis* Trevis) to five species. We follow the nomenclature of Kondrat'eva (1968) based upon her careful micrographic and statistical analysis of field material collected in the U.S.S.R. According to this scheme only two separate species are recognized, *Microcystis wesenbergii* Komárek and *M. aeruginosa* Kütz. emend. Elenkin, the latter being divided into three formae (f. *aeruginosa*, f. *flos-aquae* and f. *viridis*). These taxa were also adequate to describe the range of *Microcystis* colony structures represented in the meres of the English northwest Midlands, except that *M. aeruginosa* f. *viridis* has not been recorded (see Reynolds 1978*a*). Indeed, most British records of *Microcystis* can be accommodated within the same three taxa (Whitton *et al.* 1978). Both the *aeruginosa* and *flos-aquae* formae coexist in Rostherne Mere (Reynolds 1978*b*). The material from Blelham Tarn described in this paper is ascribed exclusively to f. *aeruginosa* (W.D.U. Codon 012901; Whitton *et al.* 1978).

Nevertheless, the range of colony size and structure observed in the Blelham populations has been considerable (see plate 1). We attempted to follow Kondrat'eva's (1968) subdivision of *M. aeruginosa aeruginosa* into morphological stati but our observations did not conform satisfactorily to her scheme. As a temporary expedient, we devised morphological categories identified by Roman numerals (I–VI). In practice, some further subdivision proved necessary; an annotated list of the nine 'stati' ultimately adopted is given below. Examples of each type are illustrated in figures 3 to 13, plate 1.

Status I a (figure 3). This form has no separately named equivalent in Kondrat'eva's (1968) nomenclature although it is apparently included in her st. *aeruginosus*. Initially, all colonial structures in which the arrangement of live cells was sufficiently diffuse to allow all the cells to be individually viewed in two dimensions were grouped together as st. I. This included elliptical or less regular mucilaginous bodies, having maximum linear dimensions of 40–1000 μm, in which the cell concentration was less than 1 per 1000 μm^3. Such colonies were abundant in samples of benthic populations and they were assumed to be senescent or moribund: empty cells were often visible, while the mucilage supported large numbers of bacteria and frequently contained amorphous brownish residues. They were subsequently reclassified as st. I a to distinguish them from colonies referred to st. I b, below.

Status Ib (figure 4). Small (maximum linear dimension less than 250 μm) irregular ovoid or cylindrical colonies each containing between 20 and 200 live cells at a mean concentration of less than 1 per 1000 μm^3 were observed in exponentially increasing epilimnetic populations. A separate category was introduced because, though superficially similar to st. Ia, st. Ib colonies lacked the features symptomatic of senescence. However, it has not been established that st. Ib colonies are produced as a consequence of typical seasonal growth.

Stati II *and* III (figures 5, 6). Although mutually separated from each other by a distinct morphological character, st. II and III colonies can be described together. Both categories are characterized by irregular elliptical, strap-like or more complex shapes in which the cells are always compactly arranged (more than 3 per 1000 μm^3) in a central core, such that individual cells are indistinguishable when the colony is viewed in two dimensions, and bounded by a clear peripheral mucilage layer (10–45 μm thick). St. III colonies were distinguished by their lack of fenestration, varying in form between short plates and branched cylinders, ranging between 40 and 300 μm in length. All fenestrated colonies, including punctured discs, 'doughnut rings' (cf. Wesenberg-Lund 1904), with or without side arms, and irregularly anastomosing nets, were grouped as st. II. These forms were variously segregated by Kondrat'eva (1968; see also Kondrat'eva & Kovalenko 1975) among the stati *aeruginosus, scriptus, sphaerodictioideus* and *ochraceo-sphaerodictioideus*. We suggest that these forms share a similar ontogenetic position in the life cycle of *Microcystis aeruginosa*, being typical of late post-maximal populations; they also occur among benthic, overwintering stocks.

Description of plate 1

Light microscopy of colonies of Microcystis, illustrating various morphological categories (see text)

Figure 3. Status Ia. Bright field; colony mounted in Indian ink to show extent of mucilage. (Magn. × 120.)

Figure 4. Status Ib. Reichert Anoptral contrast. (Magn. × 200.)

Figure 5. Status II. Bright field; Indian ink. (Magn. × 48.)

Figure 6. Status III. Bright field; Indian ink. (Magn. × 64.)

Figure 7. Status IV. Bright field. (Magn. × 80.)

Figure 8. Status IVn. Bright field. (Magn. × 160.)

Figure 9. Status Va. Bright field. (Magn. × 120.)

Figure 10. Status Vb. Bright field. (Magn. × 80.)

Figure 11. Status Vb. Bright field. (Magn. × 80.)

Figure 12. Status VI. Bright field. (Magn. × 120.)

Figure 13. Status VI. Bright field. (Magn. × 120.)

(Figures 5–8, 10 and 11 reproduced by courtesy of Dr H. Canter-Lund.)

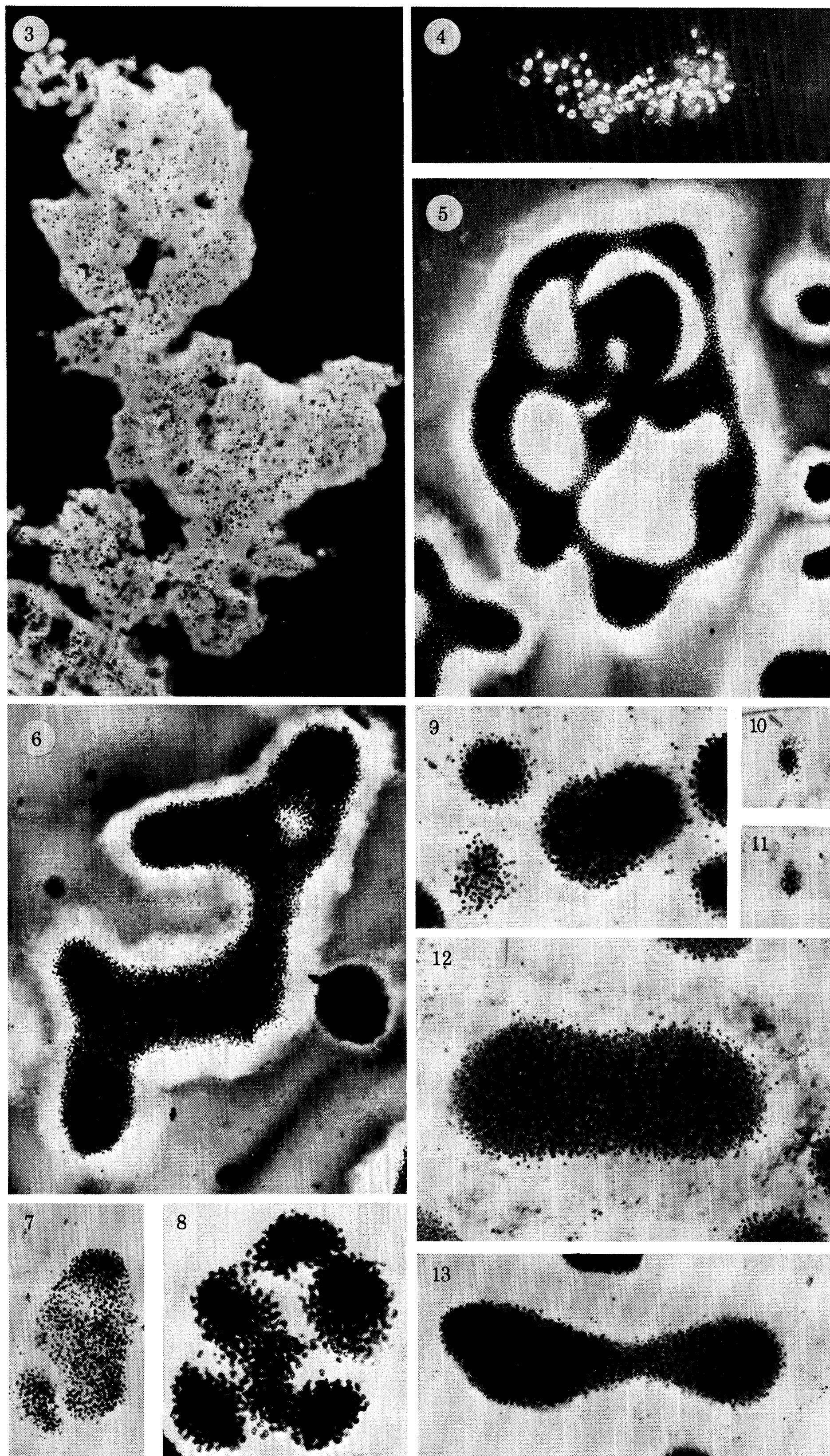
Description of plate 2

Vegetative cell ultrastructure

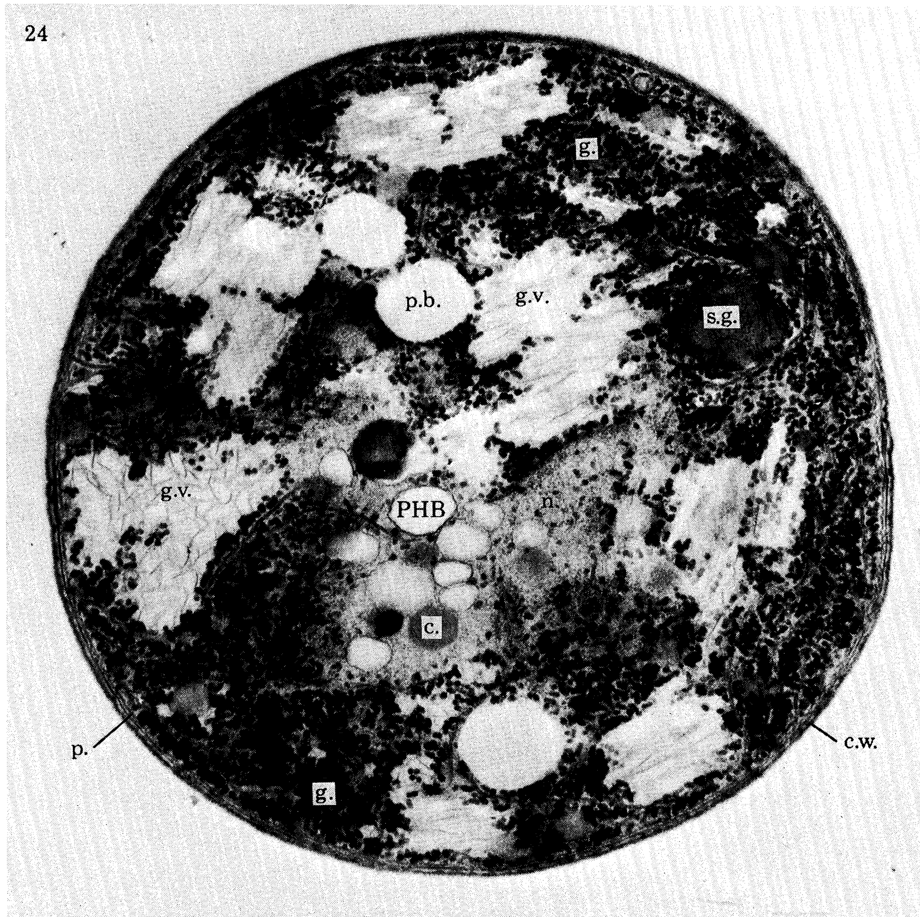
Figure 24. Median section of vegetative cell, delimited by cell wall (c.w.) and plasmalemma (p.). The nucleoplasm (n.) is packed with ribosomes and contains several types of granules. Thylakoids (arrowed) ramify throughout the protoplast, either solitary or grouped in stacks, often penetrating the nucleoplasm. Storage granules consist of polyphosphate bodies (p.b.), structured granules (s.g.), glycogen granules (g.), poly-β-hydroxybutyrate granules (P.H.B.) and carboxysomes (c.). Artefactual collapse of the gas vesicles (g.v.) is apparent (see text). (Magn. × 30000.)

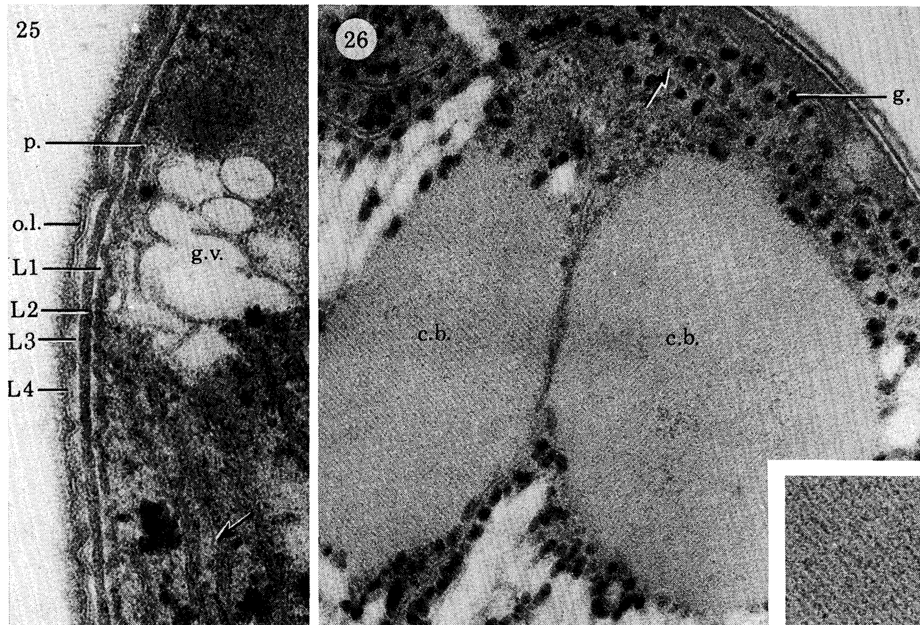
Figure 25. Detail of the quadrilayered cell wall (L1 to L4) and plasmalemma (p.); note the faint outer layer (o.l.); g.v., gas vesicles; arrow points to stacked thylakoids. (Magn. × 60000.)

Figure 26. Crystalline bodies (c.b.), seen here separated by a single thylakoid; g., interthylakoidal glycogen granules; arrow points to thylakoid. (Magn. × 50000.) Inset shows high-power detail of crystalline body. (Magn. × 120000.)

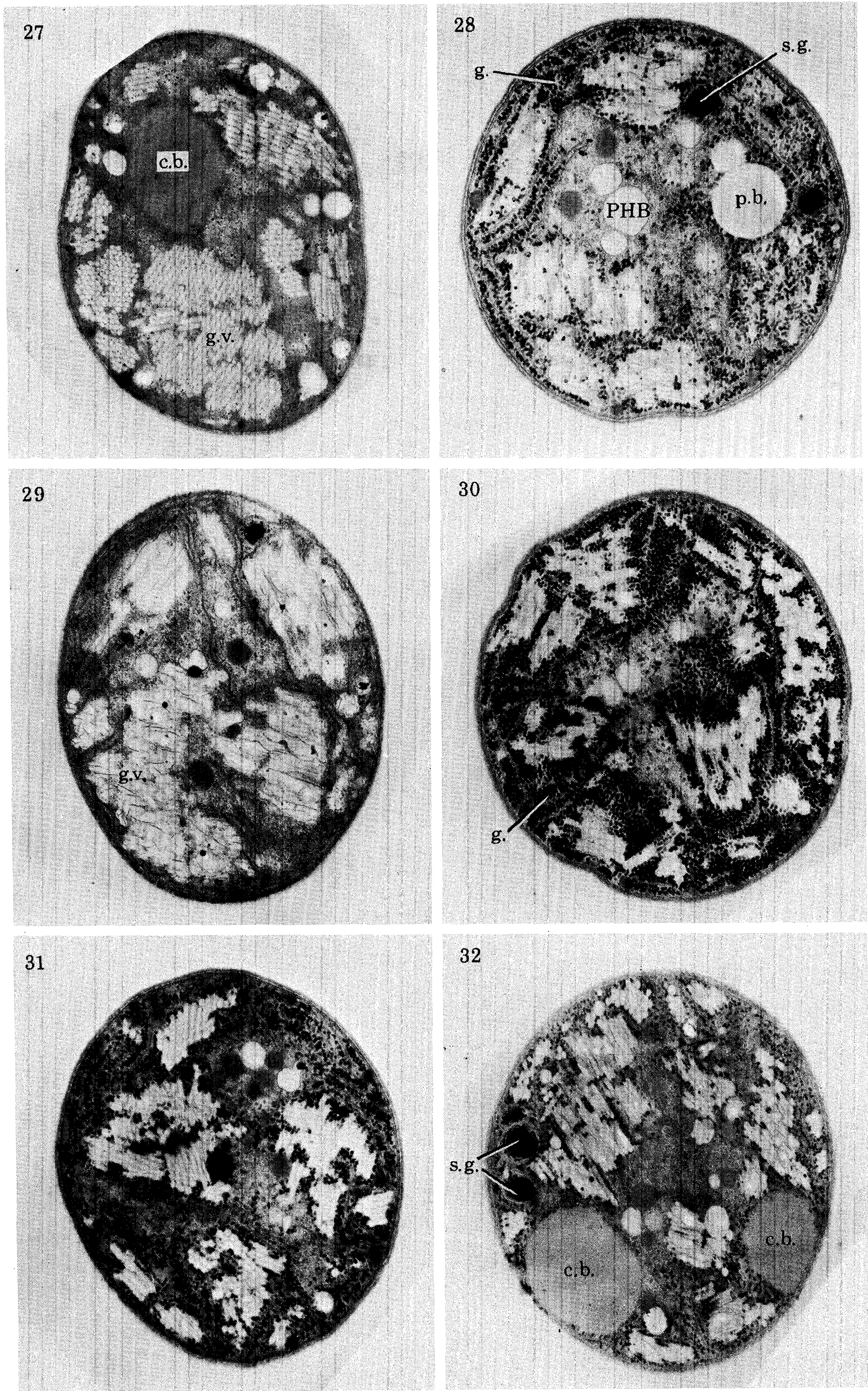


FIGURES 3–13. For description see opposite.

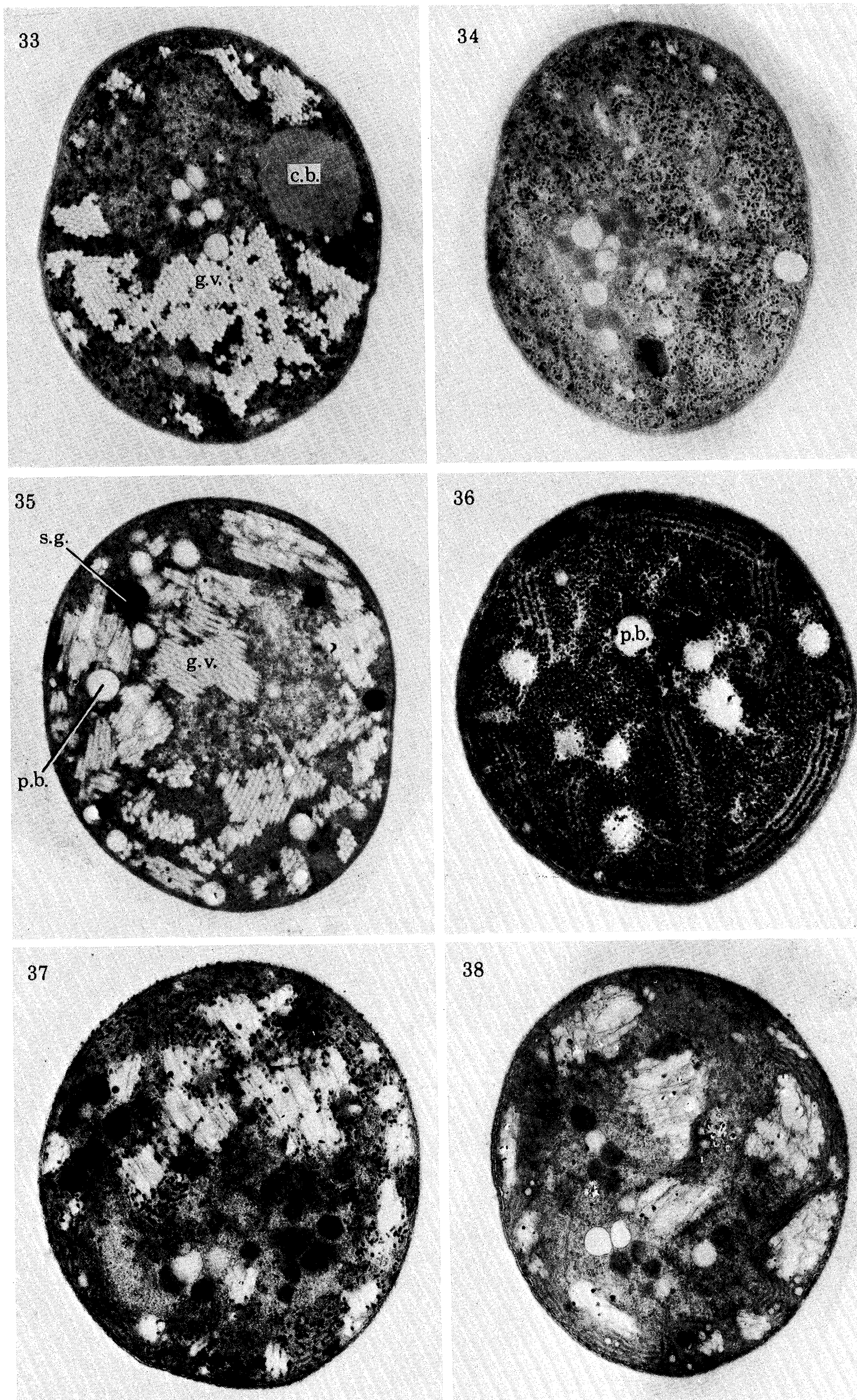




FIGURES 24–26. For description see p. 428.



FIGURES 27–32. For description see p. 429.



FIGURES 33–38. For description see opposite.

In the laboratory, wet-mounted spherical colonies (st. Va) can be induced to open out and assume appearances similar to those of st. II or III colonies by applying gentle pressure to the coverslip (cf. Reynolds 1973). This observation suggests that 'solid' *Microcystis* colonies are, in reality, convoluted plates; an increasing incidence of st. II and st. III colonies among post-maximal populations may indicate a natural tendency for st. V colonies to unfold spontaneously as they become senescent.

Status IV (figure 7). Colonies, otherwise ascribable to st. Ia, in which healthy cells formed one or more localized aggregations within the mucilage were referred to st. IV. The clusters of 20–200 cells occurred peripherally, typically as discrete swellings or mamillate projections. In this study, such colonies were found mainly in samples collected from the bottom mud, where they are believed to have been indicative of an overwintering or germination stage. Similar formations were repeatedly induced among st. Ia colonies when samples of benthic populations were incubated under suitable laboratory conditions (see §§7, 8). Although separation from the parent mucilage has not been observed, we believe that these cell clusters are eventually fragmented off, each constituting a new daughter colony (st. Vb, *q.v.*).

Colonies comprising between three and ten larger clusters of 500–2000 cells and resembling Komárek's (1958) illustrations of *Diplocystis novacekii* Komárek were distinguished as st. IVn (figure 8). They are believed to have been representative of an alternative course of maturation of st. IV colonies, fragmentation of daughters having not occurred.

Status Va (figure 9). The greater proportion of planktonic populations observed during seasonal growth was of the familiar ovoid or quasi-spherical form (st. *simplex* of Kondrat'eva (1968)). St. Va colonies were characterized by the relatively compact (3–5 cells per 1000 μm^3) and even arrangement of the cells within the mucilage, save for a clear peripheral layer. The

Description of plate 3

Seasonal changes in vegetative cell ultrastructure

Figure 27. Cell of benthic, overwintering colony; c.b., crystalline body; g.v., gas vesicles. (Magn. × 11500.)

Figure 28. Planktonic cell, logarithmic growth; note accumulation of glycogen (g.). Labels: p.b., polyphosphate body; p.h.b., poly-β-hydroxybutyrate granule; s.g., structured granule. (Magn. × 16500.)

Figure 29. Planktonic cell, logarithmic growth, pallid type; gas vesicles (g.v.) show artefactual collapse. (Magn. × 17500.)

Figure 30. Planktonic cell, mid-plateau-phase growth; note increase in number of glycogen granules (g.). (Magn. × 16000.)

Figure 31. Planktonic cell, late plateau-phase growth. (Magn. × 15500.)

Figure 32. Benthic cell, decline phase of growth. Note diminution of glycogen. Abbreviations: c.b., crystalline bodies; s.g., structured granules. (Magn. × 13000.)

Description of plate 4

Cells illustrating the results of various laboratory studies

Figure 33. Control cell containing conspicuous gas vesicles; c.b., crystalline body. (Magn. × 12500.)

Figure 34. Cells subjected to 1.1 MPa pressure showing little evidence of gas vesicles. (Magn. × 13500.)

Figure 35. Cell from batch culture, early logarithmic growth; g.v., gas vesicles; p.b., polyphosphate body; s.g., structured granule. (Magn. × 11500.)

Figure 36. Cell from batch culture, late post-logarithmic growth; note striking increase in glycogen granules. Abbreviation: p.b., polyphosphate body. (Magn. × 20000.)

Figure 37. Control cell (light-grown) containing normal level of glycogen. (Magn. × 18500.)

Figure 38. Cell after 48 h incubation in darkness; note absence of glycogen granules as in 'pallid' cells (see, for example, figure 29). (Magn. × 16500.)

mean thickness of the peripheral mucilage band varied between 3 μm (in actively growing populations from Rostherne Mere) and 35 μm (in colonies collected from a surface bloom on enclosure B in September 1979). The maximum diameter of st. Va colonies (excluding peripheral mucilage) generally varied within the range 80–300 μm, though larger colonies (maximum 570 μm) were observed occasionally.

Status Vb (figures 10, 11). Although conforming with Kondrat'eva's (1968) criteria for inclusion within the st. *simplex*, we separated small, spherical colonies (diameter less than 40 μm, excluding 2–5 μm of peripheral mucilage, and containing fewer than 100 cells) as st.

TABLE 1. RADII (ANNUAL MEANS ± STANDARD DEVIATION) AND RANGE OF CALCULATED VOLUMES OF INDIVIDUAL CELLS IN SELECTED *MICROCYSTIS* POPULATIONS

population	number of determinations	radius/μm	volume/μm³
B, 1976	13	2.38 ± 0.07	51.6–61.6
A, 1978	2	2.60 ± 0.21	57.1–92.9
B, 1978	8	2.81 ± 0.12	81.5–105.4
B, 1979	6	2.51 ± 0.14	55.8–78.0

TABLE 2. DRY MASS AND CHLOROPHYLL CONTENT OF *MICROCYSTIS* CELLS

population	date	dry mass of 10^6 cells/μg	chlorophyll *a* content of 10^6 cells/μg
B, 1978	4 Sep.	34.9 ± 1.1	0.420–0.429
B, 1979	10 Sep.	28.0 ± 4.3	0.259–0.345
Rostherne Mere, 1977†	22 Sep.	33.2 ± 10.4	0.348–0.366

† From Reynolds & Jaworski (1978).

Vb. These colonies were particularly prominent in spring and early summer collections from bottom muds and the plankton, where we believe them to be relevant to the re-establishment of summer, planktonic populations (see § 8).

Status VI (figures 12, 13). During active growth st. Va colonies eventually divide into two or more daughters. Separation is preceded by budding and lobe formation as the cells become progressively polarized within the dividing colony (figure 13). Any colony showing constriction consistent with this division process was classed as st. VI, though care was sometimes necessary in distinguishing them from st. III colonies. The distinction from other stati was useful in that the relative proportion of st. VI colonies in given planktonic populations was superficially correlated with their physiological activity and rate of growth.

(*b*) *Cell size*

The form of individual *Microcystis* cells was typically spherical, ovoid or capsule-shaped. Individual maximum dimensions were in the range 4–9 μm. Cell diameters were measured routinely during the course of four populations: the mean diameter of individual peripheral cells, one from each of not less than 30 freshly net-collected colonies, was determined weekly. The data (expressed as radii) are summarized in table 1 as the annual means of the mean values recorded in each determination; the quoted standard deviation applies only to the latter calculation. The calculated volumes assume the cells to be spherical; the ranges cover the standard deviations of the mean radius calculations. It should be noted that cells of the 1978 population in enclosure B were, on average, 40 % greater in volume than those of either the 1976 or 1979 populations in the same enclosure.

(*c*) *Dry mass and chlorophyll content*

Dry mass determinations were carried out on duplicate portions of fresh net-collected concentrates of *Microcystis*. The suspensions were air-dried to constant mass at 105 °C; the masses necessarily included the mucilage and any other organisms attached thereto. Chlorophyll *a* was extracted from further portions of the same suspensions. In table 2, masses and pigment concentration are expressed relative to the concentrations of *Microcystis* cells in the original suspensions.

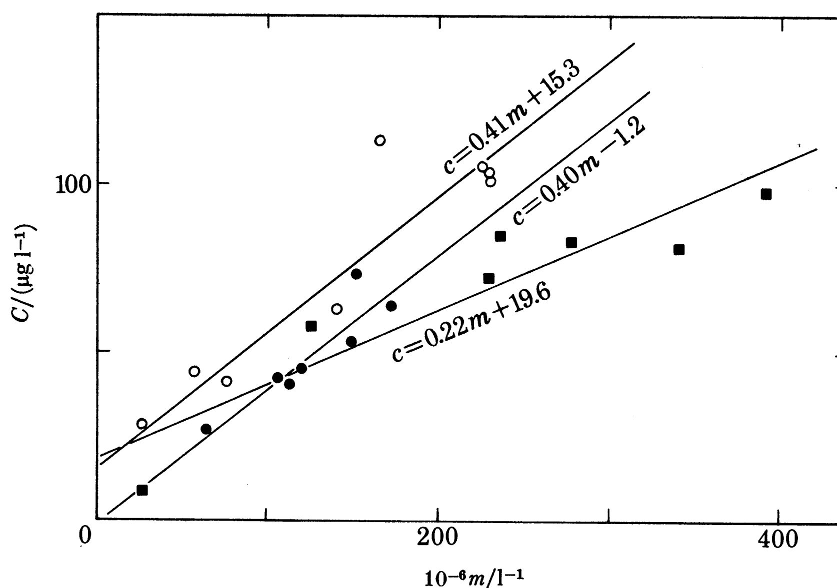


FIGURE 14. The regression of chlorophyll *a* content (C) of 0–5 m column water samples dominated by *Microcystis aeruginosa* against corresponding concentration of *Microcystis* cells, m. Data for three populations are shown separately: B, 1976 (●); B, 1978 (○), and B, 1979 (■).

The regression of weekly estimates of chlorophyll *a* concentration in 5 m column samples dominated by *Microcystis* against the corresponding estimates of cell concentration provides an alternative measure of cellular chlorophyll content. Separate regressions are fitted to the data for three different populations in figure 14: the coefficients for 1978 and 1979 are comparable with the corresponding estimates in table 2, and the differences between the two populations may be related to the differences in size (table 1).

(*d*) *Mucilage volume*

A conspicuous feature of all natural *Microcystis* colonies is the mucilage in which the cells are embedded. This contrasts with the condition of *Microcystis* cultures, in which mucilage is frequently lost as they sooner or later become unicellular. (See, for example: Lange 1976; Krüger & Eloff 1977; Rippka *et al.* 1979. See also appendix of Reynolds & Jaworski 1978.) The functions of the natural mucilage are unresolved: it may provide a specialized micro-environment around the cells in which essential nutrients are concentrated and maintained (Lange 1976); it enhances the sinking and floating response to regulated buoyancy changes (Reynolds & Walsby 1975), and the size of colonial units may be modified by the turbulence characterizing the system (Bykovskiĭ 1976). Mucilage also serves a protective role against

grazing animals by increasing the size of the colonial structure (cf. Burns 1968*a*, *b* and Porter 1977; see also Schindler 1971 and Tevlin & Burgis 1979).

These functions may be incidental: Sirenko and her coworkers (reviewed in Sirenko 1972) have shown that a low-redox microenvironment is maintained within the mucilage. They have argued that this adaptation enables cells to tolerate high external concentrations of oxygen (see also: Gusev 1962; Sirenko *et al.* 1969).

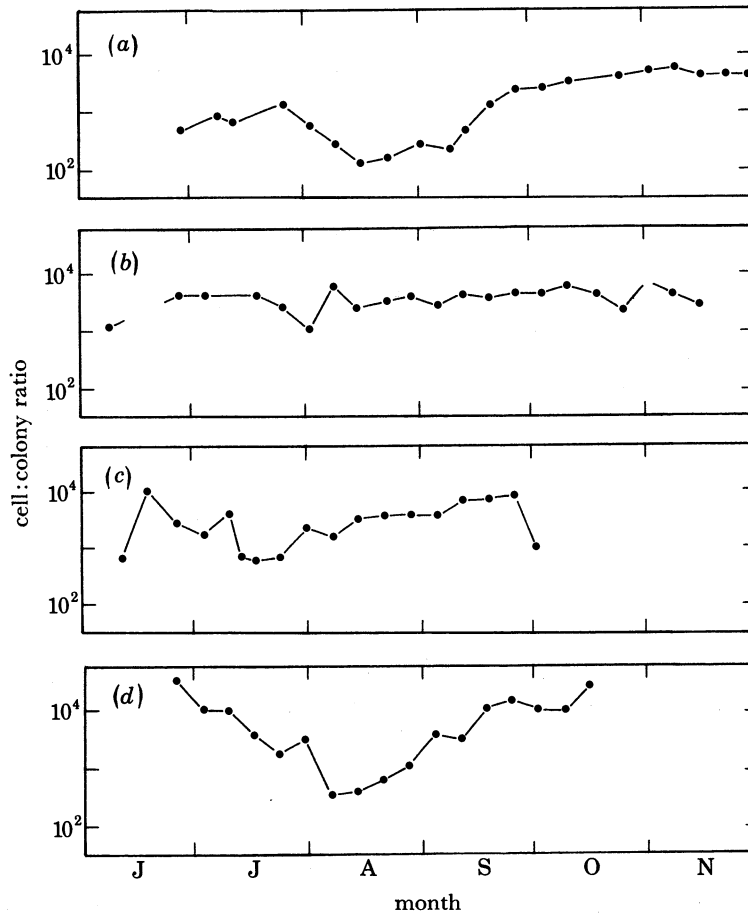


FIGURE 15. Semilogarithmic plot of the seasonal fluctuation in mean cell:colony ratio during the course of four *Microcystis* populations: (*a*) B, 1976; (*b*) A, 1977; (*c*) B, 1978; (*d*) B, 1979.

We have attempted to quantify the seasonal changes in the amount of mucilage by (i) subtracting the calculated cell volume from the calculated colony volume and (ii) direct measurement of the width of the peripheral band in st. II, III, V and VI colonies.

Temporal variations in the ratio of cell concentration to colony concentration (i) in 5 m column samples for four pelagic populations are presented in figure 15; the relation between the two quantities is shown on a logarithmic scale. Cells rarely occupied more than 20 % of the colony volume (maximum 26 %: B, 1978), but, in the 1976 (especially) and 1979 populations in enclosure B, the fraction was less than 5 % for long periods. In both populations, the cell volume/colony volume ratio declined towards the end of the phase of exponential increase, but recovered during stationary phase. A similar trend was observed in the 1978 population in the same enclosure, although the volume occupied by cells was consistently relatively greater;

this observation coincides with the larger individual cell volumes (see table 1). The major factor immediately determining the relation is presumably the relative contributions of different colony types to the composition of the population as a whole: measurements made on individual colonies indicated that cells occupy 25–33 % of the volume of st. Vb colonies but less than 1.5 % of those classified as st. Ib.

The remainder of the colony is assumed to be mucilage, which therefore occupies more than 67 % of the volume at all times. The present data may be compared with measurements made on other natural *M. aeruginosa* populations in Crose Mere (where cells occupied 9–21 % of colony volume (Reynolds 1973)) and in the Kremenchug Reservoir (45–85 % (Sirenko 1972)). The differences between these ranges may reflect alternative methods of calculation as much as any genuine phenotypic differences; certainly, Sirenko's (1972) qualitative description of the seasonal and ontogenetic variation in *Microcystis* is matched by the present observations. We believe, however, that she may have underestimated the volume of the mucilage: to occupy 80 % of a given space spheres must be packed to the theoretical upper limit before significant distortion occurs (cf. Dinsdale & Walsby 1972) whereas the packing density of cells in natural colonies is evidently well below this limit.

Variations in the width of the peripheral mucilage (ii) during the course of three *Microcystis* populations are represented in figure 16. A steady and significant increase was observed during the final growth stages of the population in enclosure B, 1979. A similar trend was evident in enclosure A, 1977, but as no record of the individual measurement was kept we cannot comment upon its significance. Mucilage thickening was evidently weaker in the B, 1978, population, but increased significantly. Non-routine measurements of the peripheral mucilage in benthic colonies distinguished between two categories: 'overwintering' colonies (st. II, III, Va) retaining thick (20–40 μm) mucilage coats, and newly 'germinated' colonies (st. Vb) in which the thickness of the mucilage rarely exceeded 3 μm. Sirenko's (1972; see also Sirenko *et al.* 1969) explanation for parallel variations in natural populations, that mucilage production was most prolific in response to high external oxygen concentrations, seems to be applicable to our observations, though it may be incomplete. In the planktonic populations observed in Rostherne Mere during 1977, the mean thickness of the peripheral mucilage was only 1.37 μm (s.d. $\pm$ 1.24); the maximum individual measurement (4.4 μm) was recorded at the time of the population maximum, in September. Re-examination of iodine-preserved material collected from populations from Crose Mere and other Shropshire meres (described in Reynolds 1971, 1973) also indicated that peripheral mucilage (3–6 μm in Crose Mere; 10–15 μm in Ellesmere and White Meres) was typically much narrower in planktonic *Microcystis* colonies from these lakes than in those from the Blelham enclosures. The surface waters of all these meres are subject to periodic supersaturation with oxygen (Reynolds 1973 and unpublished results). They also have higher dissolved organic content and ionic strengths (especially with respect to calcium and bicarbonate ions) than those of Blelham Tarn.

(*e*) *Density*

To clarify the supposed role of buoyant behaviour, not only in influencing the vertical distribution of *Microcystis* colonies in the water column (cf. Reynolds & Walsby 1975), but also in mediating seasonal migrations to and from the sediments (Reynolds & Rogers 1976), some empirical relations between the relative densities of the cells and mucilage and the relative gas vacuole volume to counter them have been derived.

Density was estimated by two methods (see §2, above; the application of a third, density-

gradient centrifugation method proved impractical). In the first approach, the mean density of *Microcystis* colonies in concentrated suspensions prepared from material drawn from the mud surface of freshly collected Jenkin cores (and previously exposed to a pressure of *ca.* 1.1 MPa to collapse gas vacuoles) was calculated from the mass of the suspension ($\rho = 998.9$ kg m^{-3})

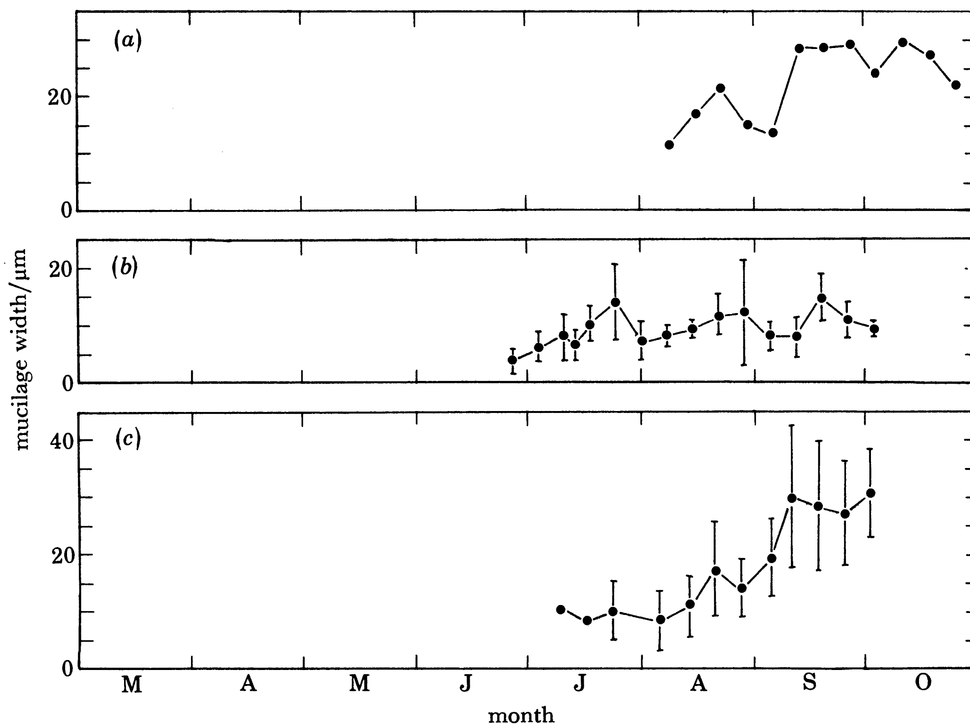


FIGURE 16. Variations in the mean width of clear (cell-free) peripheral mucilage in st. Vb and VI *Microcystis* colonies during the course of three populations: (*a*) A, 1977; (*b*) B, 1978; (*c*) B, 1979. Standard deviation of means are shown where known.

and the proportion of its volume occupied by colonies, according to equation (1). Results are presented in table 3. With the assumption that the calculated colony density (ρ') has two components, that of the mucilage (ρ'_x) and that of the cell (ρ'_y), these components were evaluated from the relative volumes of the mucilage (x) and cells (y), and by simultaneously solving equations (2) and (3)

$$0.914\rho'_x + 0.086\rho'_y = 1001.04, \tag{2}$$

$$0.951\rho'_x + 0.049\rho'_y = 1000.42. \tag{3}$$

By solving to eliminate ρ'_y,

$$\rho'_x = 999.59 \text{ kg m}^{-3}. \tag{4}$$

By substituting (4) in equations (2) and (3), the solutions to ρ'_y are 1016.45 and 1016.53 kg m^{-3}; simultaneous solution of (2) and (3) to eliminate ρ'_x gives

$$\rho'_y = 1016.36 \text{ kg m}^{-3}. \tag{5}$$

In the second approach, the densities of pressure-treated st. V colonies were first calculated from the observed sinking rates from the Stokes equation (see methods, §2). In figure 17,

mean values for each determination are regressed against corresponding estimates of the relative volume of the cells; density estimates for the 1976 and 1978 planktonic populations as well as the 1976–1977 benthic population are distinguished; the ranges of evaluations for planktonic colonies from Crose Mere (Reynolds 1973) in 1971 (29 points) and in 1972 (20 points) are also represented. Regressions have been fitted separately to all (72) points and to those describing the 1976 planktonic population only. In either case, significant ($p < 0.05$) correlations were found: $r = +0.422$, $+0.814$ respectively. In figure 17 are shown 95% confidence intervals, calculated for standard error determinations of the regression coefficients (Snedecor & Cochran 1967). Although their slopes are different, their confidence intervals overlap along the greater part of their lengths. Their intercepts with zero cell fraction predict that the

TABLE 3. THE MEAN DENSITY OF *MICROCYSTIS* COLONIES (ρ') AND OF THE WATER (ρ) IN PREPARED SUSPENSIONS AND THE RELATIVE VOLUMES OF COLONIES OCCUPIED BY MUCILAGE (x) AND CELLS (y)

date	source	$\rho'/(\text{kg m}^{-3})$	$\rho/(\text{kg m}^{-3})$	x	y
4 Nov. 1976	B, mud	1001.04	998.97	0.914	0.086
30 Nov. 1976	B, mud	1000.42	998.90	0.951	0.049

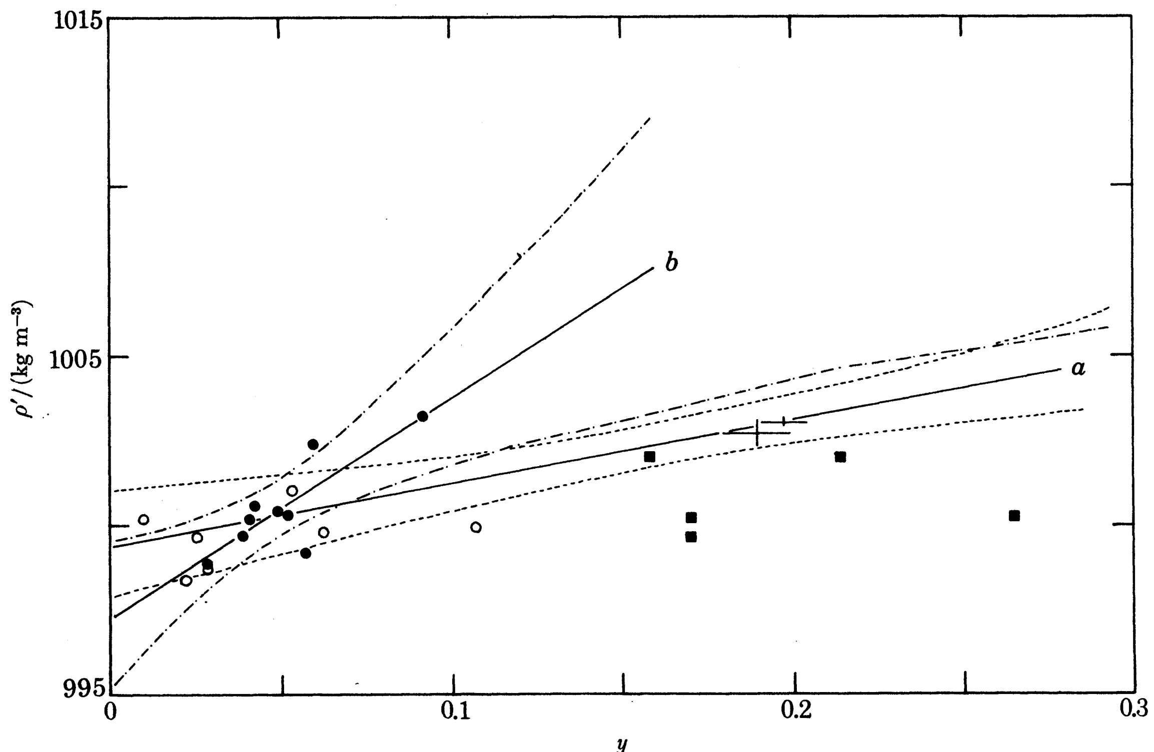


FIGURE 17. The mean density (ρ') of st. V *Microcystis* colonies in pressure-treated suspensions, calculated from sinking rate according to the Stokes equation, plotted against the fraction of the colony volume (y) occupied by cells (as opposed to mucilage): ●, for the planktonic population in B, 1976; ○, for the benthic population in B, autumn–winter 1976; ■, for the planktonic population in B, 1978. The range of individual points (total 49) obtained for Crose Mere populations in 1971 and 1972 is shown by the intersecting perpendicular bars. The regression equations plotted (together with their 95% confidence limits) are (a) for all 72 points and (b) for the 1976 planktonic populations only. The regression equations (both significant at 5% probability level) are: (a) $\rho' = 999.4 + 18.4\,y$; (b) $\rho' = 997.3 + 64.2\,y$.

density of the mucilage (ρ'_y) is in the range 997.76–1000.99 kg m^{-3} (when all points are considered) and 995.08–999.47 kg m^{-3} (1976 points only). The regression equations also respectively predict that cell density (i.e. when $y = 1.0$) will be 1017.8 (1010.0–1025.7) and 1061.5 (1022.6–1100.0).

According to this treatment the density of the mucilage does not differ significantly from that of the suspending media (filtered lake water) in which the experiments were conducted (ρ = 998.1–998.6 kg m^{-3}). The mean mucilage density predicted by all the points, 999.4 kg m^{-3}, exceeds the water density by 0.8–1.3 kg m^{-3}, comparing favourably with the 0.7 kg m^{-3}, obtained by the first method. The distribution of points towards one end of the fractional scale (cell volumes less than 25 % colony volume) inevitably biases against extrapolation of cell density; nevertheless the available data suggest that the density of cells is more variable than that of mucilage.

The mean relative gas-vacuole content required to render colonies neutrally buoyant can be derived from these data. There will be no unique critical value for all colonies at all stages of their life cycle (cf. Reynolds & Walsby 1975). Neutral buoyancy will be determined by the balance of vacuole volume against cell density, the relative fractions occupied by cells and mucilage and changes in water density, mediated principally by temperature fluctuations.

As an example, for the colonies described by equation (2) to become neutrally buoyant, their density must fall to 998.9 kg m^{-3}; thus the cell density is required to fall to 990.5 kg m^{-3}, which could be achieved through the provision of sufficient gas-filled space to increase cell volume by 2.6 %. Following parallel reasoning, an increase of 3.1 % would be required to the colonies described in equation (3) to reach neutral buoyancy.

5. Seasonal variations in spatial distribution of the *Microcystis* populations

Seasonal changes in the relative abundance of morphological stati in the six populations for which data are available are presented in figures 18–23. Each figure illustrates: (*a*) the occurrence and vertical distribution of *Microcystis* colonies in the water column; (*b*) the composition of the total suspended population in terms of four morphological categories (namely I, II + III, IV + Vb, Va + VI); (*c*) the areal recovery rate of colonies in either (1976) upward-pointing Tauber sediment traps at selected depths or (all except 1976) in upward- and downward-opening jar traps (see §2 for details) suspended about 150 mm above the mud surface; and (*d*) the areal density of colonies recovered from the mud–water interface in fresh Jenkin cores, and classified into the same categories as used in (*b*).

Although differing from each other in detail, the sequences of morphological and behavioural changes were broadly similar. During the planktonic phase, colonies were usually more numerous towards the top of the water column. Planktonic populations were, at most times, dominated by st. Va and VI colonies. During the growth phases, however, st. Ib colonies became more or less abundant, representing a maximum of 18–95 % of the colonies, but never containing more than 40 % and rarely more than 5 % of the total cell concentration. St. II and III colonies were subdominant throughout the course of most populations but were relatively and absolutely more abundant during stationary and decline phases. The 1977 population in enclosure A (figure 22*b*) was exceptional in this respect where st. II and III colonies were together dominant for long periods, accounting for a maximum 83 % of all

colonies present; similar colonies were briefly dominant in enclosure B in 1976 when the suspended population was augmented by colonies recruited from the sediment (figure 18*a*; see also Reynolds 1978*c*).

Seasonal trends of fluctuations in the areal concentration of colonies estimated to be present at the sediment surface (generally within the range 600–12000 cm^{-2}) alternated between net decline in the early summer and net increase during the late summer (the 1977 population in enclosure A was again the exception). Variations on shorter time scales were evident in both enclosures (figures 18*d*, 19*d*, 21*d*, 23*d*) and were, to some extent, reciprocal to changes in the

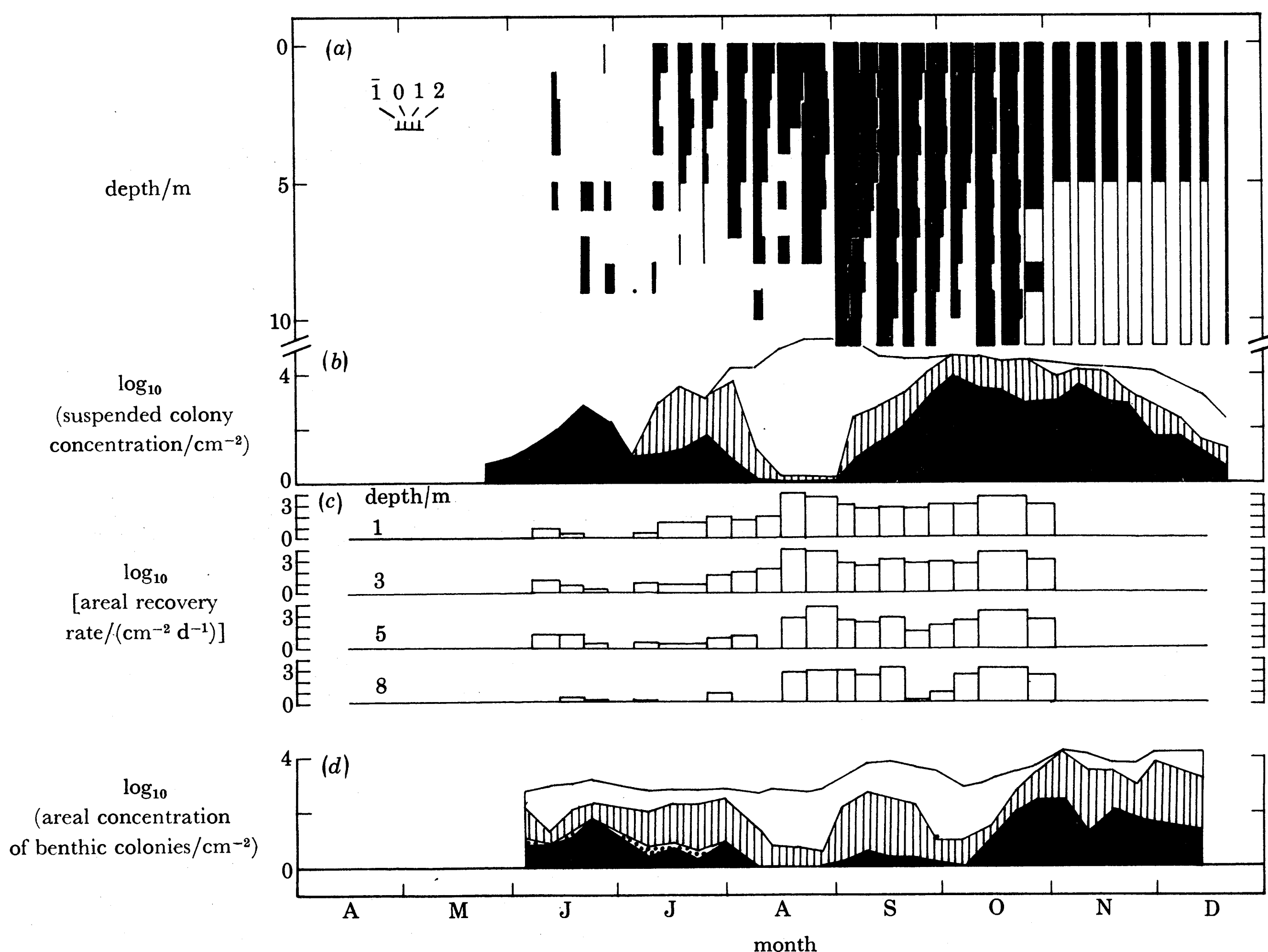


FIGURE 18. Seasonal changes in the vertical distribution, morphology and buoyant behaviour of the *Microcystis* population in enclosure B, 1976. Vertical distribution (*a*) is shown as a series of profiles whose left edge corresponds with the date; colony concentration (ml^{-1}) in 11 successive 1 m layers is represented on a logarithmic scale (inset); unshaded sections of profiles indicate that population was extrapolated and not directly measured (for full details of method of presentation see Reynolds & Wiseman (1981)). Changes in the areal density of colonies (summated in the vertical profile (*b*)) are represented on a logarithmic scale; differential shading shows the *proportions* of the population composed of st. Va and VI colonies (solid black), st. II and III colonies (hatched) and st. I colonies (unshaded). Recoveries (number of colonies per unit area per day) in Tauber trap placed at *ca.* 1, 3, 5 and 8 m below the water surface are represented in (*c*). The areal concentration of colonies at the mud–water interface are represented in (*d*); *proportions* of the population composed of st. Va and VI colonies (black), st. IV and Vb (stippling), st. II and III (hatching) and st. I (unshaded) are shown by differential shading.

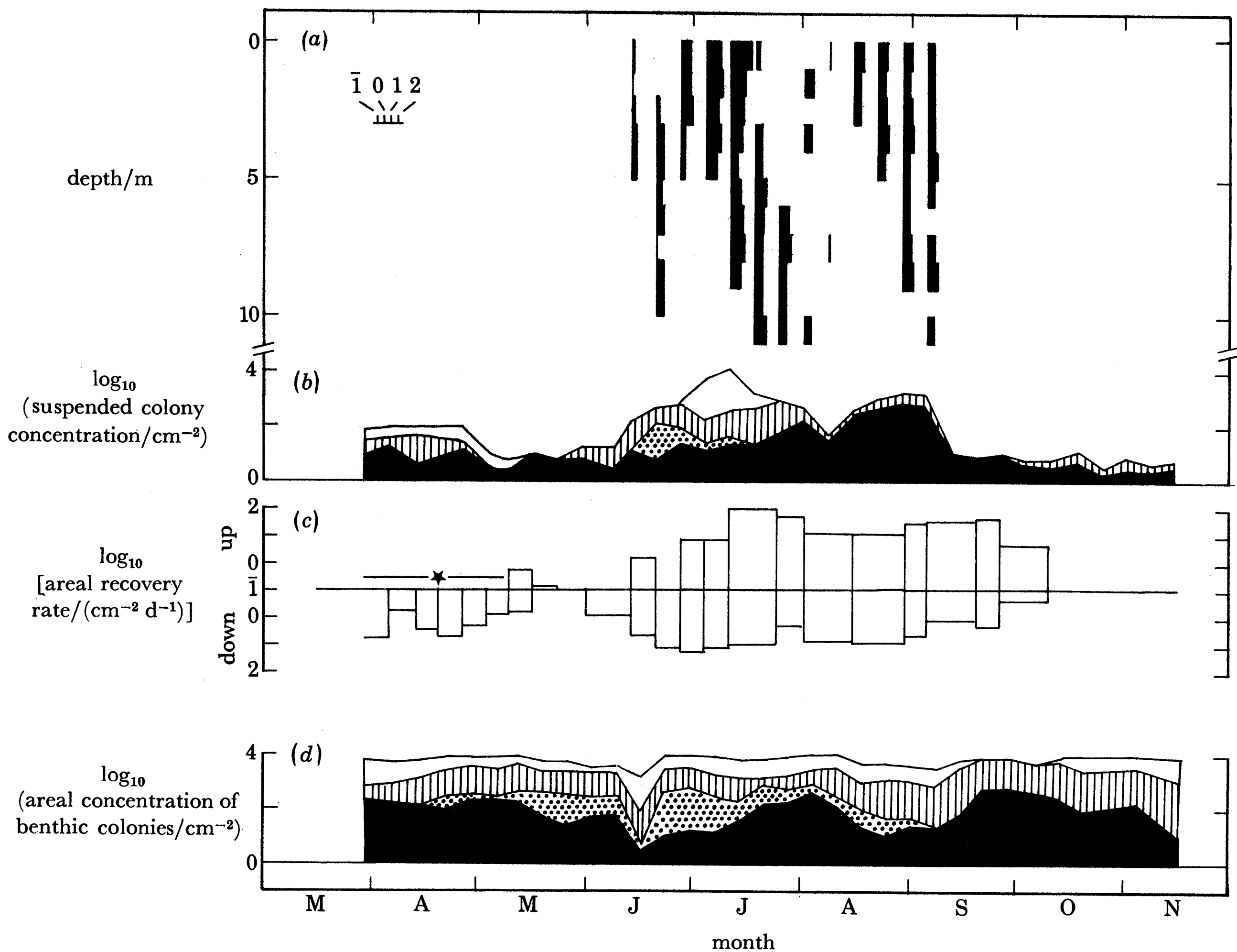


FIGURE 19. Seasonal changes in the vertical distribution, morphology and buoyant behaviour of the *Microcystis* population in enclosure B, 1977; (*a*), (*b*) and (*d*) constructed as corresponding parts of figure 18. Catches in jar traps, with apertures pointing both up (above the base line) and down (below the base line), are represented in (*c*). The symbol ★ denotes no traps set during this period.

planktonic concentration and relative composition. Where both upward- and downward-opening jar traps were in operation over the entire season (1978, 1979: figures 20*c*, 21*c*, 23*c*), apparent imbalances in collecting rate are consistent with the net vertical direction of transfer. Striking examples indicative of a net descent of colonies to the sediments are represented at the end of the 1978 (both enclosures, see figures 20, 23), 1977 and 1979 (enclosure B; see figures 19 and 21) seasons.

Seasonal changes in the composition of the benthic populations were also apparent. During the spring and summer of 1977 (both enclosures; see figures 19*d*, 22*d*) and 1979 (enclosure B, figure 21*d*), the areal concentration of st. Ia, II and III colonies increased absolutely and as a proportion of the recoverable population. The compensatory decline in the proportion of Va colonies was absolute in most instances; this trend was not reversed until late in the season, when areal colony density was rapidly increased, arguably by the settling out of planktonic colonies (cf. Reynolds & Rogers 1976; Reynolds & Wiseman 1981). Two explanations for the decreasing abundance of st. Va colonies may be proposed: either (i) it is the effect of

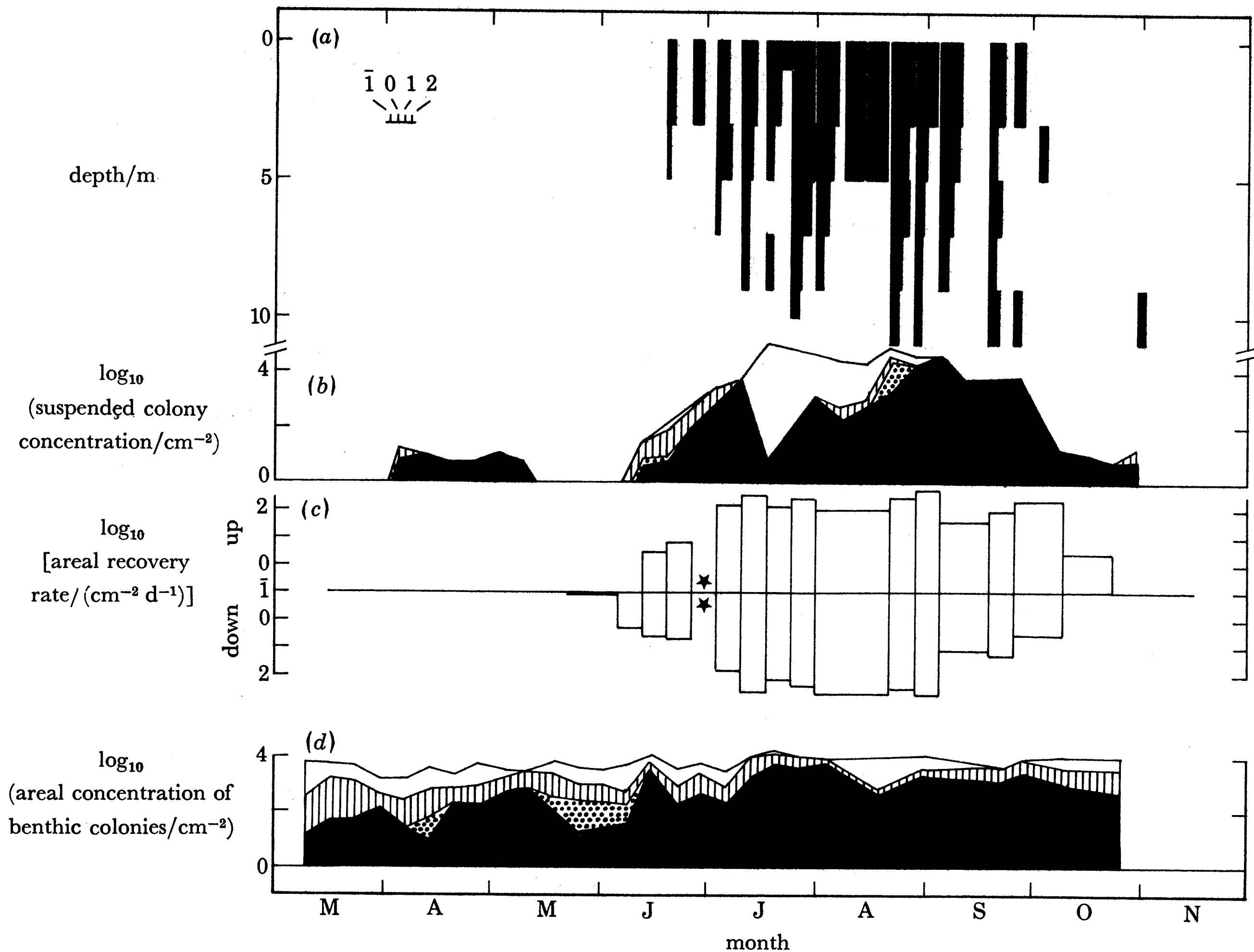


FIGURE 20. Seasonal changes in the vertical distribution, morphology and buoyant behaviour of the *Microcystis* population in enclosure B, 1978. Construction as in figures 18 and 19.

progressive depletion of benthic stocks as such colonies re-enter the plankton; or (ii) it is the consequence of physiological deterioration, manifest as a change in physical appearance (Va → II/III → Ia; cf. §4). Colonies were initially detected in the 5 m column samples at concentrations of 10–20 colonies per litre (see figure 1, 5–10 colonies per square centimetre). In those years for which full data are available the early recoveries of colonies in downward-opening traps were consistent with an input of a similar order from the sediments and exceeded contemporary catches in upward-opening traps, at least while the planktonic populations were small. However, no significant decrease in the total benthic population was detected. In any case, it is impossible to distinguish between 'chance' trappings of colonies dislodged from and sinking back to the mud, as opposed to directed migratory movements. Although the recovered colonies observed at these times were generally ascribable to st. Va or Vb, their depletion at the bottom does not seem attributable to migration. Thus there is no evidence to support the first suggestion (i) that benthic populations return *en masse* to the plankton. Nevertheless the trap data do not exclude the possibility that the abundant planktonic populations originate

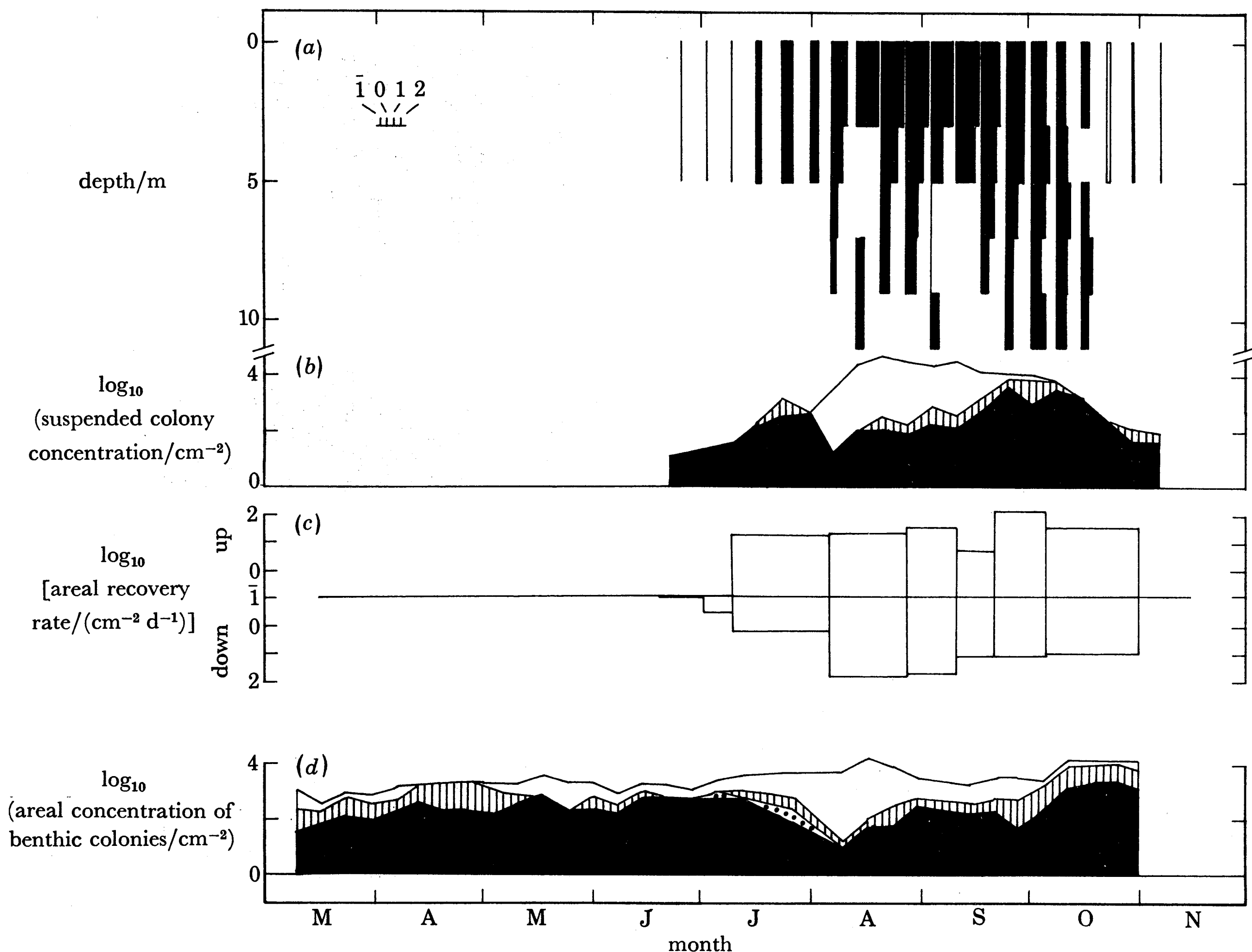


FIGURE 21. Seasonal changes in the vertical distribution, morphology and buoyant behaviour of the *Microcystis* population in enclosure B, 1979. Construction as in figures 18 and 19.

from a small inoculum of overwintering colonies migrating to the water column in spring or early summer.

The alternative explanation (ii) is complicated by the recruitment of st. V colonies to the benthic stock. Apart from the sudden autumnal settlement of colonies, already described, colonies may settle out to the mud more insidiously (cf. Vladimirova 1968). Our trap data suggest that in mid-season sinking losses roughly balance flotational gains, and this is more likely to be due to compensatory movements of individual algae (cf. Reynolds & Walsby 1975) rather than to permanent sinking losses being fortuitously balanced against fresh recruitment.

The benthic stock of st. V colonies is potentially increased by *in situ* growth of new colonies. Between April and August, during 1 to 4 months, distinctive, localized groupings of cells appeared in st. Ia colonies which, in our nomenclature, were then immediately referred to st. IV. In extremes, up to 15% of the colonies present at any one time could be so classified. Their initial appearance was followed by an absolute increase (to up to 40% of the total) in the concentration of small, quasi-spherical colonies, which we referred to st. Vb. As the season progressed, the areal concentrations of benthic IV and Va colonies decreased; similar colonies were occasionally and briefly observed in the plankton (see figures 19*b*, 20*b*, 23*b*).

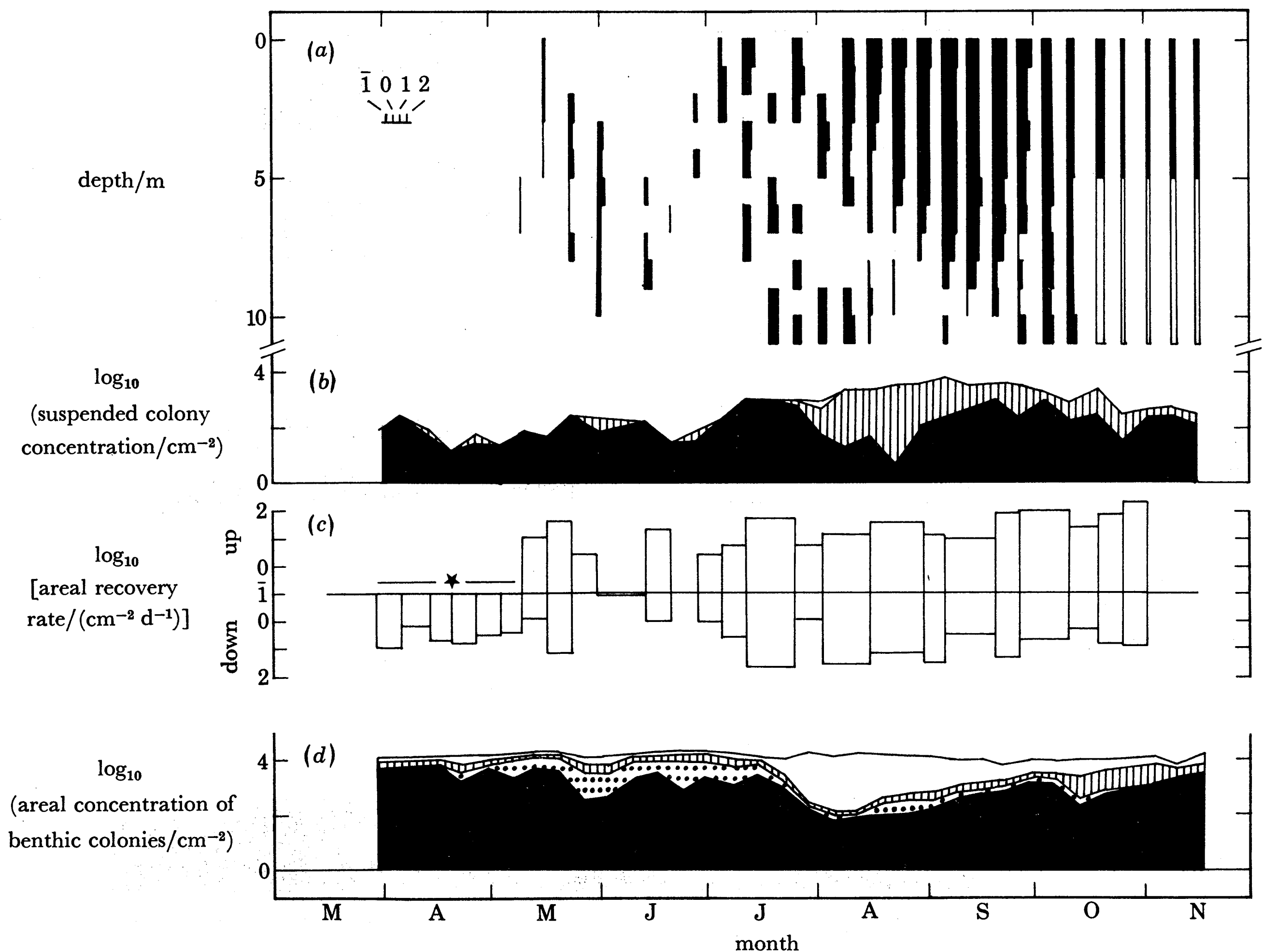


FIGURE 22. Seasonal changes in the vertical distribution, morphology and buoyant behaviour of the *Microcystis* population in enclosure A, 1977. Construction as in figures 18 and 19.

The presence of localized clumps of cells in the mucilage of old or moribund colonies has been illustrated or described by Wesenberg-Lund (1904), Chernousova *et al.* (1968) and May (1972). Our observations suggest that the clumps are products of cell division rather than of mutual convergence of cells within the mucilage (cf. Wesenberg-Lund 1904; §4 above); cell division in laboratory incubations of benthic *Microcystis* has been observed (Kappers 1976; this study) or implied (Wohlschlag & Hasler 1951; Priĭmachenko & Litvinova 1968; Chernousova *et al.* 1968; Reynolds & Walsby 1975; Tow 1979). Sirenko (1972) considered that young colonies arise from single cells in old, mature colonies lodged in the bottom deposits, and that these play an important part in the reproductive cycle of *Microcystis*. Our data support this view: it seems likely that st. IV and Vb colonies originated at the bottom and were not recruited from the plankton. Moreover, comparison of parts (*d*) of figures 18–23 with figure 2 shows that the appearance of st. IV and Vb colonies in the bottom material always coincided with periods of high water transparency (shown by the Secchi disc record) and simultaneous oxygen depletion of the lower hypolimnion. The converse (that is, that such conditions were always followed by the appearance of these colonies), however, is not true. Nevertheless, we

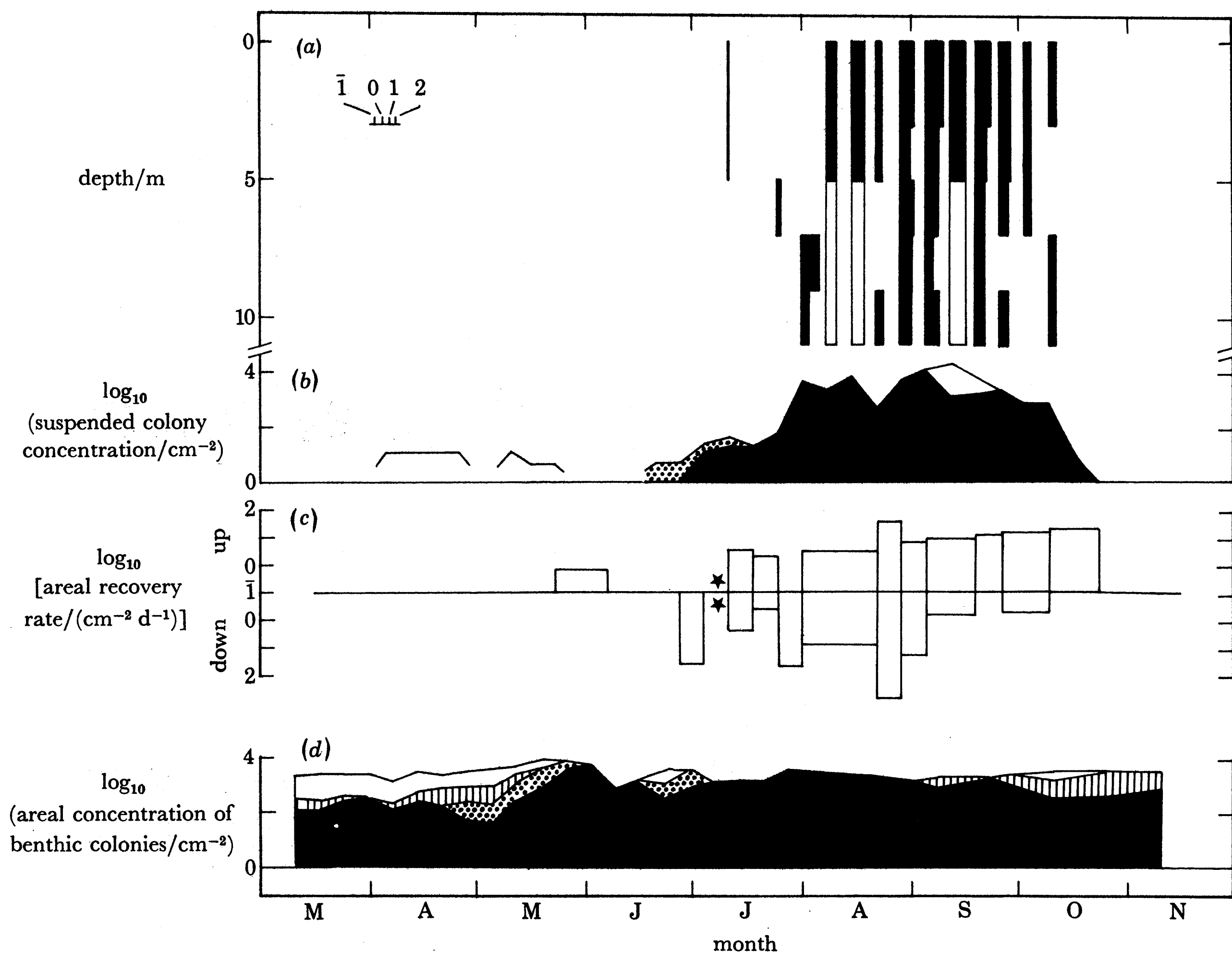


FIGURE 23. Seasonal changes in the vertical distribution, morphology and buoyant behaviour of the *Microcystis* population in enclosure A, 1978. Construction as in figures 18 and 19.

hypothesize that the development of large planktonic *Microcystis* populations is founded upon changes in the physiological activity, growth and buoyant behaviour of a relatively limited number of cells in a limited number of overwintering colonies, recruited to the sediments the previous autumn, and that these changes are stimulated by very specific environmental factors.

6. Ultrastructural characteristics of the *Microcystis* populations

(a) *Ultrastructure of the vegetative cell*

Some aspects of the ultrastructure of various species of *Microcystis* have been described elsewhere with use of material both from cultures (Jost & Zehnder 1966; Smith & Peat 1967; Jones & Jost 1970; Golecki & Drews 1974; Kessel 1978) and from natural sources (Kessel & Eloff 1975; Stewart *et al.* 1977; Belikova 1978; Kessel 1978). The present results confirm previous accounts and add several new observations for this genus.

Ultrastructural details of a 'typical' cell of *M. aeruginosa* are shown in figures 24–26, plate 2,

demonstrating the low state of differentiation found in all blue-green algae (see: Lang 1968; Shively 1974). The protoplast is delimited by a tripartite plasmalemma outside which lie the four wall layers numerically designated L1 to L4 by Jost (1965) and shown to be characteristic of the Gram-negative bacteria (De Petris 1967; Costerton *et al.* 1974). A faint single-track proteinaceous outer layer recently recognized in *Microcystis* by Kessel (1978) lies superficial to the membrane-like L4 layer. Its appearance in our material supports the contention (Kessel 1978) that this is a consistent feature within the genus, and it has since been observed (Cmiech 1981) also in the coccoid blue-green alga *Gomphosphaeria naegeliana* (Unger.) Lemm.

Ribosomes and carboxysomes (Shively *et al.* 1973; Codd & Stewart 1976), formerly the 'polyhedral bodies' of Jensen & Bowen (1961), are visible within the reticulate nucleoplasm. Other cell inclusions are lipid droplets, polyphosphate bodies, structured granules and gas vesicles. Prominent 16 nm × 33 nm glycogen granules lie in close association with the thylakoids and represent the first visible product of cyanophycean photosynthesis (see Lang 1968). The absence of phycobilisomes (Gantt & Conti 1969) on the outer face of the thylakoids reaffirms their instability with non-specific fixation techniques (Cohen-Bazire & Lefort-Tran 1970).

In addition to these usual components, the uniquely prokaryotic storage product poly-β-hydroxybutyric acid (PHB), which is commonly found in bacteria (Dawes & Ribbons 1964; Shively 1974) but has been recorded only infrequently in the blue-green algae (Carr 1966; Jensen & Sicko 1971), is abundant. It may be distinguished from polyphosphate by the presence of a 3 nm limiting monolayer (Jensen & Sicko 1971) upon which PHB synthetase has been shown to be located in *Bacillus megaterium* (Griebel & Merrick 1971). Also frequent are large crystals with a precisely ordered substructure which appear as striations with a repeat distance of 10 nm. These bodies are not delimited by a membrane, but close association with regions of the thylakoids is regularly observed.

(*b*) *Ultrastructure of seasonal changes in the vegetative cell*

Results presented here are based upon material collected over a period of 3 years (1976–1978) from Blelham enclosures A and B. The ultrastructural changes that accompany the various phases of growth follow a similar pattern each year and can therefore be described in general terms (see also figures 27–32, plate 3).

The ultrastructure of cells shortly after their migration to the bottom muds in late autumn is shown in figure 27. Two notable features recur constantly among benthic colonies. First, a diminution in the numbers of glycogen granules allows clearer visualization of the ribosomes which are commonly masked by the reserve product in light-grown material; the cytoplasm of these cells thus displays a characteristic granular quality. Secondly, every cell examined contains gas vesicles, an apparent incongruity in view of the negative buoyancy of over-wintering colonies. The resulting image in the electron microscope is one of reduced density as compared with that of light-grown cells. This characteristic appearance, hereafter referred to as 'pallid', persists throughout the overwintering period essentially unchanged.

On first emergence into the water column, two ultrastructurally distinct colony types can be discerned, and exist in parallel for a short period before plateau-phase growth. These consist of (i) aggregates of cells containing appreciable quantities of interthylakoidal glycogen granules (figure 28) and (ii) aggregates of pallid cells that strongly resemble contemporary benthic material (figure 29). Structured granules, polyphosphate bodies, carboxysomes, PHB granules and lipid droplets are common in both cell types.

Post-exponential growth is marked by a sharp increase in glycogen levels, glycogen thus becoming the primary granular reserve, supplanting the larger storage bodies (figure 30). Subsequently, during the post-maximal growth phase, similar cells additionally display increased numbers of structured granules, polyphosphate bodies and PHB granules (figure 31). Crystalline bodies appear intermittently throughout the year.

Over the period of summer growth, comparable mud colonies range from pallid to glycogen-rich. During population decline in late autumn, benthic cells are mainly of the latter type; however, their ultrastructure gradually changes through a series of intermediates (figure 32) and finally reverts to the pallid form, remaining as such throughout the overwintering period.

(*c*) *Laboratory studies of specific ultrastructural variations*

(i) *Gas vesicles*

Since thin sections of cells known to contain gas vacuoles in the living material occasionally display aggregates of collapsed vesicles within electron-transparent spaces (figure 24) a simple experiment was performed to determine whether this observation represents true vesicle collapse or destruction through chemical fixation.

The gas vacuoles of live cells were artificially collapsed by application of 1.1 MPa in a pressure chamber, after which they were no longer visible in the light microscope. This material was then fixed, embedded and sectioned and compared with the untreated control. The latter (figure 33, plate 4) displays cells with well preserved vesicles, while the treated cells (figure 34) show little evidence of these although flattened vesicle components may be seen at higher magnifications. The disorganized appearance of gas vesicles in wild material (e.g. figure 29) can therefore be attributed to artefactual collapse during chemical fixation.

(ii) *Granular inclusions*

Samples of a batch culture of a non-axenic strain of *M. aeruginosa* were fixed at various stages of growth to determine what changes occur in the number and type of granular inclusions.

Structured granules observed during early logarithmic phase (figure 35) are quickly lost during subsequent rapid growth. In contrast, the numbers of glycogen granules are lowest during the exponential phase, after which levels increase until massive quantities accumulate in late post-logarithmic cells (figure 36). Polyphosphate bodies and carboxysomes are retained throughout transformation; gas vesicles are common in early phases but are not apparent in late post-logarithmic phase.

(iii) *Glycogen content*

Wild material was harvested from the photic zone and incubated in total darkness. Compared with control cells (figure 37) the glycogen content falls rapidly, an approximate 50% decrease having occurred by 24 h and complete loss by 48 h (figure 38). The dark-grown cells thus acquire a cytoplasmic granularity reminiscent of the previously described 'pallid' type.

(*d*) *Discussion*

The possible role of structured granules, polyphosphate bodies and carboxysomes in relation to the seasonal cycle of blue-green algae has previously been considered by Stewart *et al.* (1978), and results presented here are comparable with those obtained by these authors for populations

of *Microcystis* in Balgavies Loch. We have found a similar decline in numbers of structured granules and polyphosphate bodies over the period of thermal stratification, with subsequent re-accumulation of both these inclusions as the population declines. Carboxysomes are never absent.

The relationship between various environmental conditions and levels of glycogen granules is in accordance with the ascribed role of the glycogen granules as reserve products of photosynthesis (Giesy 1964; Weber & Wober 1975). Their rapidity of formation is demonstrated by the recovery of radioactive glucose after only 5 s exposure to $^{14}CO_2$ in the light (Kandler 1961; Kindel & Gibbs 1963); either transfer of cells into the dark or the addition of DCMU, 3′-(3,4-dichlorophenyl)-1′,1′-dimethyl urea, a specific inhibitor of photosystem II, to an illuminated culture leads to a mobilization of glycogen (Lehmann & Wober 1976), thought to involve the glycolytic pathway to pyruvate in *Chlorobium* (Sirevåg 1975). Since glycogen constitutes a labile pool that appears in the light and disappears quickly in the dark, the light history of the cells may be inferred from a consideration of the glycogen content in thin sections, an observation supported by our own laboratory experiments (figures 37, 38) and in agreement with the biochemical findings of Gibson (1975, 1978; see also §10).

It is known from studies on heterotrophic bacteria that glycogen accumulation takes place if cell division has ceased, e.g. under conditions of nutrient deficiency in the presence of a utilizable carbon source (Preiss 1969; Dawes & Senior 1973). Similar observations have been made on blue-green algae (Wolk 1973; Lehmann & Wober 1976) and are supported by our own observations of growth in batch culture (figures 35, 36).

The presence of pallid cells within the plankton during spring strongly suggests recruitment from the aphotic zone, while the accumulation of glycogen observed during post-exponential growth, together with decreased levels of structured granules and polyphosphate bodies, is indicative of nutrient deprivation. Reappearance of the larger storage granules during the later phases of growth occurs at the time of a recorded rise in exogenous nutrients in the epilimnion and could therefore be due to nutrient uptake in the photic zone, although absorption of nutrients at depth with subsequent cell recruitment to the epilimnion would also explain the observations. Glycogen-rich cells found within the lower layers during plateau phase and decline do indeed suggest a downward transport of cells out of the photic zone (see Gibson 1978); once out of the light the glycogen is quickly mobilized. The present ultrastructural studies do not demonstrate re-recruitment from the bottom muds; however, comparable studies undertaken on a shallow eutrophic lake in Yorkshire provide strong evidence that this occurs (Cmiech 1981).

According to epifluorescence microscopy benthic cells remain viable for considerable periods after the glycogen pool has been depleted (see §§7, 8). The role of bacterial PHB in providing carbon skeletons and energy for the synthesis of other cell constituents under conditions of nutrient starvation is well known (see Dawes & Senior 1973) and it is tempting to suggest that sustained dark survival of *M. aeruginosa* may be related to the PHB content of the cells.

Species-specific crystalline lattices, similar to those seen in our material, have been observed in many bacteria (see Shively 1974), where they have been linked with sporulation (Yousten & Rogoff 1969) and enterotoxin production (Duncan *et al.* 1973). Their occasional occurrence in other blue-green algae (Gantt & Conti 1969; Jensen & Bowen 1970; Butler & Allsopp 1972) has been considered the result of infection by intracellular symbionts or viruses (Jensen & Bowen 1970). The antagonistic effects of *M. aeruginosa* against algae and other organisms are

well documented (Hughes *et al.* 1958; Bishop *et al.* 1959; Vance 1965) and it might therefore be suggested that there is an association between the suspected toxicity of the Blelham populations (see § 9) and these crystalline inclusions.

7. The overwintering phase

(*a*) *Distribution on the sediments*

Since Wesenberg-Lund's (1904) observations, viable, vegetative stocks of *Microcystis* have been found on the sediments of a range of eutrophic lakes spanning several continents and differing morphometries (Gorham 1958; Chernousova *et al.* 1968; Topachevskiĭ *et al.* 1969;

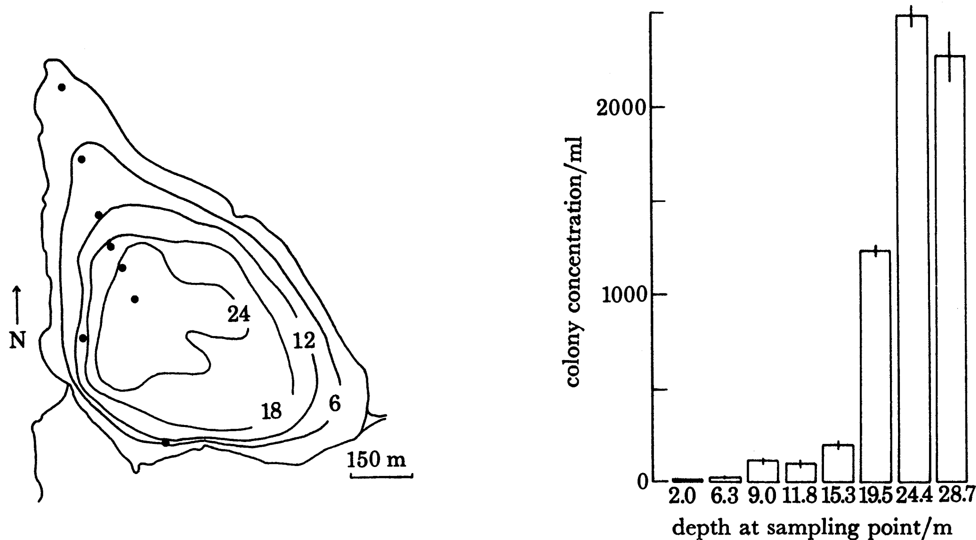


Figure 39. The concentration of *Microcystis* colonies (number per millilitre of surface mud sample) collected on 25 March 1976 at the various depth locations indicated in Rostherne Mere.

Sirenko *et al.* 1969; May 1972; Goulder 1974; Reynolds & Rogers 1976; Kappers 1976; Tow 1979). Formation of these stocks depends upon net recruitment to the sediments of erstwhile planktonic algae, perhaps abetted by 'sediment focusing' (Likens & Davis 1975), and upon the physiological capacity for survival under such conditions.

These factors also influence the relative concentrations of the various stati (st. I a, II, III and V a) making up the benthic population in the Blelham enclosures. However, the populations retained their characteristics through the winter months; it is striking that clear autumnal differences in the absolute and relative abundance of stati between the two benthic populations (cf. figures 19*d* with 22*d* and 20*d* with 23*d*) were preserved during the winter equilibration period, when the enclosures were opened to the tarn circulation and their waters mixed indistinguishably (cf. Reynolds & Butterwick 1979). We conclude that the populations observed by us were subject to minimal recirculation or lateral transport from depths greater than 11 m. In contrast, the horizontal distribution of benthic colonies in Rostherne Mere in March 1976 (shown in figure 39) indicates differential distribution, with the greatest concentrations at depths greater than 20 m. It can be attributed to (i) limited sedimentation in the immediate sublittoral zones, (ii) secondary resuspension from shallows and redeposition in deeper water (sediment focusing), or (iii) poor survival in oxygenated, shallow water (as hypothesized by

Sirenko (1972)). We cannot establish which of these processes dominated the others, although the data of Reynolds & Rogers (1976) suggest prolific recruitment at the deepest part of the mere during the autumns of 1972 and 1973. It is also relevant that, since *Microcystis* produced only a limited growth in 1975 (see Reynolds 1978*b*), a large proportion of those colonies recovered in March 1976 must have survived for at least 1 year. The observed distribution could then be interpreted as coinciding with that of permanently anaerobic, fine detrital muds in the same lake (cf. Brinkhurst & Walsh 1967), which is in line with the observations of Chernousova *et al.* (1968) and Topachevskiĭ *et al.* (1969).

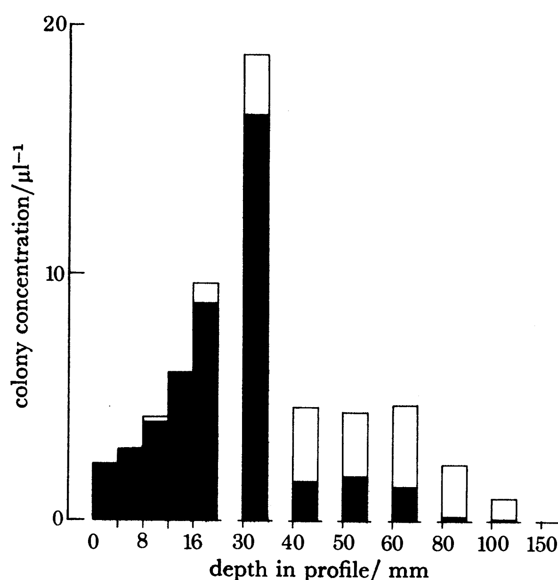


FIGURE 40. The vertical distribution and viability of *Microcystis* colonies in selected 4 mm slices of a Jenkin mud core from Rostherne Mere, March 1976. The length of the bars represents the colony concentration; the solid black areas correspond in length with the number of colonies per microlitre from which chlorophyll fluorescence was recorded.

(*b*) *Physiological condition*

The viability of *Microcystis* colonies (judged by chlorophyll fluorescence) in successive sections of a Jenkin core taken from near the deepest point in Rostherne Mere is represented in figure 40. Though *Microcystis* colonies remained recognizable throughout the core (cf. Livingstone & Cambray 1978), viable cells were largely confined to the upper 40–50 mm. It is likely that these colonies sedimented between 1972 and the end of 1974 (see above and also Livingstone & Cambray 1978). In spite of their age and a long history of light deprivation, colonies nevertheless had evidently retained their photosynthetic apparatus for one or more years. The ability to have survived long periods of darkness is presumably based upon endogenous respiration of stored carbohydrate (cf. Stanier & Cohen-Bazire 1977); uptake of exogenous carbon sources has not been specifically tested.

Colonies isolated from the surface of the Blelham enclosures on 8 February 1977 were similarly examined; fewer colonies possessed cells that fluoresced, even though most of them would have been on the sediments for only two or three months. However, qualitative differences in the fluorescence transmitted by individual cells and from colonies of different status were

evident. Strong fluorescence was observed in a relatively small number of cells in colonies (st. I*a*, II, III (see table 4)) previously considered to have been senescent.

(*c*) *Buoyancy*

A striking feature of the overwintering cells examined by electron microscopy is their high relative gas-vacuole content. It would be difficult to examine sufficient sections of sufficient individual cells to make a statistically acceptable estimate of typical gas-vacuole volume. It

TABLE 4. CHLOROPHYLL FLUORESCENCE IN *MICROCYSTIS* COLONIES RECOVERED FROM THE SEDIMENTS OF THE ENCLOSURES, FEBRUARY 1977

	enclosure A			enclosure B		
colony status	I a	II/III	V a	I a	II/III	V a
number of colonies examined having:						
(*a*) no fluorescence	3	3	31	10	15	—
(*b*) weak local fluorescence	—	—	—	12	10	10
(*c*) uniform weak fluorescence	—	1	33	—	—	2
(*d*) intense local fluorescence	5	—	—	—	7	—
total number of colonies examined		76			66	

TABLE 5. *IN VITRO* SINKING RATES OF BENTHIC OVERWINTERING COLONIES AND CALCULATED MEAN RELATIVE GAS-VACUOLE VOLUME

from	date	mean sinking rate (± s.d.) μm s^{-1} (*a*)	mean sinking rate (± s.d.) μm s^{-1} (*b*)	mean radius μm	cell fraction	$\frac{\text{gas vacuole vol.}}{\text{cell vol.}}$ (%)
B	4 Nov. 1976	2.5 ± 3.4	42.0 ± 39.3	107	0.086	2.1
B	30 Nov. 1976	8.1 ± 5.8	37.0 ± 4.0	103	0.049	2.9
B	21 Dec. 1976	6.5 ± 4.7	24.7 ± 15.1	87	0.065	1.9
Rostherne	2 Mar. 1977	18.3 ± 11.7	110.2 ± 46.6	117	0.190	1.8

(*a*) With vacuoles; (*b*) after vacuoles destroyed.

can be stated, however, that gas vesicles were present in all the apparently viable cells examined and that they seemed more numerous than at any other stage in the annual cycle. The colonies were, nevertheless, negatively buoyant: when disturbed from the mud–water interface of freshly collected Jenkin cores, colonies typically sank slowly back to the mud surface. Even the gentle 'washing' of colonies, in a current of lake water generated with a bulb pipette, to remove adherent particles, failed to overcome sinking.

Three interpretations may be proposed: (i) that the vesicles might contain not gas, but water; (ii) that the accumulation of storage products of high density (polyphosphate bodies, condensed carbohydrates, proteinaceous structured granules) elevates mean colony density above that of the water; or (iii) that only a small proportion of cells are viable and vacuolate.

The first explanation (i) seems unlikely, since the inner surface of the vesicle membrane is now known to be hydrophobic (Worcester 1975; for a recent review of vesicle structure and function, see Walsby 1978). In any case, we have found that the application of external pressures of 0.7–1.1 MPa (i.e. sufficient to collapse the gas vacuoles) always increased the sinking potential of overwintering colonies. Direct measurements of sinking rate in benthic st. V a colonies are summarized in table 5. The column on the right gives an estimate of original relative gas-vacuole volume, calculated from the change in mean sinking rate (see §2) and

the relative cell and colony volumes (see §4*d*). These calculations suggest that the mean relative gas vacuolation is not especially large, and agree with the calculated vacuole volume needed to achieve neutral buoyancy (§4, above). It should be noted that the gas-vacuole content of the Rostherne material was similar to that of the Blelham algae in spite of its higher vacuole-free density (1003.1 kg m^{-3}, as against 1000.4–1001.0 kg m^{-3}, given in table 3); the greater density is presumably a function of the relatively greater cell fraction. Because rela-

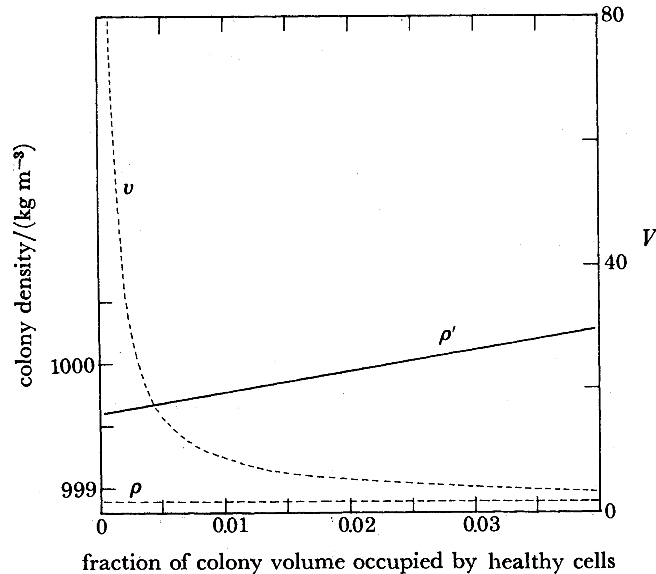


FIGURE 41. The theoretical relation between the fraction of colony volume occupied by healthy cells and the relative gas vacuole volume of those cells required to achieve neutral buoyancy. It is assumed that the density (left scale) of the water (ρ) is 998.9 kg m^{-3}, and that the mean density of the colonies (ρ') is contributed by the density of the mucilage (ρ'_x = 999.6 kg m^{-3}) and cell material (ρ'_y = 1016.4 kg m^{-3}), so that ρ' varies directly with the volume of the cellular fraction; the increased proportionate volume of gas-filled space in the colony to reduce overall ρ' to ρ is expressed as a percentage of the fractional healthy cell volume (plotted as v, right scale).

tively more of the Rostherne colony is capable of erecting and maintaining gas-filled space, the mean gas-vacuole content required to gain neutral buoyancy is proportionally smaller. This can be verified by substituting appropriate values for x and y in e.g. equation (2) and evaluating ρ_x and ρ_y according to equations (4) and (5) respectively; the calculated colony density (ρ' = 1002.8 kg m^{-3}) would be reduced to that of the water at 15 °C ($\rho' = \rho$ = 998.9 kg m^{-3}) by a mean gas-vacuole content equivalent to only 2.1 % of cell volume.

The agreement of the colony densities approximated from the Stokes's equation with those calculated from interpolated values suggests that the latter has not greatly underestimated cell density (ρ'_y = 1016–1017 kg m^{-3}). If this is so, and it applies to overwintering cells with generally abundant storage products, then the second possibility (ii) cannot be invoked to explain the apparently high relative gas-vacuole content of the cells in negatively buoyant benthic colonies. The remaining possibility (iii) is that many of the cells present, because they are either moribund or inactive for some other reason, do not maintain an adequate volume of gas vacuoles. The mean relative gas-vacuole volume (expressed as a percentage of healthy cell volume) required to attain neutral buoyancy of the colonies increases as the fractional complement of healthy cells is reduced (see figure 41). If the third explanation is correct, such

that less than 0.01 of the colony is occupied by healthy cells, then the relative volume of their gas vacuoles can exceed 8% without the colony as a whole becoming buoyant.

Maintenance of gas vacuoles in negatively buoyant overwintering colonies has been observed previously by Reynolds & Rogers (1976), who commented that colonies were nevertheless 'scarcely buoyant'. The present considerations support these earlier findings, perhaps indicating that the control of gas vacuolation is as important in the biology of the alga in winter as it is in summer. However, there must be different processes controlling gas vacuolation: neither the 'turgor collapse' nor 'growth dilution' mechanisms (see Reynolds & Walsby 1975) could be expected to function in benthic populations. That vacuoles are maintained at all is remarkable since, as is argued later, autumnal elimination of gas vacuoles from the cells of suspended *Microcystis* colonies is an essential prelude to overwintering. Maintenance of a slight subbuoyant condition may assist the alga in remaining at, or close to, the sediment surface, where it is better placed to respond to environmental conditions in the water column yet still derive maximum advantage from the sediment.

8. The initiation of seasonal growth

(a) *Benthic growth: morphological characteristics*

We have already argued that the preliminary stages of seasonal growth take place on the bottom mud, manifest in the formation of st. IV and Vb colonies. It seems likely that a short series of cell divisions gives rise to the cell clusters that characterize st. IV colonies. Alternatively, cluster formation could be preceded by multiple fission and release of propagules from specialized reproductive cells. Canabeus (1929) recorded the release of what she termed nannocytes from *Microcystis* cells, but her observations have been either ignored or discounted. However, interest in nannocytes has been resurrected by their recent 'rediscovery' by Pretorius *et al.* (1977) in a cultured strain of *Microcystis*. We have not wittingly observed any structure intermediate between individual single cells and clusters of 20 or more, nor have we been aware that significantly smaller cells contribute to their formation. In no way does this discount a possible intermediate nannocytic stage; we believe that the possibility merits further investigation.

Our observations (see §5) indicate that the development of st. IV and Vb colonies in the field was closely related to the simultaneous occurrence of high light penetration through the overlying water column and incipient hypolimnetic anoxia. Some experiments to test this hypothesis are now described.

(b) *Light*

The first experiments established the role of light in stimulating growth. A suspension of colony-rich surface deposit and bottom water was prepared from Jenkin mud cores collected at the deepest point in Rostherne Mere on 25 March 1976. The suspension was thoroughly stirred and apportioned between 250 ml Erlenmeyer flasks, which were then transferred to a culture cabinet thermostatically controlled at 20 ± 1 °C and either exposed to continuous 'daylight' fluorescent illumination from below, rated at 0.6 or 1.8 klx (7.6 or 22.7 $\mu E\ m^{-2}\ s^{-1}$) at the flask surfaces, or wrapped in three layers of aluminium foil to exclude light. Four replicates of each treatment were included. Subsequent changes in the concentration of colonies were compared with that concentration in the initial suspension (120 colonies per millilitre

comprising st. III and Va colonies in approximately equal proportions). During the next 17 days, the numbers of colonies in the illuminated flasks approximately doubled (figure 42); the proportion of st. Va colonies increased to 67–74% of the total and there was a perceptible increase in their mean diameters (from 52.6 ± 16.7 to 87.7 ± 43.8 μm). There were more colonies at the higher light intensity but, by day 31, there was no significant difference between mean colony concentrations at 0.6 and 1.8 klx. Neither the concentration nor the size of colonies in the darkened flasks had changed significantly by day 17, though the algae had become paler in colour.

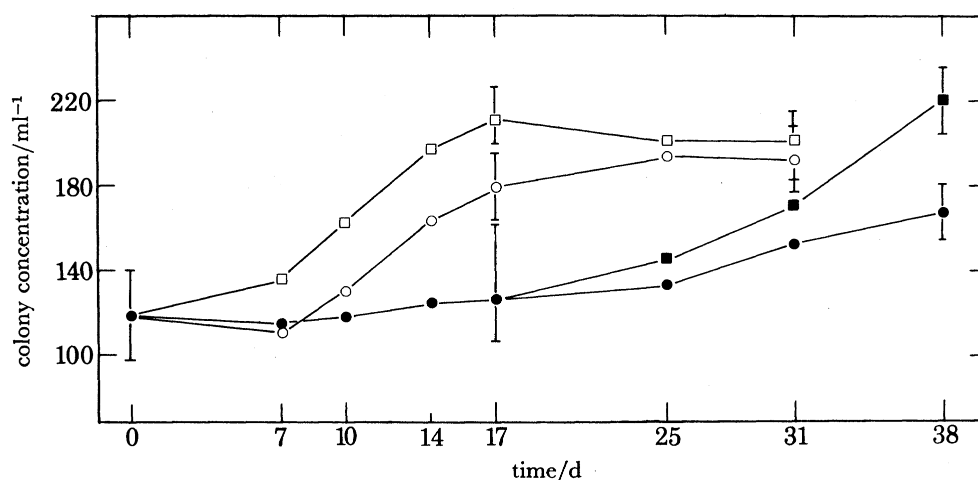


FIGURE 42. Changes in the concentration of *Microcystis* colonies in suspensions prepared from material collected at the mud–water interface of Rostherne Mere, March 1976, under different conditions: continuous dark (●), continuous illumination at 0.6 (○) or 1.8 (□) klx; vertical bars give the range of concentrations in four replicate treatments. On day 17, two of the dark flasks were transferred to continuous illumination at 1.8 klx: subsequent changes in concentration are represented (■).

Two of the darkened flasks were then uncovered and exposed to the higher (1.8 klx) light intensity. By day 38, colony concentration had more than doubled, and the mean diameter of the st. V colonies increased to 78.4 μm (range ± 16.3 μm). At the same time, the colonies in the darkened flasks had begun to deteriorate and fragment (st. Ia, II and III) contributing to the observed increase in colony concentration.

In later experiments designed to test the effects of added nutrients upon growth initiation (see below), darkened 'controls' (unmodified lake water) were maintained for periods of 2–5 months at 10 °C; in none of these was any algal growth detected; the only change consistently observed was the decline in the relative abundance of st. Va colonies. This deterioration was least in intact Jenkin core samples taken from enclosure B in November 1976 and stored in darkness at 10 °C until April 1977.

In a second series of experiments, carried out between March and November 1977, 40–45 ml of material syringed from the mud–water interface of intact Jenkin cores taken from the Blelham enclosures was routinely transferred to 100 ml Erlenmeyer culture flasks. Flasks were incubated for 6 days at 18 ± 1 °C and exposed to continuous illumination from below, equivalent to 1.2 klx (15 μE m^{-2} s^{-1}). At the end of each period, the flasks were thoroughly stirred and subsamples were fixed and sedimented for enumeration. The results are presented in figure 43. In 40 cases (out of 48), there was a significant increase in the concentration of

colonies, with a maximum rate of increase of ln (concentration) over 6 days equivalent to $0.317\,d^{-1}$. Increase was generally greater in the 'cultures' from enclosure B material (mean ± s.d., $0.109 \pm 0.100\,d^{-1}$) than in those from enclosure A ($0.063 \pm 0.052\,d^{-1}$). Changes in the composition of the cultured populations are also represented in figure 43: the relative proportions of st. IV and Vb colonies showed the most conspicuous increases in the earlier part of the year, when the faster overall rates of increase were recorded; towards the end of the

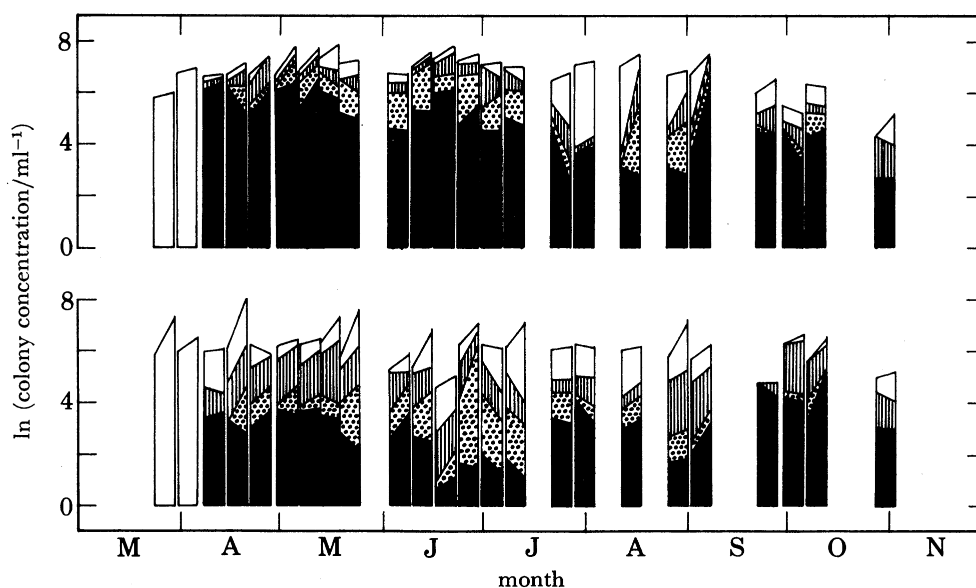


FIGURE 43. The *in vitro* development of *Microcystis* colonies in 1977 Jenkin surface mud samples incubated for 6 days at 10 ± 1 °C and exposed to 1.2 klx continuous illumination. In each case incubations commenced on the day of collection, and the concentration is represented on vertical logarithmic scale against that date; the right-hand apex of each individual figure represents the colony concentration on day 6 of the experiment. The differential shading represents changes in proportional composition of morphological stati: st. Va + VI, solid black; st. IV + Vb, stippled; st. II + III, hatched; st. I, unshaded. Upper row, incubation of enclosure A material; lower row, enclosure B incubations.

season, however, st. Va colonies typically showed the greatest relative increase. The relative proportions of stati in the cultured population may have depended upon the initial composition of the samples. Thus, the development was not necessarily always mediated by the production of cell clusters (st. IV) and young colonies (st. Vb), from which we conclude that the initiation of *Microcystis* growth is not limited to a single strategy. However, these experiments show that growth in benthic populations can be stimulated at almost any time of the year in the laboratory. Low light appears to be an essential requirement but the experiments do not discount the possibility of temperature involvement (see below).

(*c*) *Oxygen*

The 'culture' of benthic colonies was repeated during 1978, but the oxygen content of the cultures was additionally monitored by means of an E.I.L. Dissolved Oxygen Probe Model 8012.1. Positive growth responses were observed within 6 days in 29 out of 30 individual experiments and the changes in population composition generally conformed to the pattern of the previous year. The changes in oxygen concentration in individual flasks varied between

net rapid decline to occasional net increases of up to 20 mg l^{-1}, apparently depending upon the quantity of the healthy material originally included, the extent of the growth response and the relative abundance of organic detritus in the flasks. Increases in the numbers of colonies, however, were more consistently related to the initial oxygen concentration (see figure 44). The faster rates of increase observed (rates of increase of ln (concentration) greater than 0.2 d^{-1}) coincided with initial oxygen concentrations of 1–4 mg l^{-1}; outside this range, the rate of increase was less than 0.1 d^{-1} on all but three occasions.

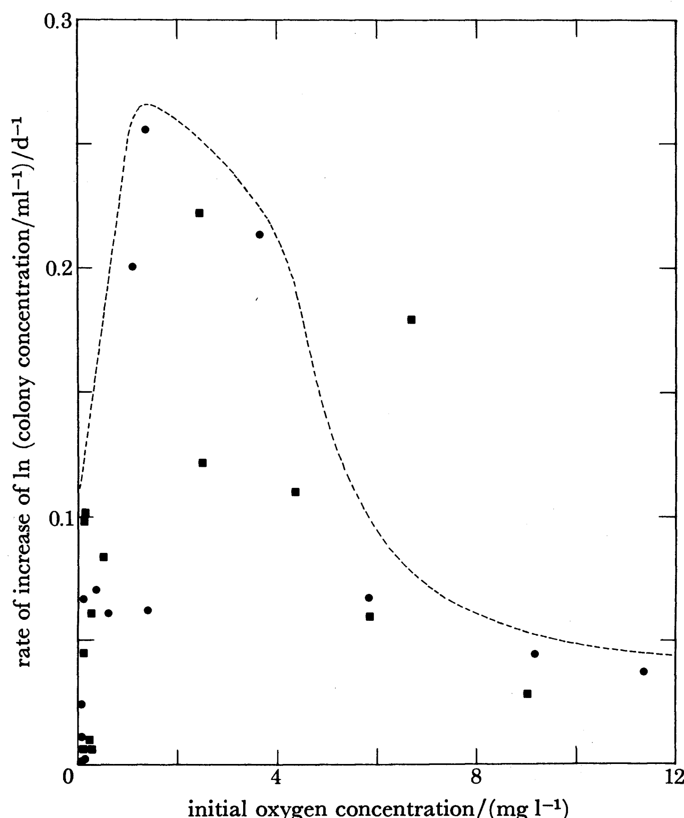


FIGURE 44. The rates of *in vitro* increase of *Microcystis* colonies in 1978 Jenkin surface mud samples incubated for 6 days at 18 (±1) °C and exposed to 1.2 klx continuous illumination, plotted against initial oxygen concentration of the well mixed sample. The results for material from enclosure A (●) and B (■) are separated. The curve is advanced as a tentative 'boundary', and has no mathematical significance.

The relation is superficial in that the oxygen concentration in the immediate vicinity of the cells doubtless differs from the measured concentration. A low initial oxygen content could merely facilitate the earlier attainment of the critical growth requirements. Moreover, the growth response is also influenced by the quality of the cells present: thus it is equally possible that the relation implies that the colonies capable of growth were relatively more abundant in the samples taken in mid-spring before the hypolimnetic oxygen concentration had fallen to zero.

On the other hand, the experiments do confirm that, either directly or indirectly, declining oxygen concentration potentially benefits the growth of benthic *Microcystis* colonies. They thus support the more thoroughly researched findings of other workers that the level of physiological activity in *Microcystis* cells (Sirenko *et al.* 1968), and, indeed, in other species of blue-green algae

(see, for example: Gusev 1962; Stewart & Pearson 1970; Weller *et al.* 1975), is intimately related to low oxygen concentrations and low redox potential external to the cell. An ultimate shift to photoautrophy, however, is indicated by the occasional increases in oxygen concentration observed in the laboratory growth experiments.

(*d*) *Chemical factors*

Chemical speciation and solubilities of biologically active compounds, especially those involving iron and sulphur, are also closely related to redox potential in fresh water. Thus, it is possible that the initiation of seasonal growth is chemically stimulated by one or other of the

TABLE 6. MEAN COLONY CONCENTRATION (NUMBER OF COLONIES PER MILLILITRE ± S.E.) IN *MICROCYSTIS* SUSPENSIONS WITH ADDED IRON (AS EDTA COMPLEX) OR SULPHUR (AS SODIUM SULPHIDE)

treatment	initial	after 60 days in darkness	after a further 14 days in light
control	76 ± 18	65 ± 6.5	101 ± 11
+ Fe		70 ± 12	112 ± 13
+ S		62.5 ± 8.5	92.5 ± 9.5

species whose presence in the hypolimnion is also seasonal. In view of the stated importance of iron (Gerloff *et al.* 1952; Lange 1974; Morton & Lee 1974; Box 1977) and sulphur (see, for example: Gerloff *et al.* 1952; Volodin 1970), we tested the effect of selective 'spikings' of soluble iron (ferric chloride and sodium ethylene diamine tetraacetate in equimolar proportions; effective concentration 1 mg of iron per litre) and sodium sulphide (effective concentration 80 μg of sulphur per litre) upon the benthic growth response. The material was from Jenkin mud cores from enclosure B, taken in January 1977. The enriched suspensions were placed in flasks and their surfaces sealed with liquid paraffin. The flasks were dark-incubated at 10 ± 2 °C for 60 days and finally transferred to the warmer, illuminated (20 ± 1 °C, 0.6 klx) conditions of the culture cabinet. In neither treatment was any growth detected during dark incubation; nor was there any significant difference in algal concentration between flasks in either test set and the controls (see table 6).

Though inconclusive, these results suggest that neither iron nor sulphide was critically controlling the growth response, perhaps because they were already freely available in the unmodified medium. Indeed, this inference may apply to other nutrients which would be freely available in the surface deposits or in solution in the interstitial water of eutrophic lakes. These would include phosphate phosphorus and ammonium nitrogen, both of which have been shown to stimulate renewed cell growth in old colonies (see, respectively: Pomiluiko 1968; Georgieva 1973).

(*e*) *Other factors*

Many authors have drawn attention to the apparent correlation between the onset of seasonal *Microcystis* growth and higher surface water temperatures, above a minimum variously suggested to be in the range 15–20 °C (see, for example, Hammer 1964; Reynolds 1971; Okino 1973). However, Reynolds (1973) argued that higher epilimnetic temperatures may be significant only in that they were correlatives of thermal and chemical stratification. Although growth is undoubtedly influenced by temperature the present evidence shows that higher

temperatures are not responsible for its seasonal initiation: the changes leading to the re-establishment of a planktonic population take place at the enclosure bottom at temperatures lower than 7–8 °C.

(*f*) *Reinvasion of the water column*

The presence of viable colonies on the sediments provides a potential source of planktonic populations. However, both the field observations and the laboratory experiments suggest that relatively few benthic colonies re-enter the plankton, and that those which do so may often be the direct products of cell growth and new colony production on the bottom. The apparent light dependence of the initial cell growth suggests that shallower sediments (for instance, those located just below the metalimnion) might furnish proportionately more new colonies than do deeper sediments. In the uniformly 'deep' enclosures A and B there are no shallow sediments; there, the apparent rate of recruitment of colonies to the plankton in spring is meagre when compared to its 'sudden appearance' (Belcher & Storey 1968) in the plankton of Rostherne Mere.

Although Topachevskiĭ *et al.* (1969) recognized that 'foci of infection' (cf. Sirenko 1972) provided the basis of summer populations, they stated that the transition to the planktonic phase was mediated 'by unexplained specific gravity changes'. Our studies (see §7, above) indicate that cells are already gas-vacuolate and that colonies are close to neutral buoyancy. A small increase in relative gas-vacuole content of the cells would be sufficient to render st. V colonies buoyant. If gas-vesicle production is resumed at the same time as autotrophic growth, the attainment of positive buoyancy would depend upon the relative kinetics of vesicle formation and cell division, as it does in the planktonic phase (Walsby 1970), and upon the 'ballast' provided by mucilage and dormant cells (cf. figure 41). At the generally low-light levels obtaining at the sediment surface, the turgor-collapse mechanism regulating buoyancy (Dinsdale & Walsby 1972) would be biased towards increasing flotation potential. That the capacity for buoyancy regulation is functional at the early stages of planktonic *Microcystis* populations has been shown previously by Reynolds (1973, 1978*c*).

9. Planktonic growth

(*a*) *Growth rate and standing crop*

Increase in the concentration of planktonic *Microcystis* populations occurred in almost every year shortly after the first appearance of colonies in the plankton. The exception to this statement applied to both enclosures in 1977, where background populations in the range 10–200 colonies per square centimetre were maintained during the period of vernal isothermal mixing (figure 1). Even on these occasions, however, the inception of sustained increase closely coincided with the simultaneous occurrence of high light penetration and anoxia in the water adjacent to the sediments.

Whereas the initial increase in the planktonic population is dependent upon benthic growth and recruitment, there is little evidence that the subsequent increase is not the result of autotrophic growth and cell division, accompanied by fragmentation of the colonies: populations contributed to higher dissolved oxygen tension and to higher pH levels; cells were typically pigmented, with prominent thylakoids and intracellular deposits of glycogen (see figure 37). Colony morphology of the actively increasing population varied between a preponderance of

TABLE 7. *IN SITU* RATES OF EXPONENTIAL INCREASE OF *MICROCYSTIS* CELL CONCENTRATIONS IN ENCLOSURES A AND B, AND A COMPARISON WITH OTHER PUBLISHED DATA

site	period	rate of increase of ln (concentration/ml^{-1})/d^{-1}	mean doubling time/d
A	27 Jun.–29 Aug. 1977	0.048	14.4
A	14 Aug.–4 Sep. 1978	0.240	2.9
A	6 Aug.–27 Aug. 1979	0.264	2.6
B	8 Jul.–2 Aug. 1976	0.188	3.7
B	13 Jun.–11 Jul. 1977	0.147	4.7
B	12 Jun.–10 Jul. 1978	0.145	4.8
B	10 Jul.–24 Jul. 1978	0.070	9.9
B	9 Jul.–3 Sep. 1979	0.114	6.1
Rostherne Mere†	21 Jun.–24 July 1973	0.166	4.2
Rostherne Mere‡	20 Jun.–30 Jul. 1977	0.172	4.0
Crose Mere§	29 Jun.–20 Jul. 1971	0.127	5.5
Crose Mere§	5 Jul.–25 Jul. 1972	0.211	3.3

References
† Reynolds & Rogers (1976).
‡ Reynolds (1978*b*).
§ Reynolds (1973).

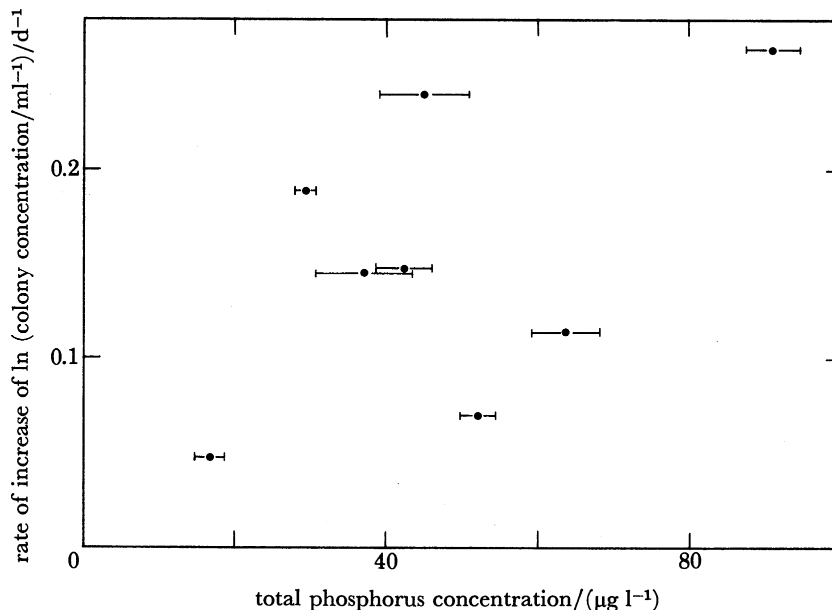


FIGURE 45. *In situ* rates of increase in cell concentration in the experimental enclosures (from table 7) plotted against total phosphorus concentration (mean ± range) over the corresponding periods.

st. Va and VI colonies to one of almost complete dominance by st. Ib colonies (see parts (*a*) of figures 18–23).

In each case, exponential increase in the number of cells present in the upper 5 m of the water column was sustained for a period of 3–8 weeks (see figure 1). Exponential constants for net increase during these periods varied between 0.048 and 0.264 d^{-1} (see table 7). In figure 45 the exponential growth constants are plotted against the mean (± s.d.) concentration of 'total' phosphorus over the corresponding periods. The results suggest that there is a general relation between growth rate and P availability, but other factors presumably obscure the fit of the data to a Monod model.

One such factor may have been the ingestion and (to some extent) consumption of st. Ib colonies by *Daphnia*. Although it is unlikely that *Microcystis* often forms the major food of crustacean zooplankton in temperate lakes, it is incorrect to assume that the alga is 'ungrazed' (see, for instance: Schindler 1971; Gras *et al.* 1971; Burgis *et al.* 1973; Tevlin & Burgis 1979). *Microcystis* is known to have formed part of the food of *Daphnia hyalina* in enclosure B during July 1978 (Ferguson *et al.* 1981) although at most times colony size apparently conferred some immunity from ingestion by zooplankton (cf.: Burns 1968*b*; Gliwicz 1970; Porter 1977). At such times, the true rate of growth probably approximates to the observed (net) increase rate.

The maximal seasonal standing crops of *Microcystis* in the enclosures are represented in figure 1. Their sizes were probably not determined by nutrient concentration: except for enclosure A in 1977 and B in 1976, major nutrients were added each week (Reynolds & Butterwick 1979). The populations in enclosure B during 1978 and 1979, however, may have been ultimately limited by self-shading. From the mean concentration of chlorophyll *a* in Friedinger samples integrated over the surface 3 m layer (late August 1978, 160–290 $\mu g\ l^{-1}$; September 1979, 98–105 $\mu g\ l^{-1}$) and the approximate euphotic depth, estimated as 2.3 times (cf. Reynolds & Walsby 1975) the corresponding Secchi disc readings (figure 2: 0.6–1.0, 1.5–1.7 m respectively), the euphotic chlorophyll *a* contents at the times of these maxima were in the order 360–400 mg m^{-2}. These compare with theoretical upper limits on net phytoplankton production (200–300 mg m^{-2} (Steeman Nielsen 1962)) and observed values for natural *Microcystis* populations (Ganf 1972, 1974*a*). Clearly the biomass produced within the experimental enclosures was ultimately limited by the interaction of several factors.

(*b*) *Bioassay*

During 1977–1979, the capacity of enclosure B water to support growth of laboratory strains of *Microcystis* was investigated by an algal bioassay procedure (modified after Lund *et al.* 1971). Bioassay with use of the diatom *Asterionella* as a test organism showed that the fertilization scheme followed in 1977 and adopted as standard in both enclosures during 1978 and 1979 maintained fertility throughout the period of isolation; if the water was not fertilized, algal growth therein soon became subject to limitation, first by phosphorus and later by chelated iron (Reynolds & Butterwick 1979). When *Microcystis* was used as test organism in parallel bioassays it was found that, in spite of frequent fertilization, laboratory growth in filtered enclosure water was confined to the mid-year period (June–August 1977, June–July 1978 and May–June 1979: figure 46). Comparison with figures 1*c*, *e*, *g* shows that natural growth was confined to the corresponding periods in 1977 and 1978 but not 1979. Spiking of the filtered water with phosphorus and nitrogen either singly (1977, 1978) or in combination (1977, 1978, 1979) did not invoke increase in the *Microcystis*. The 1977 results indicate that chelated iron (EDTA–Fe in figure 46) was the major limiting factor: its presence either enhanced, or was essential to, the growth with respect to that in the unenriched water. Apart from two occasions in spring 1978, this effect could not be repeated in the subsequent assays. However, there was a continuing positive response to the presence of uncombined chelate (sodium ethylene diamine tetraacetate, or EDTA–Na) even when chelated iron failed to stimulate growth. EDTA was not simply rendering colloidal iron biologically available.

The immediate interpretation is that a suitable chelating agent is required to support growth in lake water for a function that is not clear. The original purpose of adding the chelate was to maintain iron and essential metals in solution (cf. Provasoli *et al.* 1957). If one sets aside any

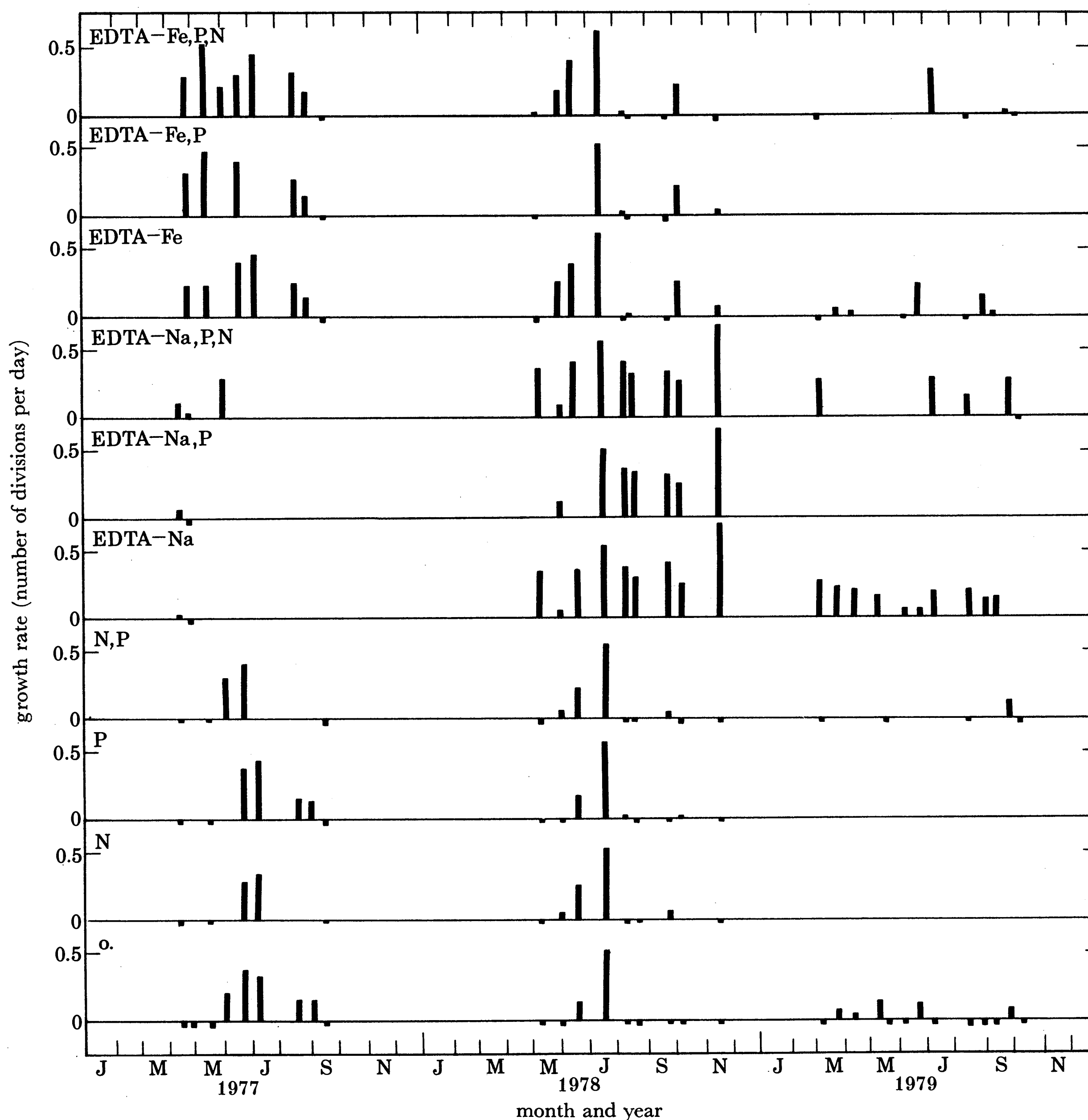


FIGURE 46. Bioassay of enclosure B water, 1977–1979, with *Microcystis* L155 or L305 as test organisms. Cell yield expressed relative to inoculum in divisions per day. Assays were performed on filtered lake water left unenriched (*o*) or enriched with nitrate nitrogen (N), phosphate phosphorus (P), sodium ethylene diamine tetraacetate (EDTA–Na), EDTA-chelated iron (EDTA–Fe) and in combinations thereof.

possible differences in the behaviour of strains L155 and L305, it is apparently the excess concentration of chelate that is important, either to assist in the uptake of other limiting metals (which at times may include iron), or possibly to inactivate toxic or other inhibitors present in the water. On the other hand, growth in the unmodified lake water may be dependent upon natural substances that are present only seasonally and whose effect is directly imitated by EDTA.

Further experiments were carried out in an attempt to resolve these possibilities. In the first

experiment, selected metals (iron, copper, zinc and manganese) were added to filtered lake water, together with equimolar concentrations of EDTA–Na. Only EDTA–Na and chelated manganese enhanced growth (table 8). Owing to the weak affinity between EDTA and Mn, the latter result could be attributable to the presence of free chelate. This view was supported by a further series of assays in which manganous chloride solution was added to test media

TABLE 8. THE EFFECT ON THE GROWTH OF *MICROCYSTIS* L305 OF ADDING METALS WITH EQUIMOLAR PROPORTIONS OF CHELATE (EDTA) TO FILTERED WATER FROM ENCLOSURE B, APRIL 1979

treatment	number of cell divisions in 14 days
control	1.08
EDTA–Na	3.10
EDTA–Fe	0.39
EDTA–Cu	0.27
EDTA–Zn	0.91
EDTA–Mn	2.79
EDTA Fe/Cu/Zn/Mn	0.69

TABLE 9. THE EFFECT ON THE GROWTH† OF *MICROCYSTIS* L305 OF 'CHELATED MANGANESE' COMPARED WITH MANGANOUS CHLORIDE SOLUTION (Mn: 340 $\mu g\ l^{-1}$) AND FREE CHELATE (EDTA–Na)

date	8 May	5 Jun.	19 Jun.	28 Aug. 1979
control	0.97	0	0.80	0
+ $MnCl_2$	0.81	0	0.64	0
+ EDTA–Mn	1.22	0.67	0.73	1.20
+ EDTA–Na	1.07	0.52	0.52	1.45

† Cell divisions in 7 days.

without chelation; again free chelate seemed to be the critical additive (see table 9). If the EDTA does serve to chelate a metal, not only have we failed to identify it, but it has to be proposed that growth of *Microcystis* is critically limited by a substance or substances hitherto regarded as a trace and which is normally omitted from culture media except as an impurity. We think it likely that EDTA serves another function.

It is important to stress at this point that apparent nutrient deficiencies in the assayed media may be the result of preparation and do not necessarily testify to a limiting condition in the original water (see, for example: Lee 1973; Reynolds & Butterwick 1979). For instance, much of the iron available to the natural algal population at a given time will already be within, or attached to, the cells (Elder 1977); it is thus removed by filtration from the assayed medium. A water sample from Rostherne Mere, collected on 22 September 1977 at the peak of almost unialgal *Microcystis* population (*ca.* 300000 cells per millilitre), was analysed for total iron before filtering, after filtration through phytoplankton net (*ca.* 50 μm mesh) and again after filtration through Whatman GF/C paper (*ca.* 0.45 μm). The results are presented in table 10. More than one-third of the total iron in the original sample was removed with the *Microcystis*, either in or attached to the algae, or simply present in tripton particles more than 50 μm across. A further 16% was present as smaller particles. Almost half the original iron remained

in the GF/C filtrate, either dissolved or in suspension as fine (smaller than 0.4 μm) particles. This filtrate was assayed with *Asterionella* as the test organism and shown to be iron deficient, according to standard interpretation (see table 11). The interpretation might not have applied to *Microcystis* in the original sample had the iron removed by the phytoplankton not been intracellular and available for assimilation.

TABLE 10. THE 'PARTITIONING' OF TOTAL IRON IN A WATER SAMPLE FROM ROSTHERNE MERE, COLLECTED DURING A PERIOD OF *MICROCYSTIS* ABUNDANCE (0.3×10^6 CELLS PER MILLILITRE)

	total Fe/(μg l^{-1})
unfiltered lake water	498
net (50 μm) filtered	323
GF/C (0.45 μm) filtered	243

TABLE 11. BIOASSAY OF GF/C FILTRATE (TABLE 10) WITH *ASTERIONELLA FORMOSA* AS TEST ORGANISM

treatment	number of cell divisions in 7 days	number of cell divisions in 10 days
control	0	—
+N	1.5	—
+EDTA–Fe	7.4	10.1
+EDTA–Fe+N	7.8	10.3

That the bioassays indicated that the fertility of unmodified lake water with respect to *Microcystis* was restricted to the summer season agrees with the earlier findings of Box (1977) for two English Lake District waters (Esthwaite, Blelham Tarn). He observed that the restoration of fertility coincided with vernal diatom maxima and the development of thermal and chemical stratification in the lakes. Box (1977) postulated that this may have been due to a chemical 'conditioning' of the water by a substance or substances, 'probably organic', released into the spring epilimnion by the vernal diatom populations. If this interpretation is substantially correct, then it is implicit that EDTA–Na can be substituted when the 'conditioner' is present in suboptimal quantities.

These considerations inevitably impinge upon the 'traditional' (cf. Reynolds & Walsby 1975) link between blue-green algae and dissolved organic content of the water, which has still not been adequately investigated. The variety of organic compounds present at low concentrations in natural lake waters and of their possible interactions with organisms as well as inorganic substances is dauntingly complex. Liberation of extracellular organic solutes by blue-green algae (see, for example: Fogg 1952, 1971; Hellebust 1974) provides an added complication. The possible competitive role of autogenic chelates (siderochromes: Neilands 1973; Simpson & Neilands 1976) could be critical to the outcome of interspecific competition (see Murphy *et al.* 1976).

(*c*) *Buoyancy control*

The regulation of the buoyancy imparted by gas vacuolation occupies a central role in the ecology of planktonic blue-green algae (Reynolds & Walsby 1975). The representations of vertical distribution of *Microcystis* in the Blelham enclosures (parts (*a*) of figures 18–23) show

how populations frequently become concentrated in the upper reaches of the water columns. The effect was especially noticeable when microstratified conditions succeeded interludes of wind mixing which increased the depth of the epilimnion beyond the euphotic depth (*ca.* 2.3 × Secchi depth; see figure 2). Indeed, the incidence of surface blooms was consistently associated with these conditions. The responses were the predictable consequence of positive

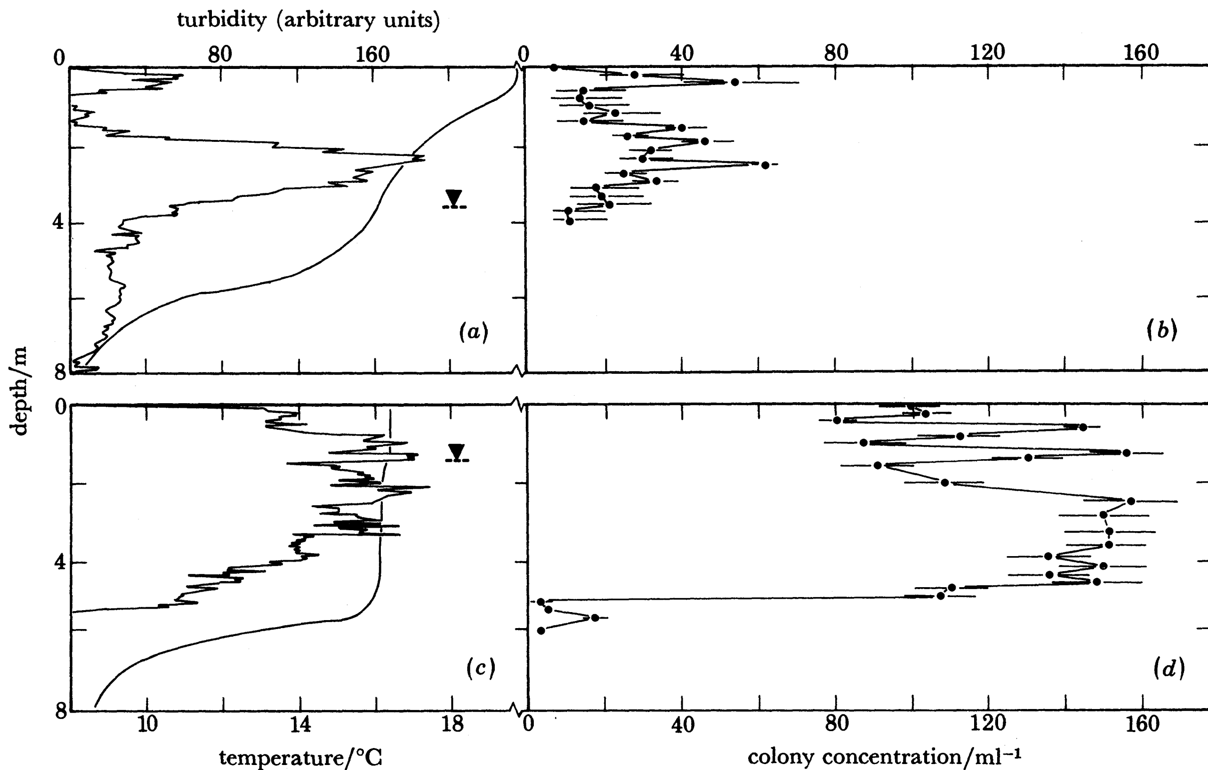


FIGURE 47. Horizontal beam transmissometric profiles (irregular line) compared with the temperature gradient (smoother line) and the depth of Secchi disc extinction (▼) on two occasions in 1978 (12 July above; 26 July below). (*b*, *d*) Colony counts (± 95 % confidence limits of count) in corresponding close-interval samples.

buoyancy. Equally, the downward migrations of the vertical modes of pre-maximal populations in enclosures A (June 1977) and B (June 1976, July 1977; see also Reynolds 1978*c*) when Secchi depth exceeded epilimnetic depth were attributable to negative buoyancy. The approximately neutral or slight positive buoyancy of the algae at most other times is symptomatic of effective control over gas vacuolation (cf.: Reynolds 1973; Konopka *et al.* 1978).

On two occasions in July 1978, the vertical profile of phytoplankton distribution in enclosure B was monitored directly by horizontal beam transmissometry (cf. Jones 1977; Harris *et al.* 1979). Since the phytoplankton was then dominated by *Microcystis* (95–98 % of biomass), the profiles represent the *in situ* distribution of *Microcystis* colonies. Enumeration of colonies in close (0.2–0.4 m) interval samples over the top 4 to 6 m showed a close similarity (figure 47). In particular, the profiles confirm the tendency for the alga to be distributed within the epilimnion (*ca.* 5.0 m on 12 July; 5.2 m on 26 July). On 12 July the weather was bright and windless; the water column was stratified to within a few centimetres of the surface; the Secchi disc reading was 3.5 m. The *Microcystis* was coarsely stratified at about 2.5 m, that is at about one-third of

the depth of the euphotic zone. The algal stratification is the consequence of regulated buoyant movement; that it is manifest at all is dependent upon the focus of buoyant movement lying within a stably stratified layer (cf. Reynolds 1978*c*). Of particular interest, however, was the evident depletion of colonies in the immediate subsurface layer; this may have been the result of direct surface avoidance.

The second profile (26 July) was obtained during a spell of cooler, disturbed weather, during which the epilimnion was wind-mixed to a depth of *ca.* 5 m. On this occasion isothermal mixing extended to a depth of 0.8 m. The *Microcystis* was distributed evenly between 1.5 and 4 m. Below 4 m, the turbidity and concentration of colonies declined to zero; the same was broadly true for the water above 1.5 m, but distribution was less even. The Secchi depth was 1.5 m. This pattern suggests that the majority of the population was approximately neutrally buoyant, as might have been expected on theoretical grounds (Reynolds & Walsby 1975), with some upward migration by positively buoyant colonies at the bottom of the layer (that is at or below the euphotic boundary) and some surface avoidance by negatively buoyant colonies.

These evidently rapid buoyant responses confirm previous experimental observations made on natural populations (see, for example: Ganf 1974*b*; Konopka *et al.* 1978). They also show that the density must be delicately balanced; this suggests that the turgor control mechanism (Dinsdale & Walsby 1972) is normally operative.

A similar conclusion may be drawn from a series of *in vitro* tests, carried out in 1976 on suspensions of natural *Microcystis* from enclosure B. The suspensions were prepared from fresh net collections which were then dark-incubated at 18–20 °C for 20–24 h. At the end of the period, the colonies that had floated to the surface were decanted off into unstoppered McCartney bottles, topped up if necessary with filtered lake water at the same temperature, and the algae were resuspended by shaking. The bottles were then exposed to daylight in the window on the shaded side of the building (light intensities reaching the bottles were not measured but may have been in the range 20–50 klx, or 250–630 $\mu E\ m^{-2}\ s^{-1}$). The distribution of colonies with bottles after 4 h was assessed by carefully pipetting off the top, middle and bottom thirds of the contents (cf. Walsby & Klemer 1974) and enumerating them separately by the iodine-sedimentation technique. The distributions, as percentages of the mean concentration of the whole bottle, are represented in figure 48. Substantial reduction in buoyancy relative to the initial condition could be implied if more than 100% of the alga failed to return to the uppermost layer; the presence of less than 33% (the fraction theoretically present in each layer at the start of the daylight exposure) would remain in the uppermost layer if the population became, on average, negatively buoyant. Rapid buoyancy reversal was obtained in the late June–early July experiments, and again in early September. The mixed depth/Secchi depth ratio (figure 2) at these times was conducive to maintenance of approximately neutral buoyancy: the absolute change in vacuolation required to effect dark flotation or sinking in the light would have been relatively small. The algae collected when the epilimnion was more turbid (mixed depth $\gg$ Secchi depth) were initially more buoyant and failed to overcome their buoyancy sufficiently to sink completely within 4 h, but some reduction in buoyancy was evident in most of the experiments. The rapidity of these responses is dependent upon a commensurate loss of gas vacuoles which is most likely to be mediated by turgor collapse.

(*d*) *Allelopathy*

The secretion by blue-green algae of metabolites inhibitory to the growth of potential competitors has often been advanced to explain the dominance and succession of blue-green algae in natural phytoplankton assemblages as well as in laboratory experiments (see, for example: Harder 1917; Le Fevre *et al.* 1952; Rice 1954; Proctor 1957; Hughes *et al.* 1958; Bishop *et al.* 1959; Vance 1965). The existence of dialysable substances that are inhibitory or fatal ('allelopathic' (Keating 1977)) to the growth of other algal species in culture (allelopathy, or antibiosis) has been demonstrated in several recent studies (Keating 1977; Lam & Silvester 1979; Vincent & Silvester 1979).

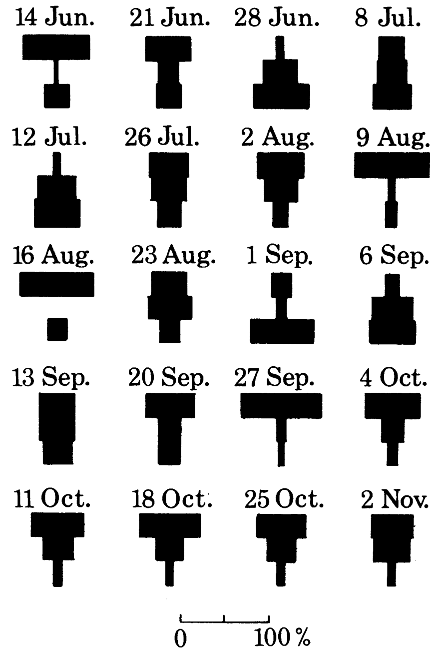


FIGURE 48. Buoyancy regulation in suspensions of *Microcystis* collected from enclosure B on the dates given in 1976: suspensions were prepared from surface aggregation that formed during 20–24 h dark incubation, and which were then redispersed in fresh medium (filtered lake water). The silhouettes represent the vertical apportionment of colonies after 4 h of standing in room lighting (> 20 klx, but no direct sunshine) between the top, middle and bottom thirds of a McCartney bottle.

A striking feature of the large *Microcystis* populations that developed in enclosure B in 1976, 1978 and 1979 and in Rostherne Mere in 1973 and 1977 (Reynolds 1978*b*) was their 'purity': the crops were virtual monocultures. This could be accepted as a predictable, if extreme, outcome of competitive exclusion by the alga best adapted to the contemporary conditions. Nevertheless, we applied a simple toxicity test to investigate possible allelopathic effects.

Water was collected from enclosure B at the time (August 1978) of the *Microcystis* maximum; 1–5 ml portions of exponentially growing cultures (*Asterionella* L306, *Eudorina* L145, *Chlorella* L289 and *Ceratium* L258: for details see Freshwater Biological Association (1979)) were separately added to duplicate flasks each containing 100 ml of GF/C-filtered water. The flasks were incubated at 18 ± 1 °C under continuous illumination (*ca.* 4 klx, *ca.* 50 μE m^{-2} s^{-1}). The experiment was terminated after 11 days, when the 'cultures' were prepared for enumeration.

In fact, inspection of algae in the flasks adequately conveyed the results (presented in table 12). The experiment was repeated on samples collected from enclosure B on 22 September, except that the 'cultures' were inspected after 3 days. These results, which are included in table 12, entirely supported those of the first, save that *Eudorina* may have increased initially. *Asterionella* and *Eudorina* did not survive the experiments; *Chlorella* and *Ceratium* were apparently inhibited.

TABLE 12. THE RESPONSE OF CULTURED ALGAL STRAINS TO LIGHT INCUBATION ($50.5\ \mu E\ m^{-2}\ s^{-1}$ AT $18(\pm 1\ °C)$ IN GF/C-FILTERED LAKE WATER SUPPORTING LARGE *MICROCYSTIS* POPULATIONS

alga	test I (11 days)	test II (3 days)
Asterionella L 292	no increase: all cells dead	all cells dead
Eudorina L 145	no increase: all cells dead	increase in cells(?), but all dead
Chlorella L 289	no increase in intact cells	decline in live cells; loss from suspension
Ceratium L 258	no increase; cells active	no increase; cells active

In a further test, begun on 29 September 1978 (when the natural *Microcystis* population was in decline), fresh, GF/C-filtered water from enclosure B was used to dilute media (modified Chu of Lund *et al.* 1975) in which the test algae were already established and increasing exponentially. In this experiment, growth in all the cultures was maintained (see table 13), suggesting either that the 'toxic' effect had been removed, or that larger inocula are relatively insensitive to inhibitory substances. It did not eliminate the possibility that *Microcystis* inhibits the growth of potentially competing organisms.

10. AUTUMNAL POPULATION DECLINE

In the observed populations, the attainment of seasonal maxima was followed by periods of varying length (15–45 days) during which the concentration of suspended colonies remained roughly constant or declined slowly. Generally, cells remained gas-vacuolate but accumulated intracellular deposits of protein (structured granules), carbohydrate (glycogen) and phosphate (polyphosphate bodies). In several years this phase of 'stationary growth' was ended more or less abruptly by a rapid loss from suspension, which reduced the epilimnetic concentration by up to an order of magnitude within 7 days. The 1977 population in enclosure A, however, declined by a similar magnitude in not less than 30 days. The onset of the decline phase was also conspicuously variable between years and populations, occurring at any time between late August (B, 1977) and early December (B, 1976). Most commonly, decline was initiated during September or October; frequently, though not always, it coincided with increased epilimnetic circulation (cf. figures 1, 2 and table 14). Decline is not solely attributable to dilution in the increased volume of the epilimnion: even complete mixing of the 12 m water column would reduce the concentration of colonies in suspension only by a factor of about two. Surface bloom formation cannot account for the losses; unless algae are destroyed (for instance by photo-oxidation) at the surface, colonies are in any case retained within the experimental enclosures and not deposited on lee shores (cf. Reynolds 1971). Reynolds & Wiseman (1981) calculated that between 28 and 50% of the standing crop of *Microcystis* in enclosure A was destroyed by lysis and photo-oxidative death following a series of surface blooms in mid-September 1978 (see figure 2). Complementary changes in the benthic population, both in areal density (table 14)

and in the relative abundance of stati (parts (*d*) of figures 18–23) indicate that settling to the sediment is the most likely explanation for the loss of *Microcystis* colonies for suspension.

The mean intrinsic sinking rates that can be calculated according to equations developed by Reynolds & Wiseman (1981) to account for the mass transfer from a partially mixed water column to the sediment surface are within the range 0.99–3.82 m d^{-1} (equivalent to 11.4–

TABLE 13. THE EFFECT OF DILUTING ACTIVELY GROWING CULTURES (IN MODIFIED Chu 10 SOLUTION†) WITH VARYING PROPORTIONS OF GF/C-FILTERED LAKE WATER SUPPORTING *MICROCYSTIS* POPULATIONS

ratio of GF/C filtrate: medium	culture	growth rate (number of divisions per day)
25:75	*Fragilaria* L 299	0.98
25:75	*Eudorina* L 145	0.97
25:75	*Chlorella* L 289	1.34
50:50	*Asterionella* L 292	1.87
25:75	*Asterionella* L 292	1.74
10:90	*Asterionella* L 292	1.66
5:95	*Asterionella* L 292	1.67

† Modifications in Lund *et al.* (1975).

TABLE 14. LOSSES FROM SUSPENSION OF COLONIES IN EXPERIMENTAL ENCLOSURES. RECRUITMENT TO THE SEDIMENT SURFACE AND CALCULATION OF MEAN INTRINSIC SINKING RATE (V')†

population	period	colony concentration eliminated from suspensions (±95% c.l.‡) cm^{-2}	k_s/d^{-1}	colony concentration recruited to sediment (±95% c.l.) cm^{-2}	epilimnetic depths§/m		mean sinking rate/(m d^{-1})
					at start	at end	
B, 1976	14–28 Nov.	3960 ± 1437	0.210	10047 ± 8138	11.9	11.8	2.24
A, 1977	18–25 Oct.	2530 ± 1605	0.361	1999 ± 1619	11.1	12.2	3.70
B, 1977	5–9 Sep.	1110 ± 215	0.370	486 ± 394	5.9	7.5	2.32
A, 1978	25 Sep.– 9 Oct.	2297 ± 531	0.323	1141 ± 924	8.9	10.8	2.98
B, 1978	25 Sep.– 9 Oct.	6685 ± 1193	0.436	5750 ± 4657	9.3	10.8	3.82
B, 1979	1–15 Oct.	8975 ± 4003	0.138	9227 ± 7473	7.2	7.7	0.99

† Calculated from the equation derived by Reynolds & Wiseman (1981):

$$V' = Z_e\,(1 - 1/e^{k_s}),$$

where e is the base of natural logarithms, k_s is natural logarithm of the rate of elimination from suspension and Z_e is the depth of the epilimnion or full isothermal circulation.

‡ Abbreviation c.l. stands for confidence limits.

§ Or full isothermal depth when column is not stratified.

44.2 μm s^{-1}). This is not excessive for *Microcystis* colonies of diameter greater than 200 μm and normal cell content (see table 5); faster sinking rates (750–1820 μm s^{-1}) were measured by Ganf (1974*b*); indeed, it is not even necessary to propose that all gas vacuoles are first collapsed.

Nevertheless, there is more or less abrupt change in buoyancy which, since colony size does not appear to alter, is presumably mediated by increase in mean colony density. This might be achieved in two independent ways.

The first might be brought about by a reduction in gas vacuolation. However, neither of the buoyancy-regulating mechanisms can be invoked: because the net growth is virtually static, growth dilution of gas vacuoles (cf. Walsby 1970) cannot apply; because of deteriorating photic conditions (shortening day, increased turbidity and turbulent mixing) the turgor-collapse mechanism (Dinsdale & Walsby 1972) is unlikely to affect 90% of the standing crop simul-

TABLE 15. ANTHRONE-REACTIVE CARBOHYDRATE CONTENT AND DENSITY OF *MICROCYSTIS* COLONIES IN ENCLOSURE B, 1978, AND THE CALCULATED DENSITY OF THE CELLS (ρ'_y), FOR A GIVEN PROPORTION OF COLONY VOLUME OCCUPIED BY CELLS (y) AND WITH THE ASSUMPTION THAT ρ'_x = 998.8 kg m^{-3}

date	17 Jul.	31 Jul.	28 Aug.		18 Sep.	
treatment (see text)	L	L	L	D	L	D
mean carbohydrate content (fraction of dry mass)	0.274	0.214	0.221	0.199	0.083	0.071
colony density	1001.96	1001.92	1001.30	1000.94	1000.71	1000.53
y	0.158	0.214	0.170	0.170	0.265	0.265
ρ'_y/(kg m^{-3})	1018.80	1013.38	1013.51	1011.39	1006.01	1005.33

taneously. However, the rapid deepening of the epilimnetic circulation by 1.5–2.0 m would expose colonies to additional hydrostatic pressure of up to 0.2 atm (*ca.* 20 kPa), which could be sufficient to reach the critical collapse pressure of most of the vesicles present (cf. Walsby 1971).

Differences in behaviour among the various populations suggest that an additional component determines susceptibility of vesicles to collapse which may be occasionally (for instance in 1976, when the water column was already well mixed) the cause of buoyancy loss. The possibility that the gas vesicle membranes are first weakened by proteolytic enzymes has been advanced on a number of occasions (see, for example: Walsby 1971; Reynolds 1973), but has not been substantiated. If valid, the process must be limited either in its physical action, or in its temporal effectiveness, because gas vacuoles are neither eliminated completely before the cells sink, nor insidiously removed from the viable cells once they have sedimented. Evidently, few vesicles are collapsed beyond those necessary to reduce buoyancy just below neutrality. Ultrastructurally, the gas vacuoles of colonies prepared for electron microscope examination in autumn showed no greater propensity towards structural disorganization (cf. figure 39) than at other times.

The second way in which colony density could be increased is through the accumulation of storage products having high relative densities: proteins (*ca.* 1.3), carbohydrates (*ca.* 1.5) and polyphosphate bodies (*ca.* 2.5). Gibson (1978) has shown that the anthrone-reactive carbohydrate content of blue-green algae fluctuates seasonally, diurnally and with their vertical position in the light gradient, within an order of magnitude (*ca.* 10–100 mg of carbohydrate per milligram of chlorophyll *a*). Attendant density changes potentially affect buoyancy and its regulation.

Accordingly, we present (table 15) some calculations of cell density derived from measured sinking rates of *Microcystis* colonies in suspensions whose anthrone-reactive carbohydrate content was also determined retrospectively (see §2). Suspensions were incubated for 24 h in darkness (D) or light (L: 1.8 klx, or 22.7 μE m^{-2} s^{-1}). Density was calculated from the mean

sinking velocity of pressure-treated colonies at 20 °C; cell density was calculated from equation (1), with the assumption that $\rho'_x = 998.8\ \text{kg m}^{-3}$ (i.e. $\rho + 0.7\ \text{kg m}^{-3}$); the carbohydrate content was analysed at the F.B.I.U. Laboratories, Northern Ireland (see §2). On a sample of six determinations, calculated cell density was correlated with carbohydrate content (figure 49: $r = 0.982$, $p < 0.01$). Although the calculated densities are unlikely to be accurate, the

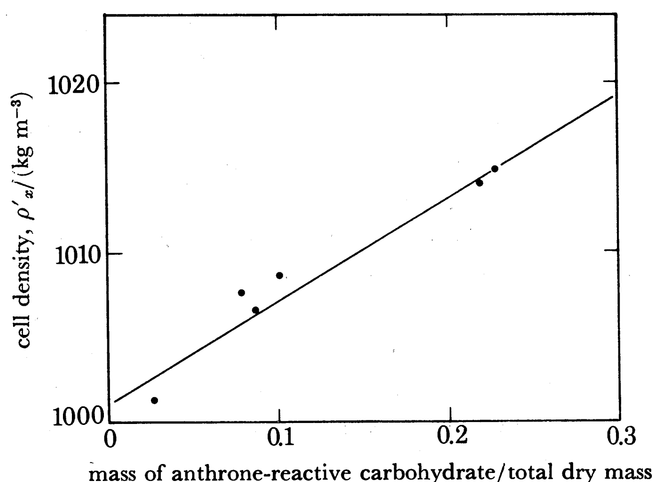


FIGURE 49. The density of *Microcystis* cells calculated from ρ', determined by Stokes's equation, and the contemporaneous mucilage and cell fractions (table 15) and with the assumption that $\rho'_x = 998.8\ \text{kg m}^{-3}$, plotted against the anthrone-reactive carbohydrate content of dried samples. The coefficient of correlation is +0.982, which is significant at the 0.1 % level.

assumptive errors are likely to be similar for each pair of variables. Thus, we can conclude that a trebling of the anthrone-reactive carbohydrate reserve may lead to a doubling of the excess density of cells over water; then satisfaction of the neutral buoyancy condition can be met either by the provision of extra gas vacuolation, or by 'dilution' of the cells in an increased relative volume of mucilage. Alternatively, the colony loses buoyancy and accumulated food reserves may well increase the potential sinking rate of the alga.

Accumulation of storage products is progressive and does not account for the sudden loss of buoyancy. If the algae first weaken their vacuoles, and hydrostatic pressure collapses a sufficient proportion thereof to render colonies negatively buoyant, the added ballast of storage products can only add to their intrinsic sinking rates. The principal function of the reserves, however, is presumably to allow the cells to perennate on the bottom sediments over the winter period. Thus the mass migration of negatively buoyant *Microcystis* colonies is an essential prelude to the overwintering phase and completion of the annual cycle.

11. Biotic factors regulating the abundance of *Microcystis*

In this section we give brief attention to factors that may limit the potential growth of *Microcystis*. Direct feeding on *Microcystis* colonies by Crustacea (Gras *et al.* 1971; Schindler 1971; Burgis *et al.* 1973; Tevlin & Burgis 1979; Ferguson *et al.* 1981), by protozoa (Goulder 1974; B. J. Finlay personal communication) and by certain fish (see, for example, Moriarty *et al.* 1973), parasitic attack by chytrid fungi (Canter 1972) and by bacteria (Daft & Stewart 1971), and lysis by 'cyanophages' (Safferman & Morris 1963; Luftig & Haselkorn 1967), all

constitute potential checks on the growth and standing crop of *Microcystis* populations. During the present observations, no fungal development was ever observed on any *Microcystis* colony examined. Neither were we aware of any changes symptomatic of attack by viral pathogens. Infestation of the mucilage by rod-shaped bacteria was frequently observed on the periphery of planktonic colonies in stationary or decline phases, and of benthic colonies throughout the year. Algae, notably *Chlamydomonas gloeophila* Skuja and *Phormidium mucicola* Huber-Pestalozzi et Naumann, were also commonly present in the plankton at about the same times; here, the relationship is presumably phoretic and not parasitic, apparently without harmful effect upon the host. This may apply equally to many of the bacteria.

Between September and November 1976 some 10% of the colonies collected from enclosure B were infected by one or, rarely, more amoeboid protozoa. 'Host' colonies were otherwise 'normal' in appearance and *Microcystis* cells were certainly identified within the protoplasts of only 2% of amoebae examined by high-power light microscopy. The main food of the animal was not identified, though the diet may have included bacteria and the mucilage itself. However, in samples collected during October and left to stand in open beakers in the laboratory, *Microcystis* colonies were observed to turn quite brown in colour and settle out. Microscopic examination showed the colour change to be due to the disappearance of vegetative cells and their replacement by a brown, ferruginous deposit within the mucilage. Amoebae were observed to be numerous and active within the colonies but, remarkably, still to be lacking recognizable *Microcystis* cells internally. Of course, the deterioration of the alga may have been independent of the presence of the amoebae. However, when a late-exponential culture of *Microcystis* L155 was inoculated with amoeba-infected colonies it too deteriorated rapidly, showing identical symptoms. If the amoebae do feed on *Microcystis* cells, digestion of cell walls must be either extremely rapid or external.

Colonies taken from the plankton of enclosure B after late November were apparently free of amoebae, though they continued to be observed in benthic colonies. Although the destruction of *Microcystis* colonies was never so extensive as in the laboratory experiments and the presence of amoebae in the other years was rarely recorded, amoeboid infestations may pose a threat to the successful perennation of *Microcystis*.

Direct zooplankton phagotrophy may be limited by selectivity among filter-feeders. Blue-green algae may be unpalatable to animals but feeding in *Daphnia* spp. is more likely to be depressed by blockage of the food groove by *Microcystis* colonies and their subsequent rejection (cf. Burns 1968*a*). Standing populations of most zooplankton species were reduced in enclosure B when *Microcystis* was overwhelmingly dominant: only *Chydorus sphaericus* increased in this period (see Ferguson *et al.* 1981). *Microcystis* was ingested by *Daphnia hyalina* during July 1978, when the algal population was dominated by st. Ib colonies, but not during August, when st. III, Va and VI colonies were prevalent. These observations support the theory of selection of food by size; indeed, colonies exceeding 20–60 μm (including mucilage) in two or more perpendicular planes probably exceed the upper limit that can be ingested by the largest *D. hyalina* individuals as well as by many other daphniids (Burns 1968*b*; Gliwicz 1970; Gras *et al.* 1971).

Some larger species of benthic ciliates (e.g. *Loxodes magnus* and *Spirostomum* spp.) that inhabit deep water sediments under aerated water columns are known to feed on live algae, including small *Microcystis* colonies or fragments of larger ones (Goulder 1974; B. J. Finlay, personal communication). In our studies we recorded ciliates only occasionally in Jenkin cores, at concentrations generally much less than 100 cm^{-2}. Since ciliates avoid anoxic water, it is

possible that the low redox potential of eutrophic sediments, even in winter, confers relative immunity from ciliate grazing upon *Microcystis* colonies, while large colony size would also contribute to their survival.

12. Conclusions: the growth and survival strategy of *Microcystis*

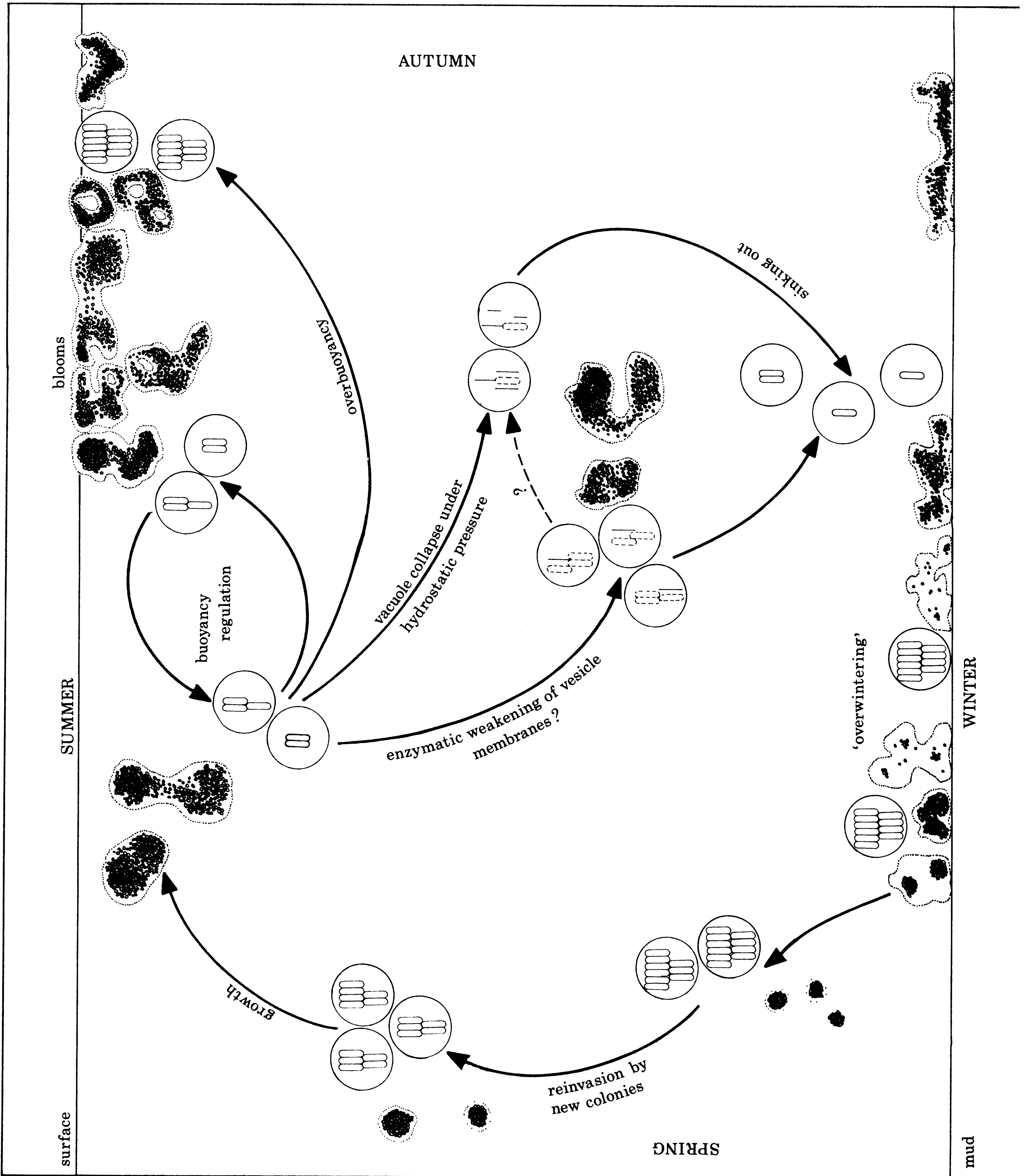
Collectively, our observations and experiments on *Microcystis aeruginosa* populations in the Blelham enclosures illustrate a series of morphological and behavioural changes extending beyond the period of abundance in the water column. These are summarized in figure 50. The main growth phase, however, does occur in the plankton. Growth is apparently photoautotrophic, occurring at the expense of inorganic carbon and simple nutrients. As with most planktonic algae, both the rate of increase and the eventual size of the standing crop are potentially limited by nutrient availability. Should nutrient limitation not occur, populations can increase to the limit imposed by the underwater light climate. Like planktonic algae, population dynamics are affected by loss processes, i.e. through grazing, sinking, death and decomposition (cf. Kalff & Knoechel 1978). Recent work on the Blelham populations (Reynolds & Wiseman 1981; Reynolds *et al.* 1981 and this study) has shown that the losses sustained by *Microcystis* during its planktonic phase are normally small. Only the smallest colonies (less than 60 μm in diameter) are grazed. Colonies regulate their buoyancy and, potentially, their vertical position in the water column, accommodating to fluctuations in turbulent mixing and avoiding prolonged exposure to supraoptimal irradiance and isolation in aphotic layers. Even in post-maximal populations, the elaborated biomass may persist in the plankton for weeks or months. Thus the combination of relatively slow rates of growth with low rates of loss may still be adequate to exceed the net rates of increase of potentially competing algae (cf. Reynolds *et al.* 1981). Allelopathy may contribute to this effect.

Mass migrations to the sediments do occur but do not necessarily constitute a loss process, functioning rather as an important transition stage in the annual cycle. The variability of the responses suggests biological mediation (see §10). Although the colonies retain their vegetative form, the cells become physiologically dormant, surviving on stored foods. In this way, a potentially viable stock is maintained over winter and provides a basis for growth in the following year. Survival is apparently enhanced if the sediments are anoxic.

Colonies re-infecting the water column the next spring are not necessarily the same ones that settled the previous autumn but are mostly new colonies that have arisen within the mucilage of the parent colony following a short phase of cell division. The renewed physiological activity is stimulated by specific environmental conditions, of which low-intensity irradiance, falling oxygen concentration and lowered redox potential of the bottom water appear to be essential. The abundance of organic solutes and the deficiency of oxygen in the immediate cellular environment might favour alternatives to oxygenic photosynthesis as a basis for growth. In spite of the assertion that *Microcystis* is an obligate photoautotroph (Stanier *et al.* 1971), future work should establish whether photoheterotrophic growth or anoxygenic photosynthesis possibly sustains the early growth of benthic *Microcystis* populations.

The water column is thus re-infected by a small inoculum of cells; there seems to be no reversed migration of colonies on the scale of the autumnal sinking. Fortuitously or otherwise, the conditions of re-infection obtain coincidentally with the capacity of unmodified epilimnetic water to support the growth of *Microcystis* in laboratory bioassays.

We have no reason to believe that the behaviour of *Microcystis aeruginosa* populations in the


AUTUMN
SUMMER
WINTER
SPRING
surface
mud
blooms
overbuoyancy
buoyancy regulation
vacuole collapse under hydrostatic pressure
?
enzymatic weakening of vesicle membranes ?
sinking out
'overwintering'
reinvasion by new colonies
growth

Blelham enclosures is in any way exceptional. Our conclusions may therefore apply generally to *Microcystis* populations in temperate and subtropical eutrophic lakes. Much of the 'miscellaneous information' (Fogg 1969) that abounds in the literature concerning the ecology of *Microcystis* (for recent reviews see: Fogg 1969; Fogg *et al.* 1973; Philipose 1972; Carr & Whitton 1973; Whitton & Sinclair 1975; Reynolds & Walsby 1975) may be accommodated within the annual life cycle that we describe. The most detailed studies hitherto have been carried out in the Ukrainian S.S.R. (reviewed in Sirenko (1972) and in Sirenko & Gavrilenko (1978)); our observations entirely support, and sometimes amplify, Sirenko's conclusions. In particular, we have been able to demonstrate the role of buoyancy and the methods of its regulation in the annual cycle, and to give circumstantial evidence for the considerable metabolic flexibility needed to alternate between the benthic and planktonic phases.

The synthesis is still incomplete and further researches are required. Nevertheless, it is possible to advance an explanation for the wide dispersal of *Microcystis* among, and its evident success in, eutrophic lakes. Its biology is tailored to the cycles of thermal and chemical stratification, especially with respect to seasonal anoxia of hypolimnia and deep-water sediments. Its ability to survive and perennate in such environments contributes to its persistence. It is therefore not surprising that *Microcystis* blooms should be so overtly associated with accelerated cultural eutrophication of our lakes.

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FIGURE 50. The annual cycle of *Microcystis aeruginosa*, showing the course of morphological variations, and the extent of gas vacuolation in healthy cells (in circles). The vertical height of the figure corresponds with the full water-column depth; the cycle, however, moves through time as well as space.

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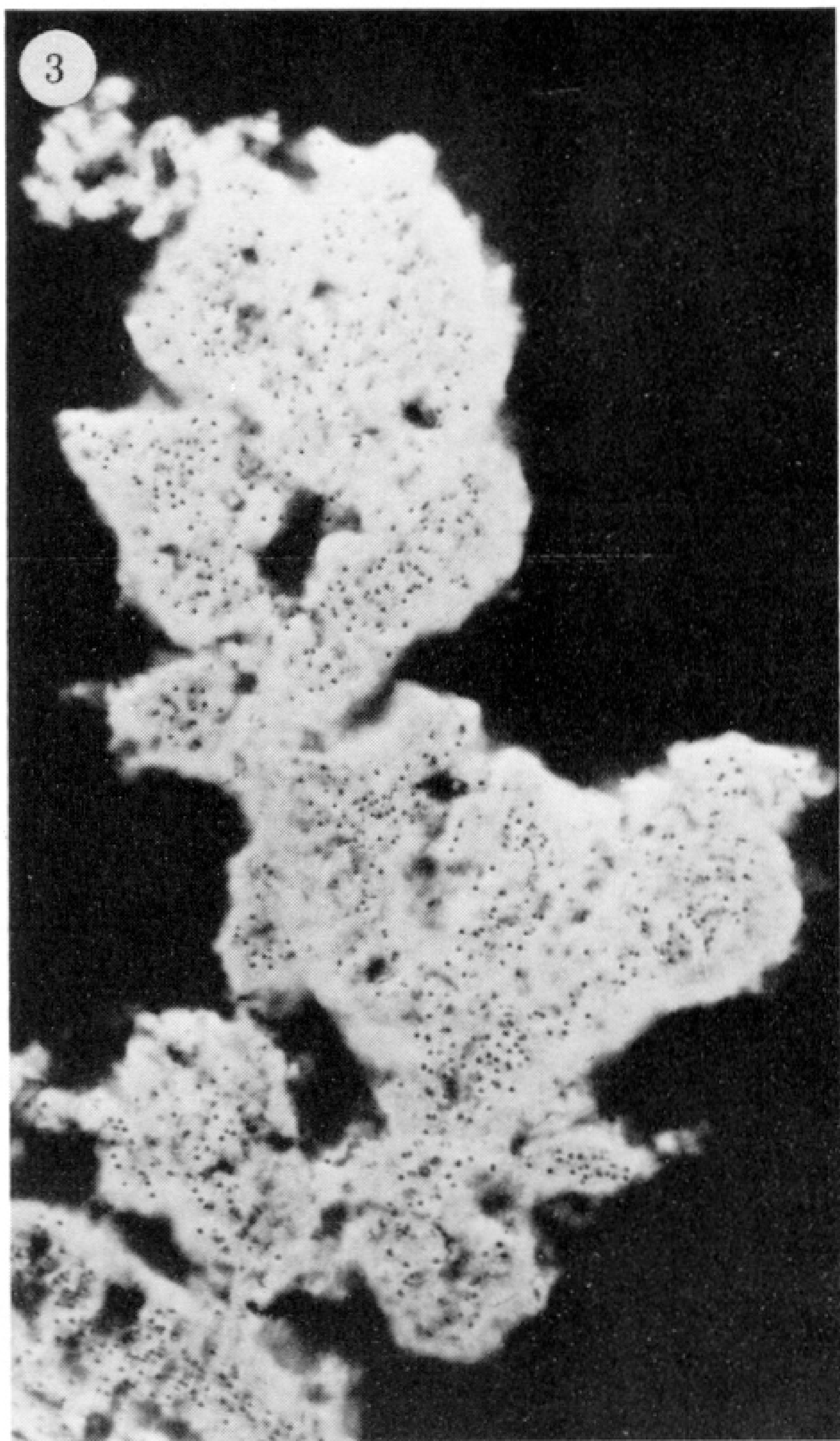
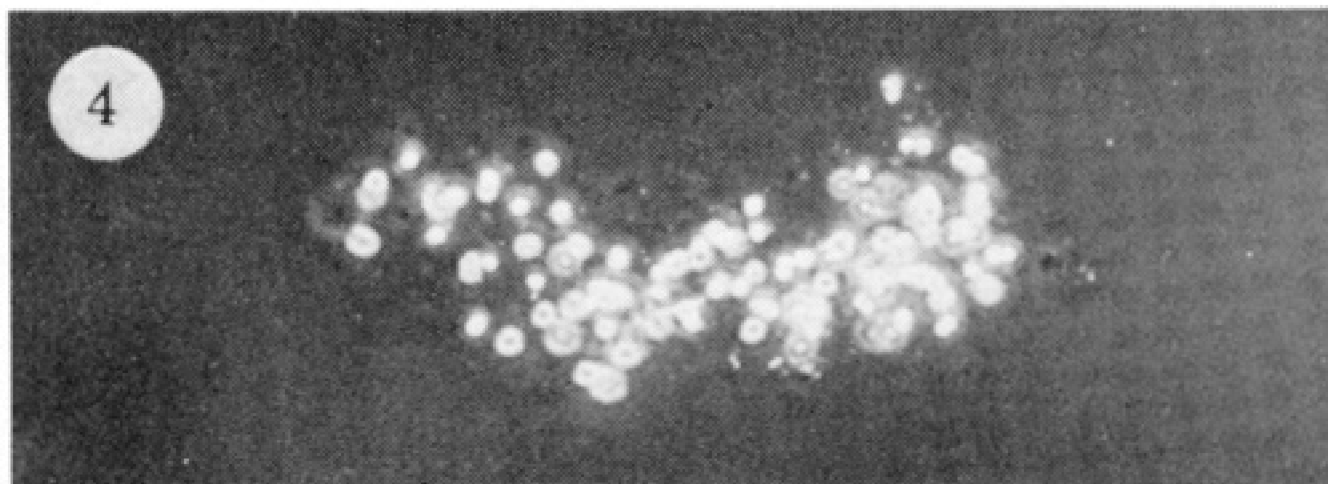
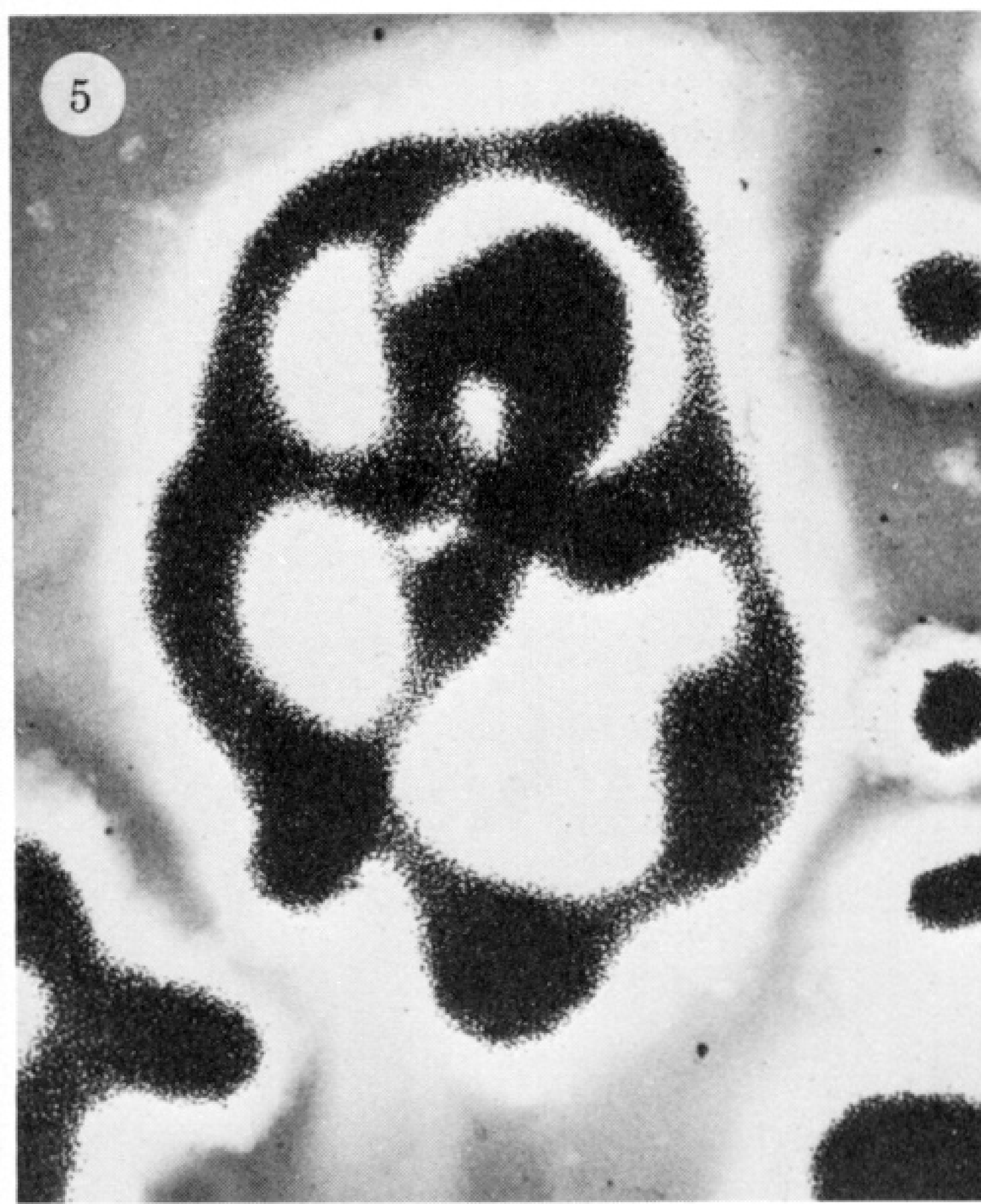
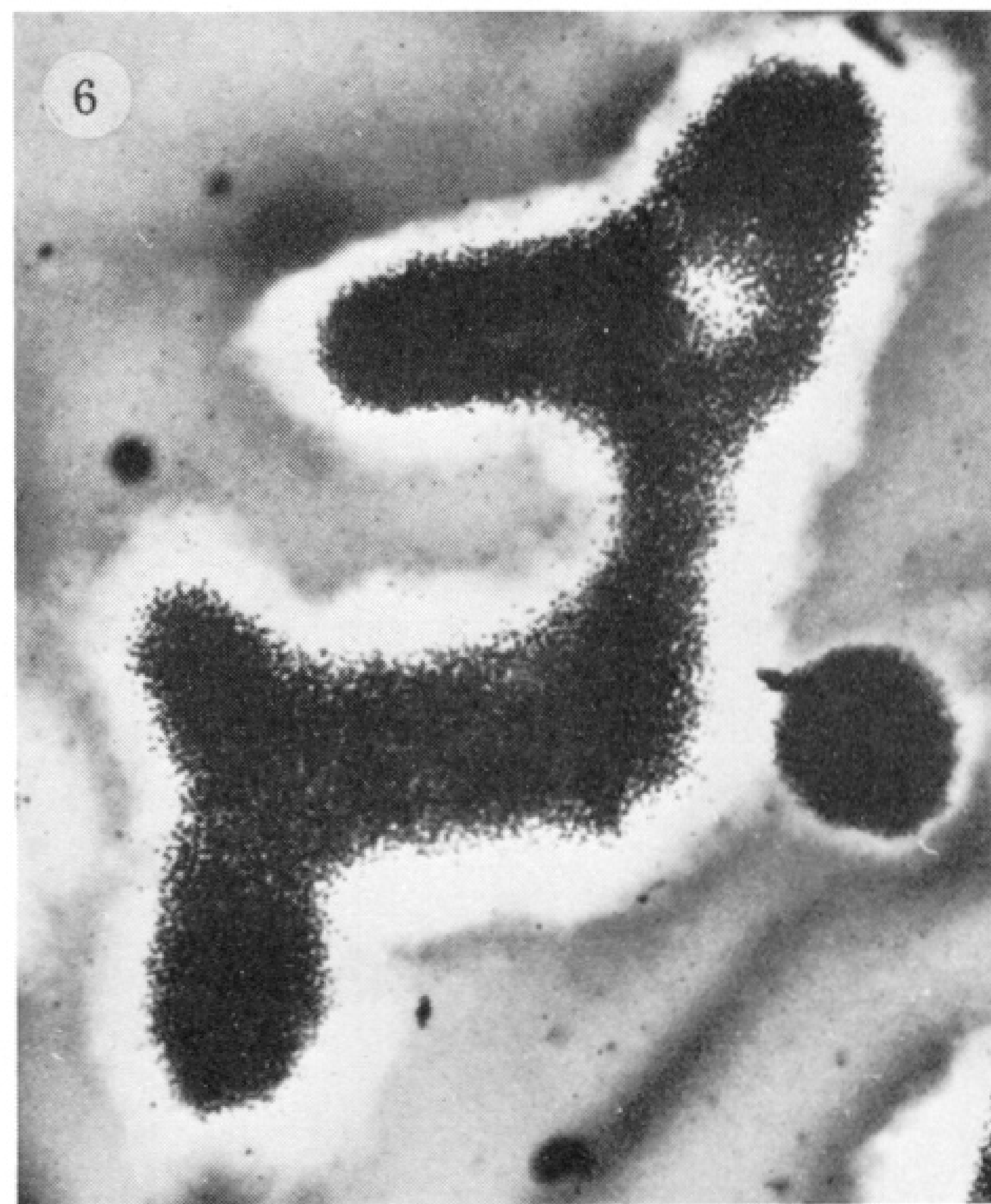
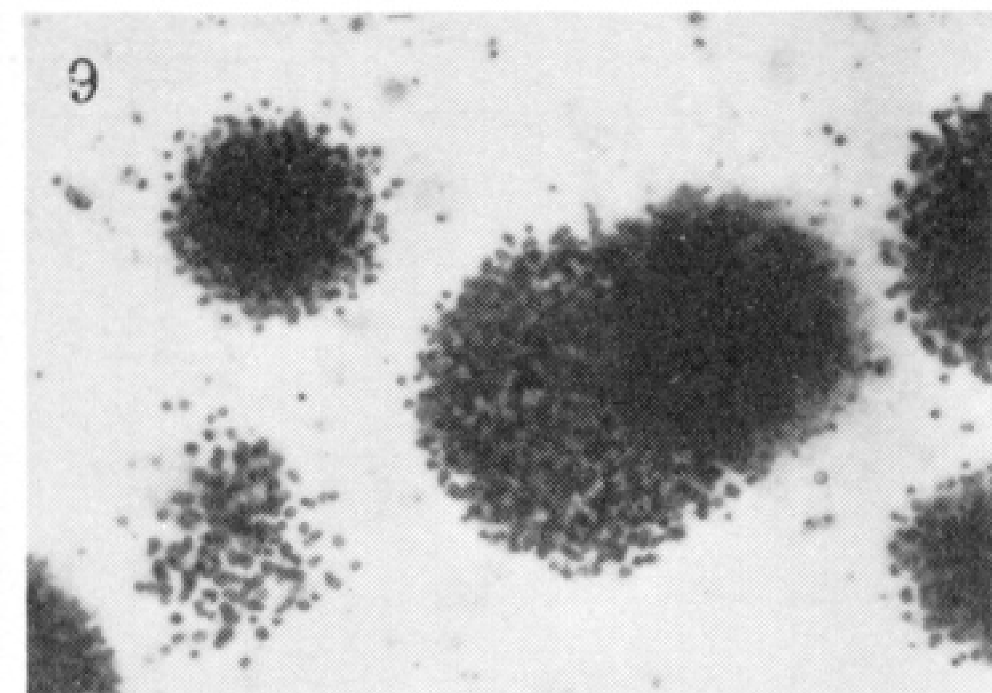
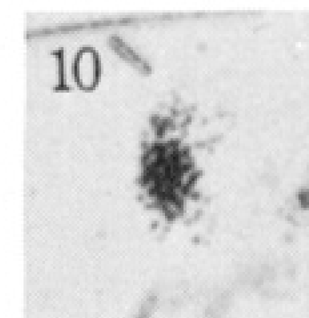
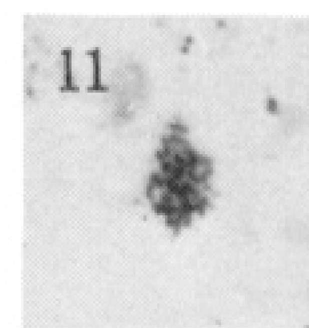
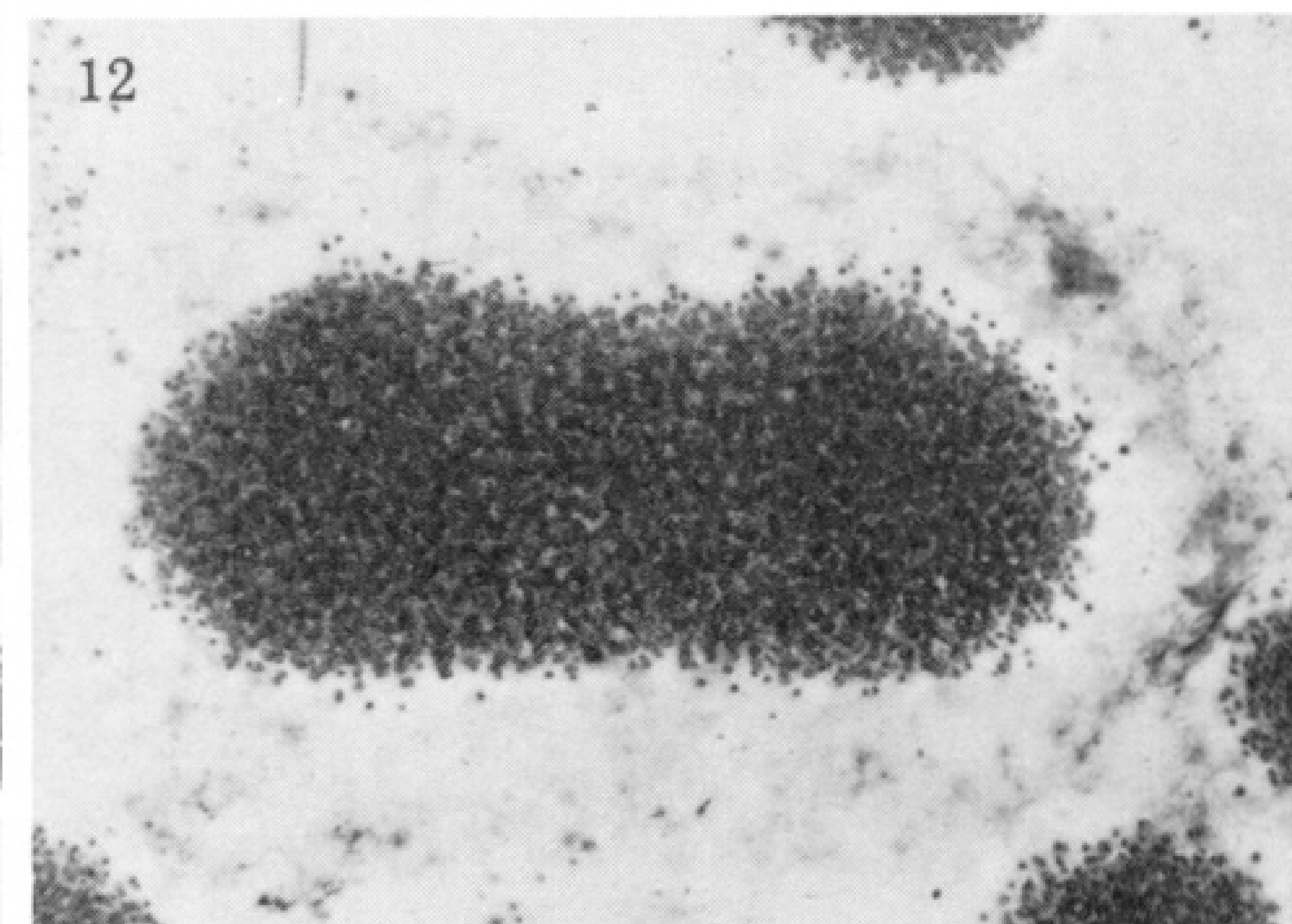
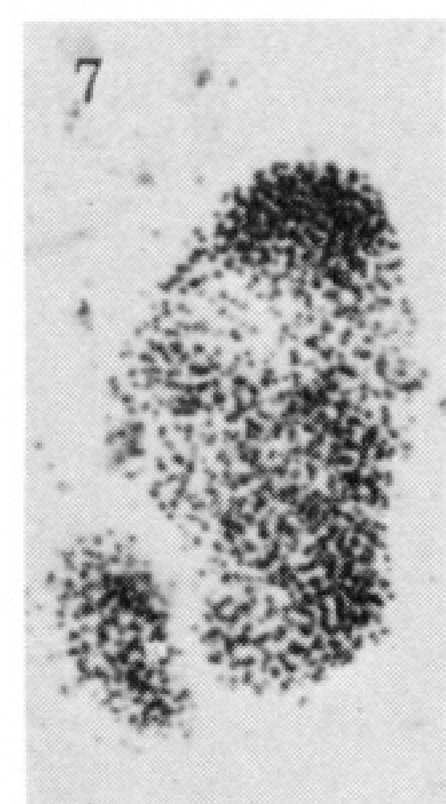
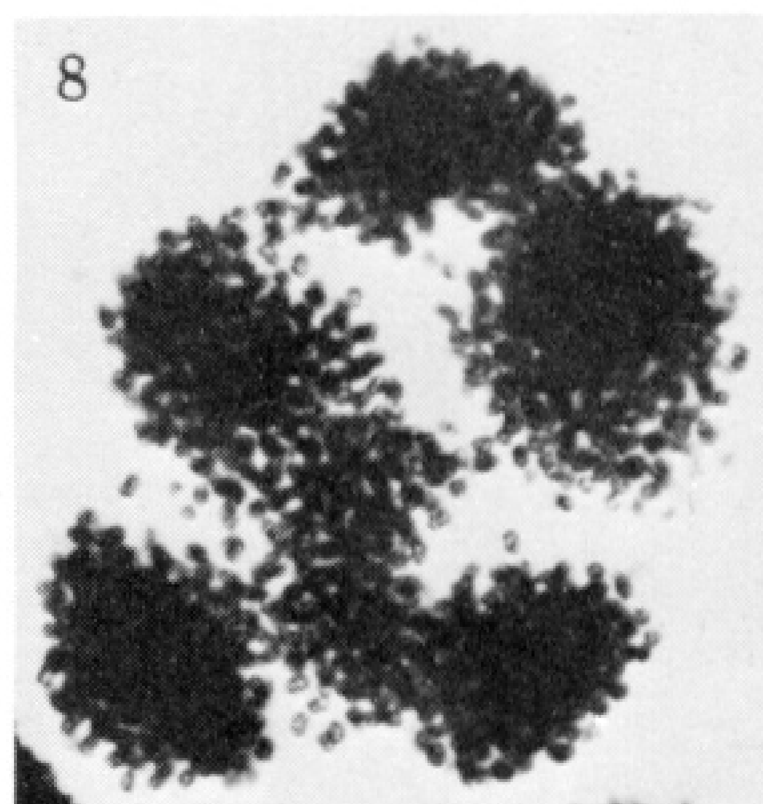
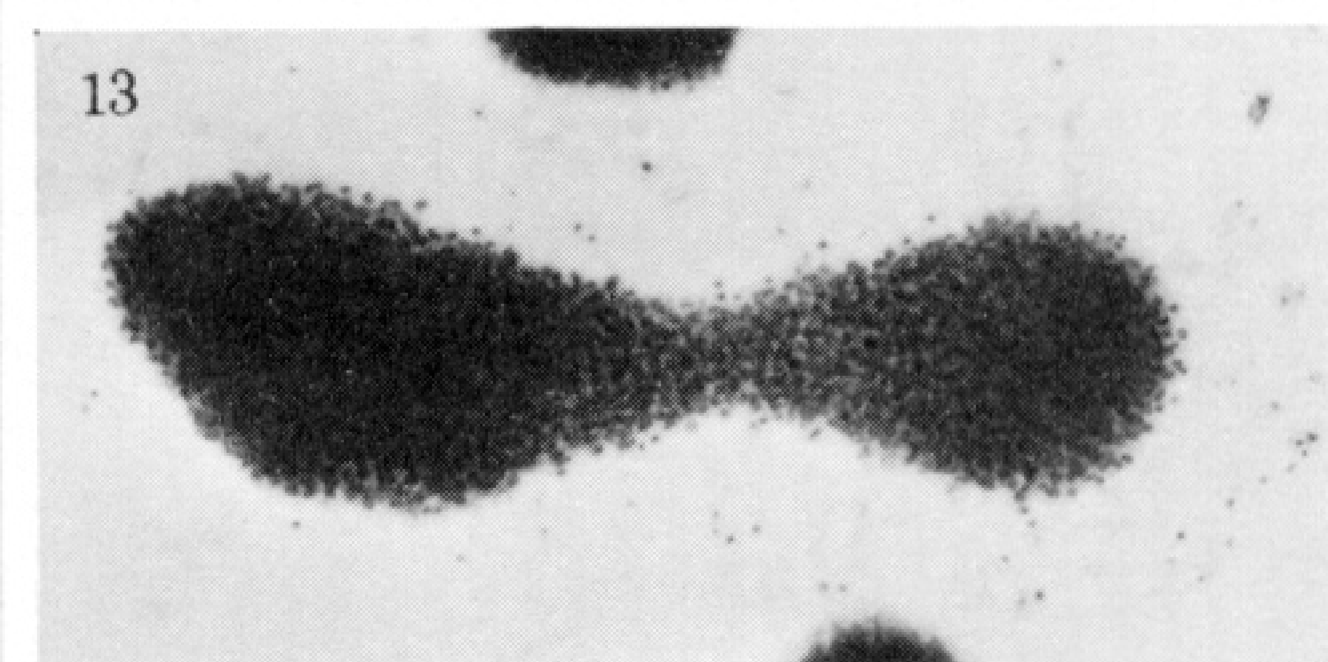
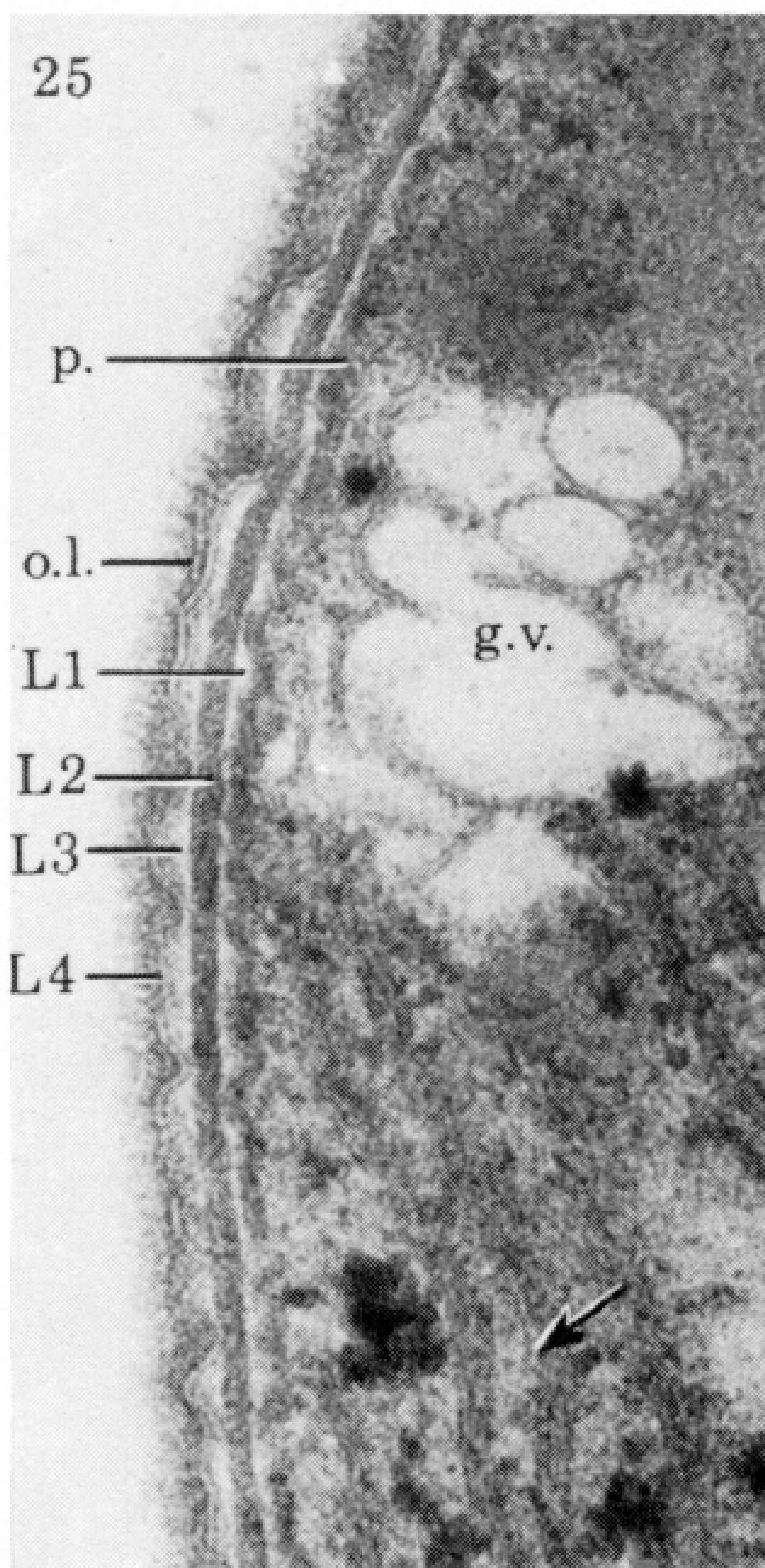
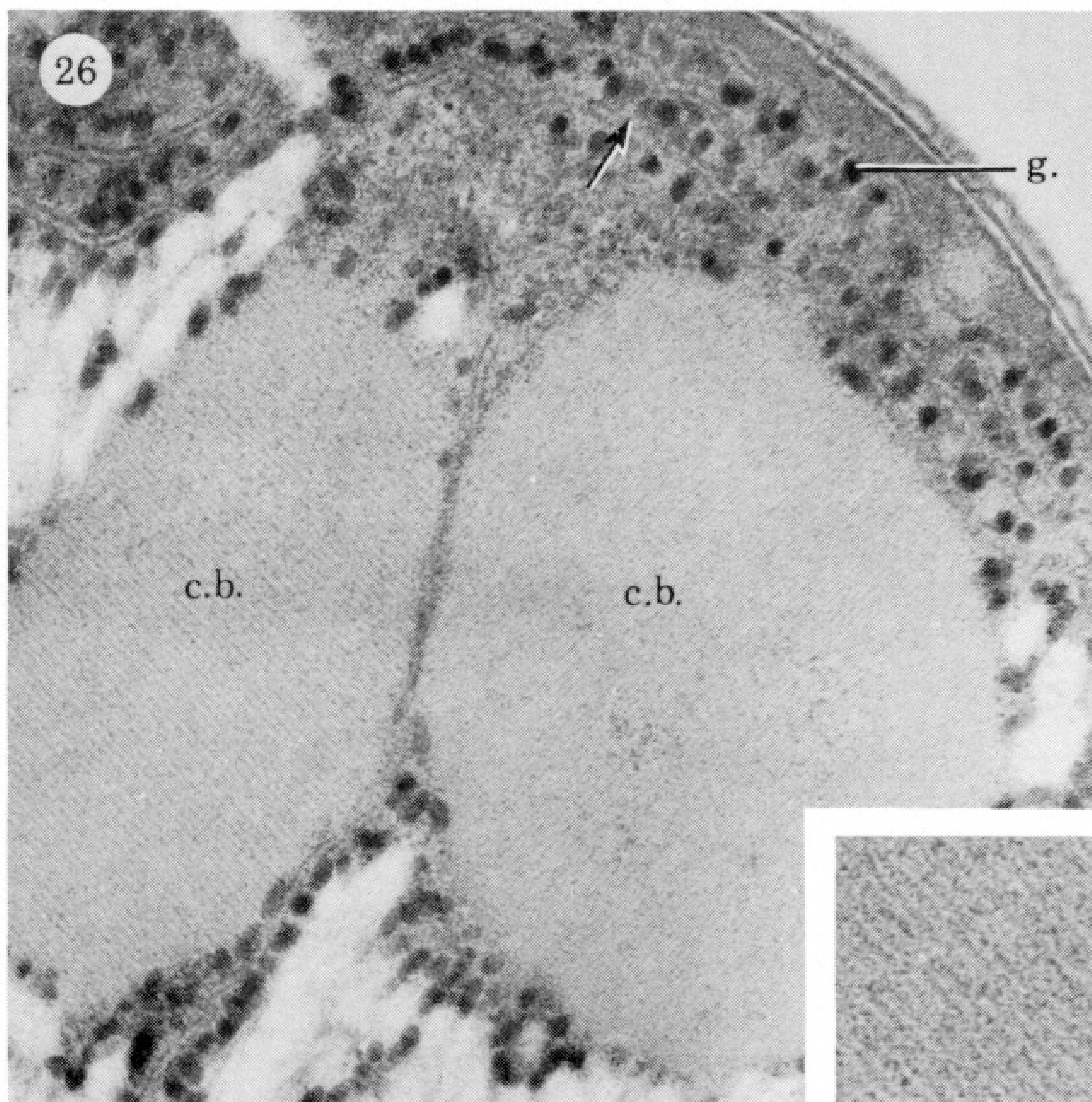
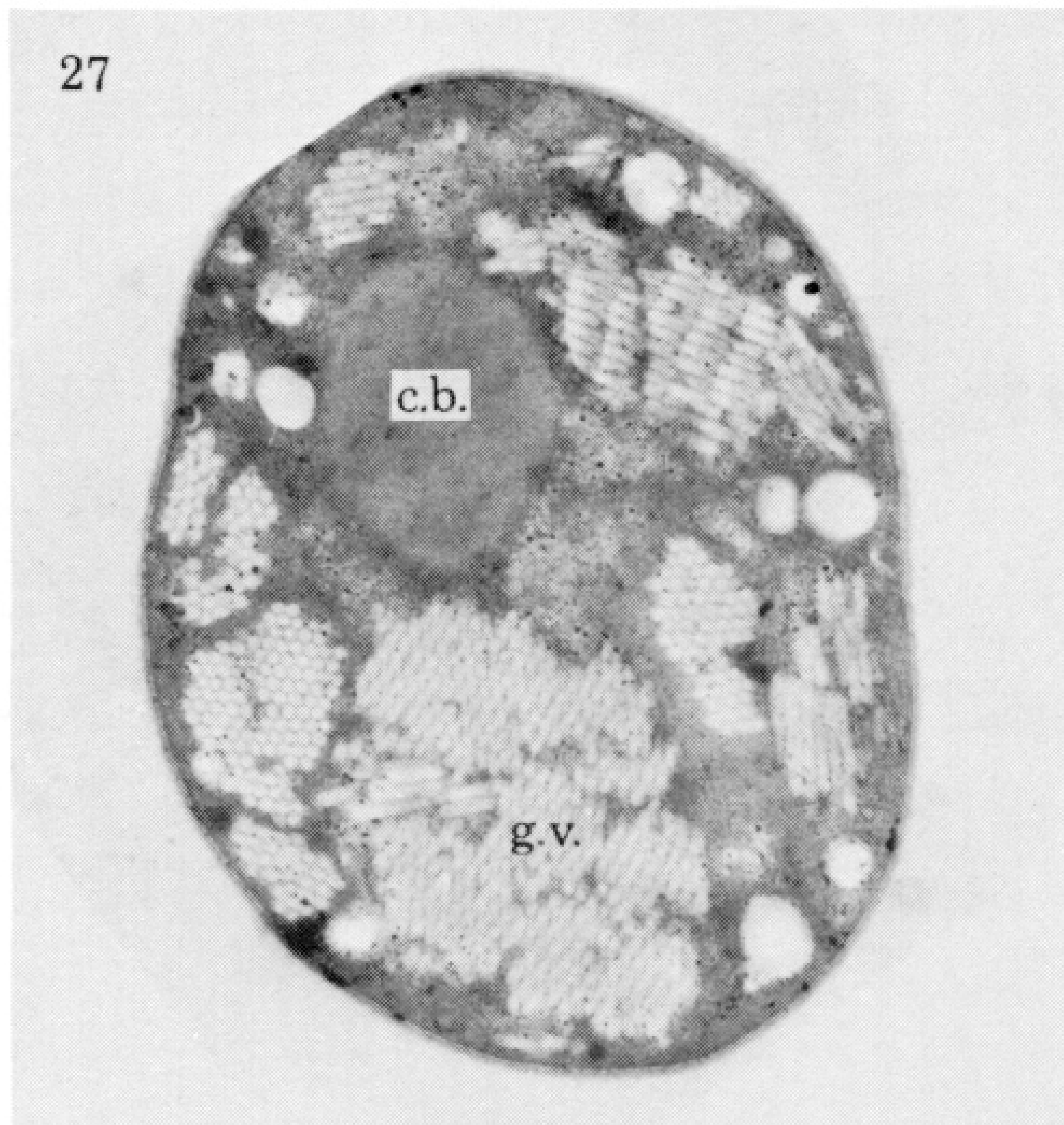












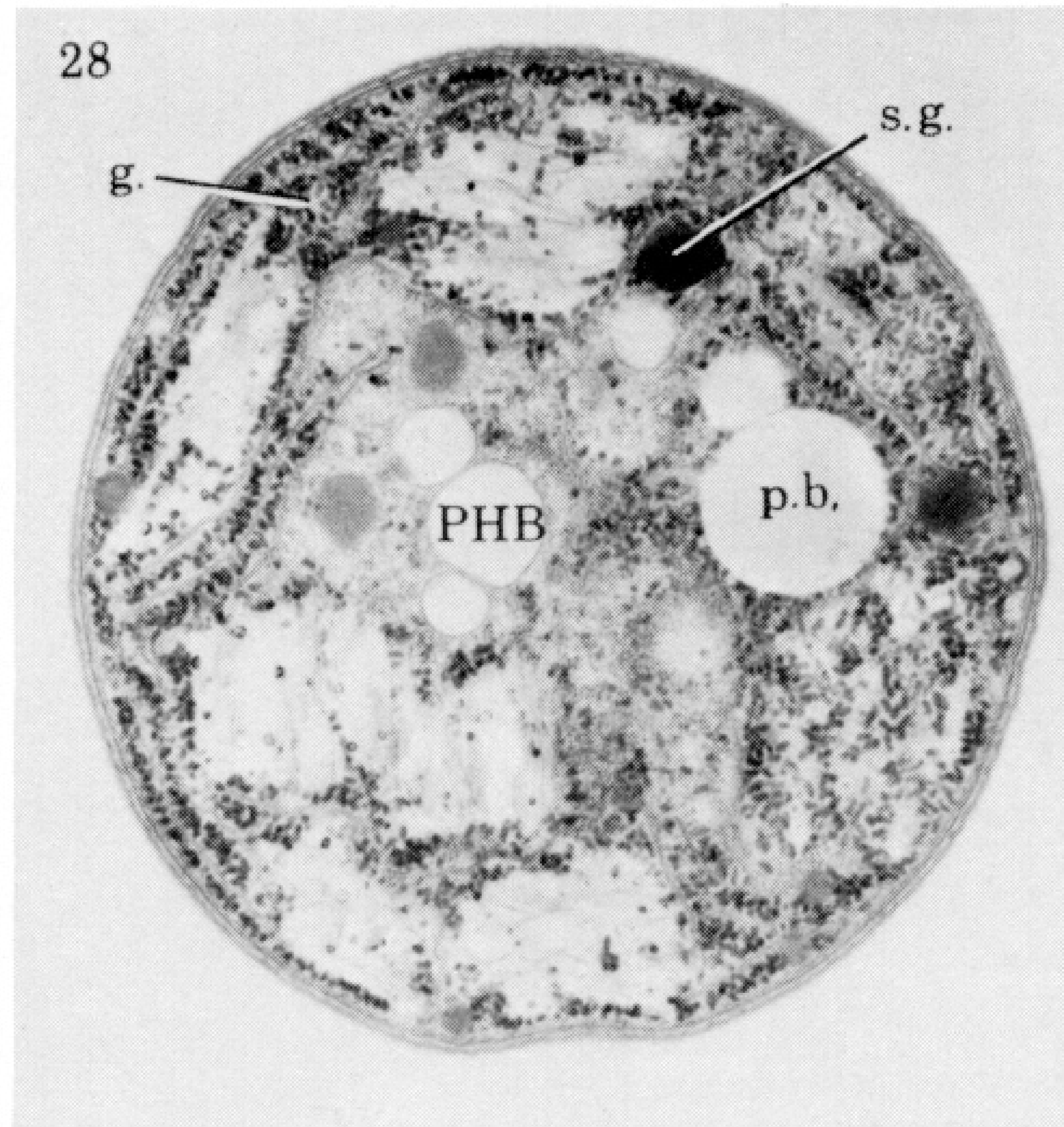
FIGURES 3–13. For description see opposite.

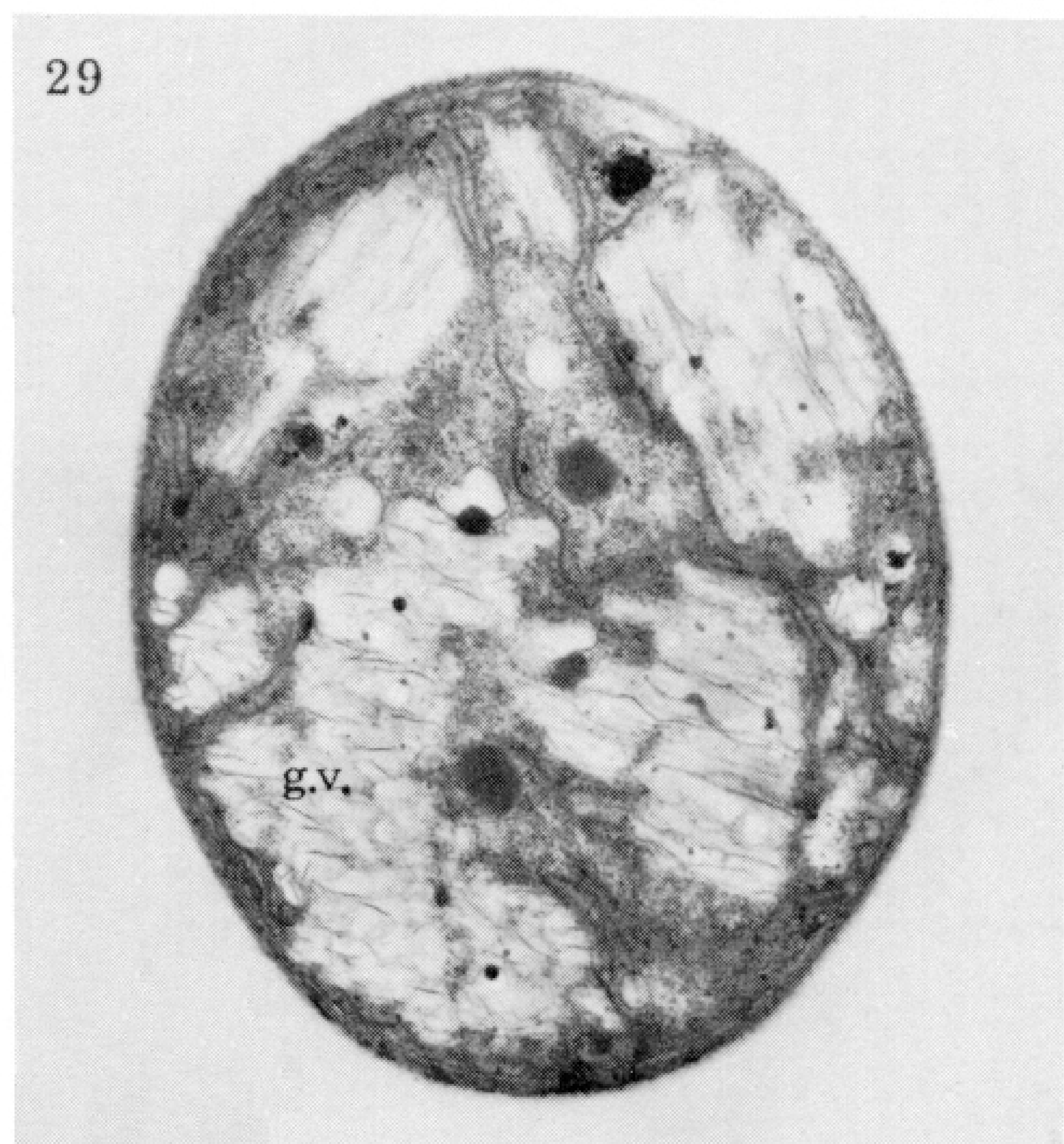


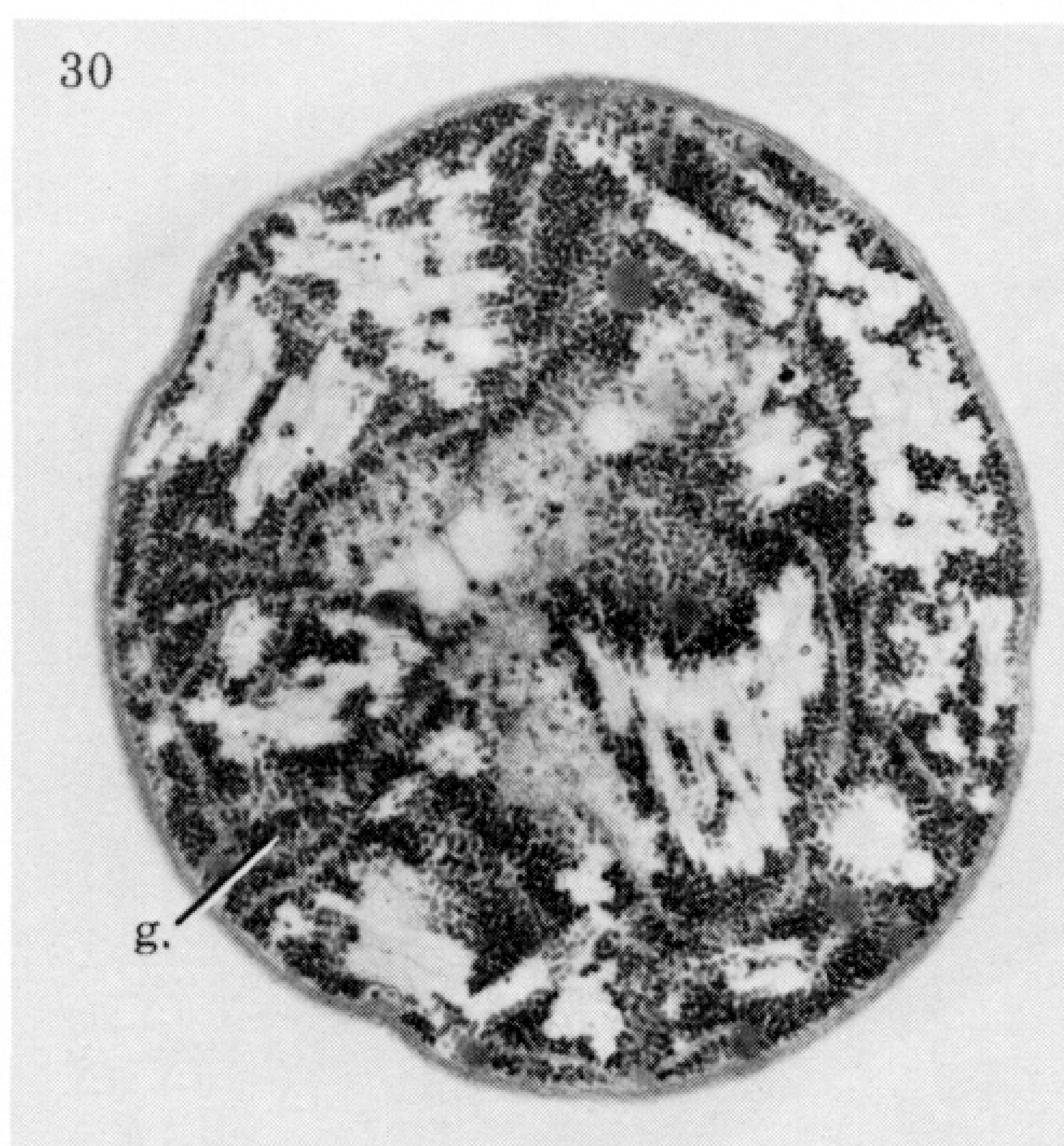


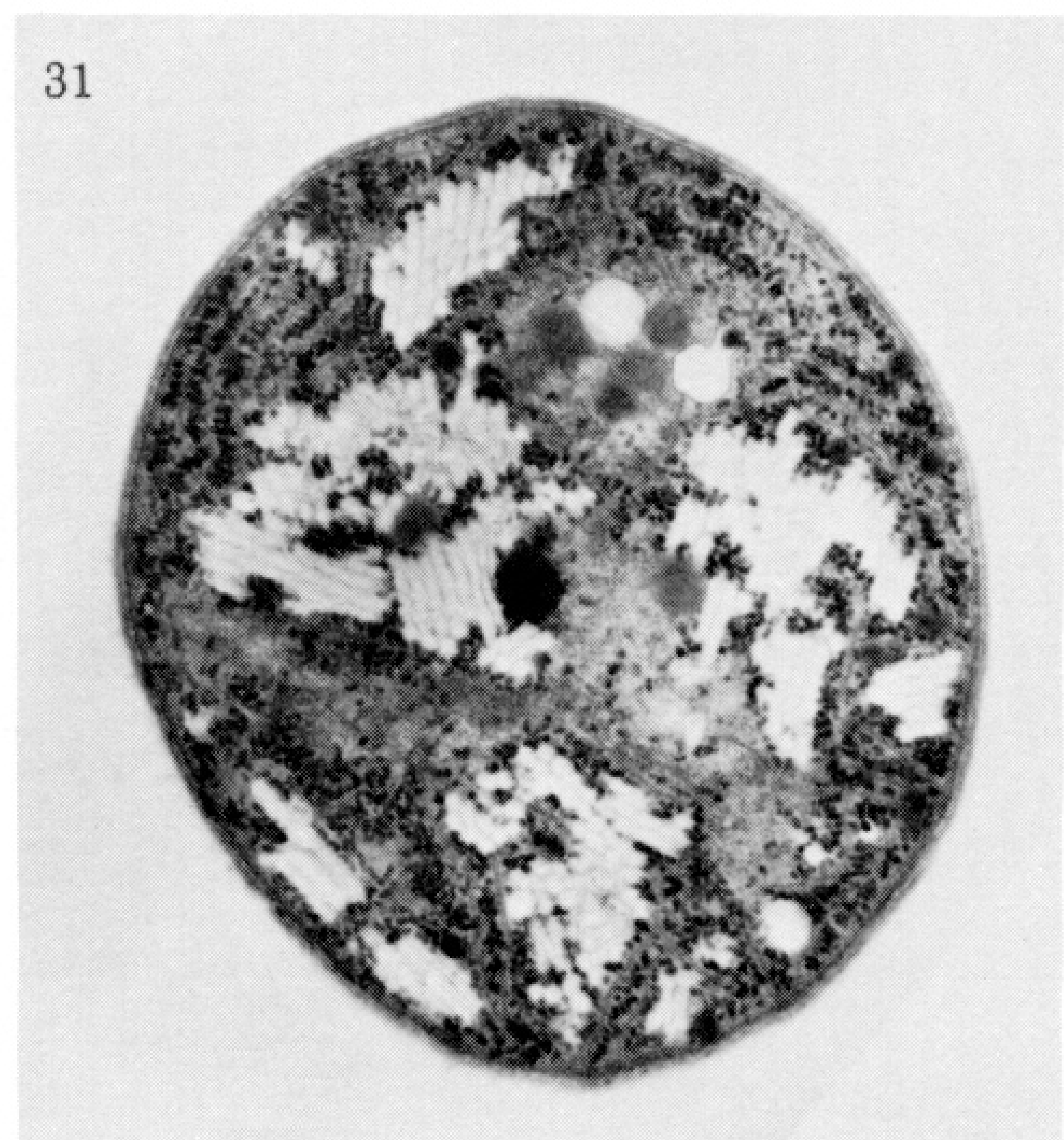
FIGURES 24–26. For description see p. 428.

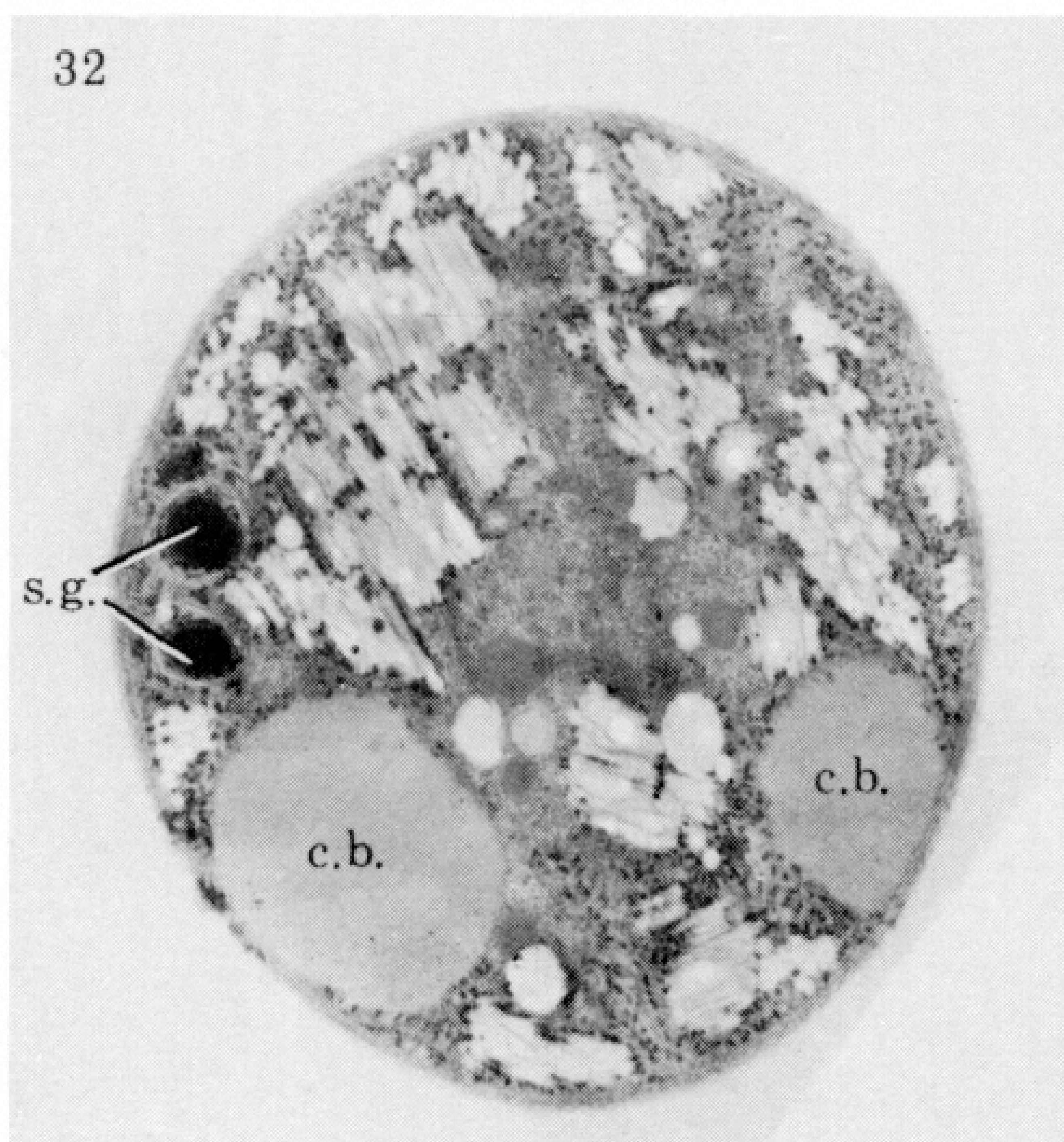




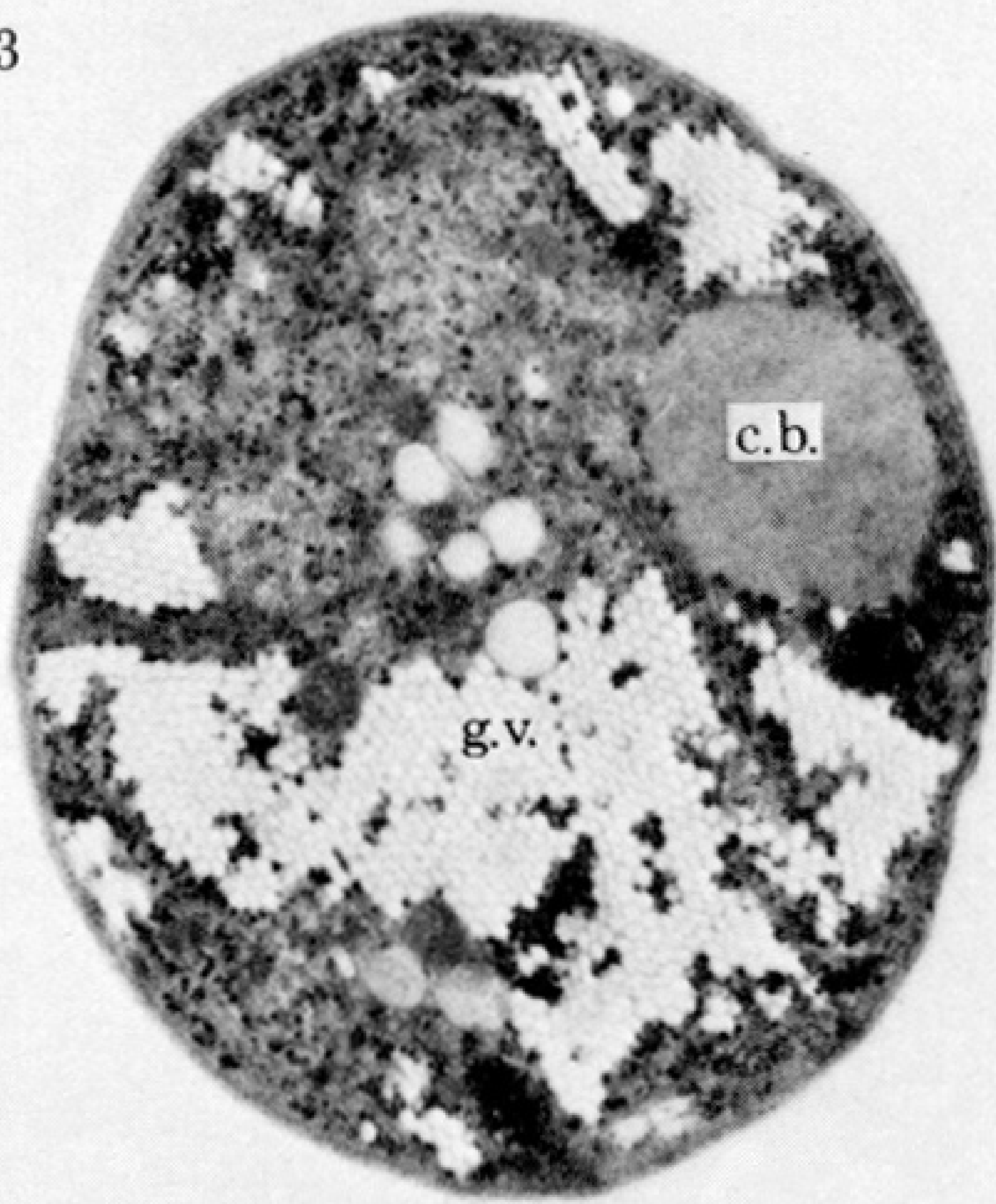


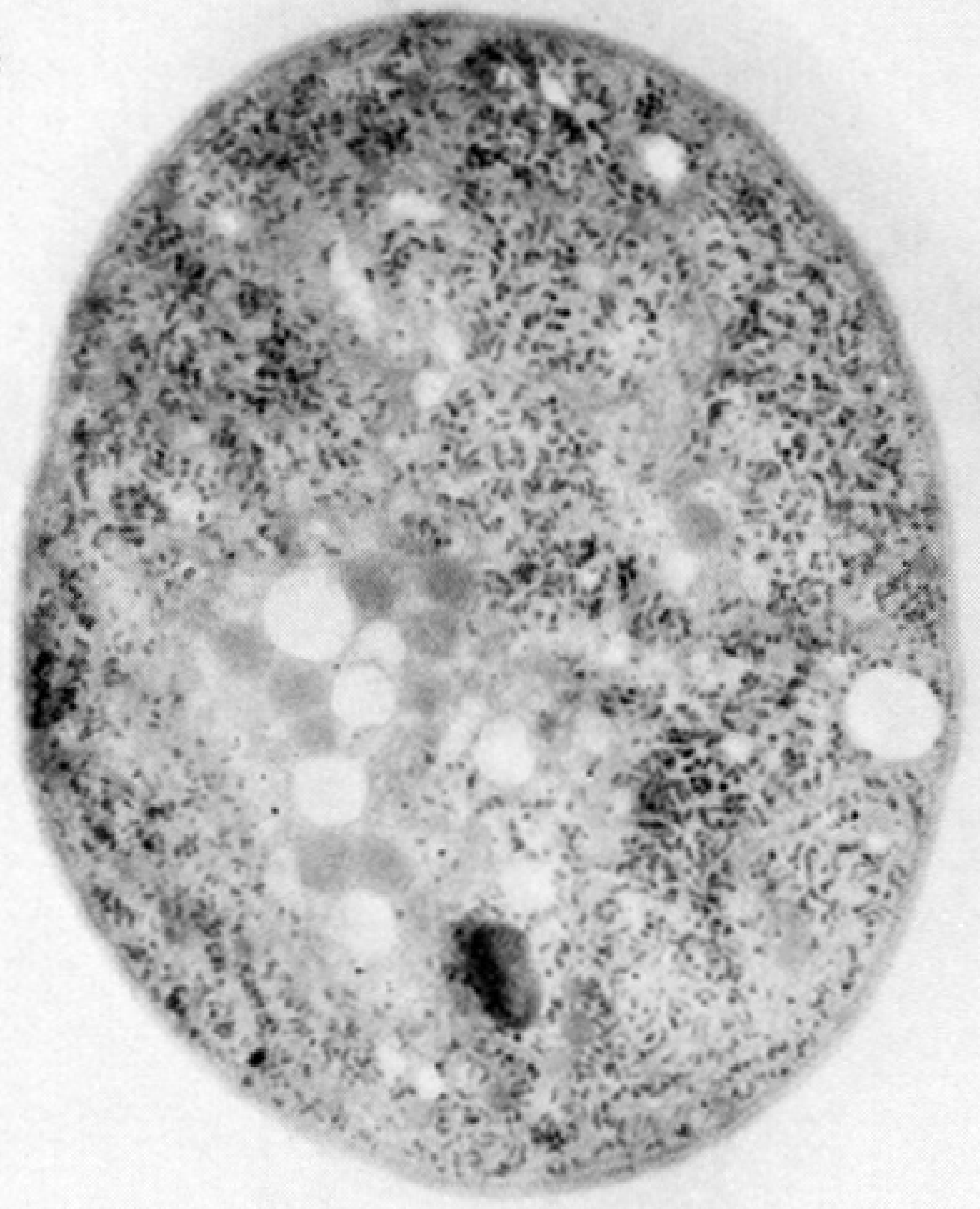


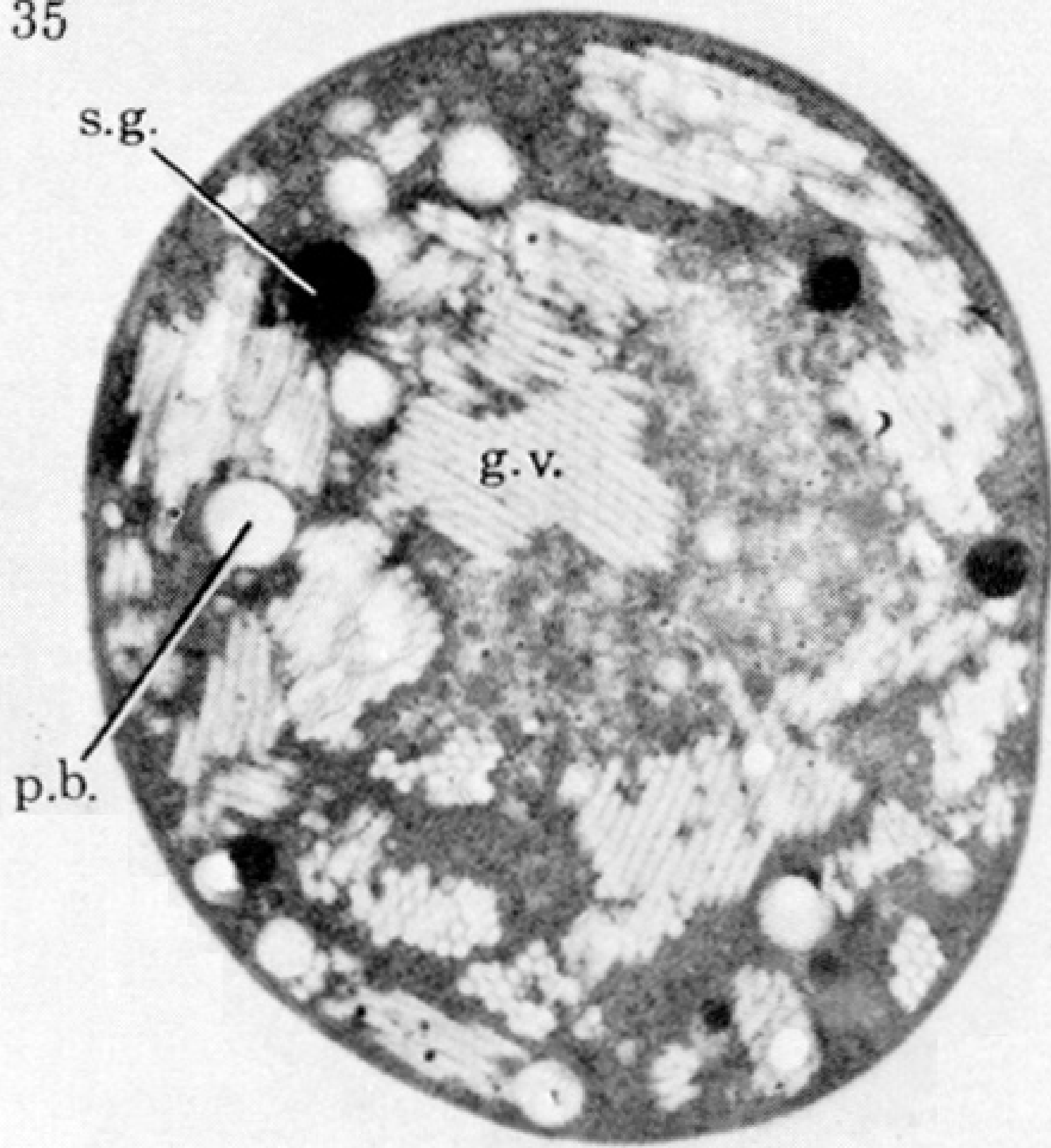


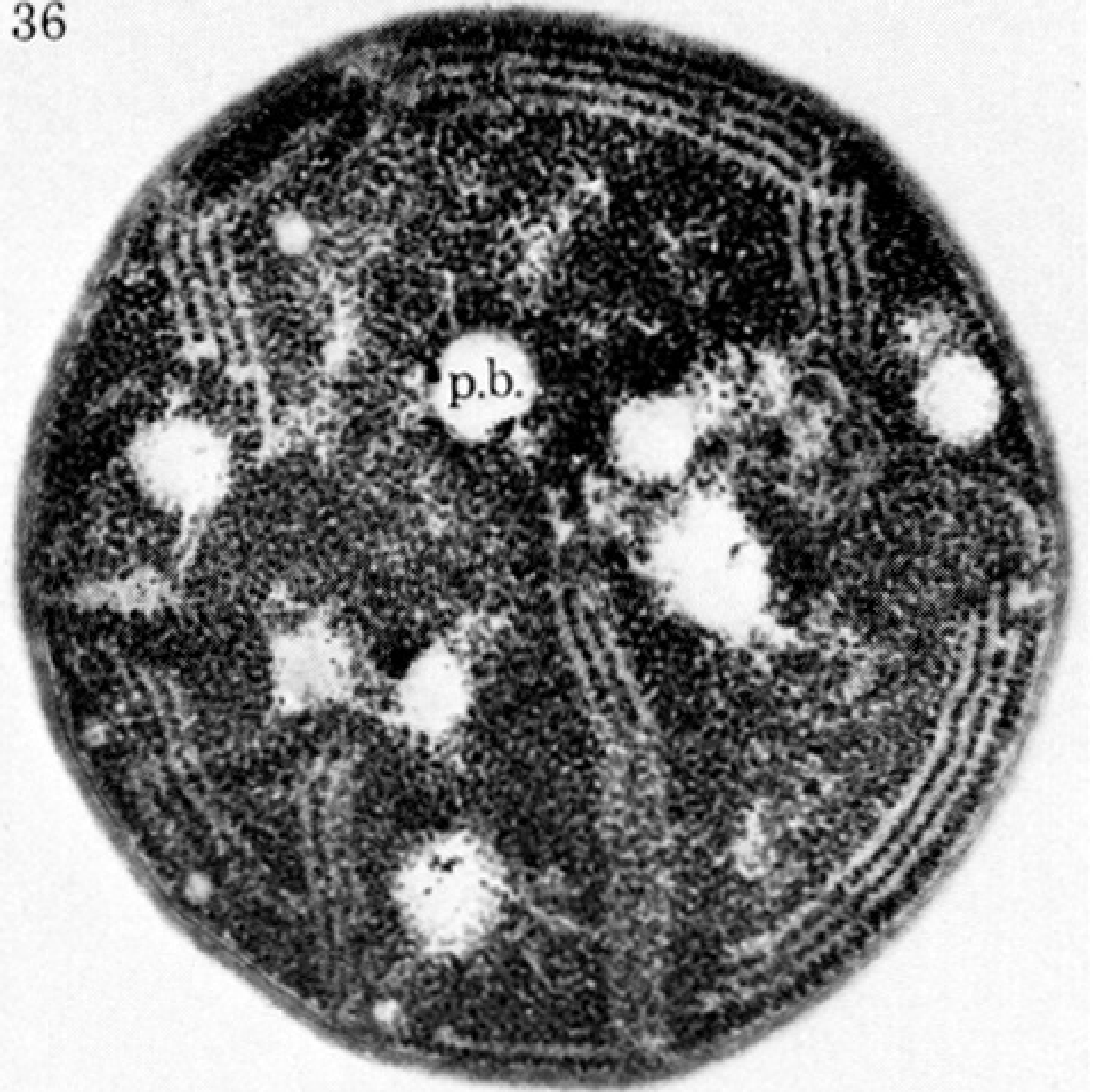


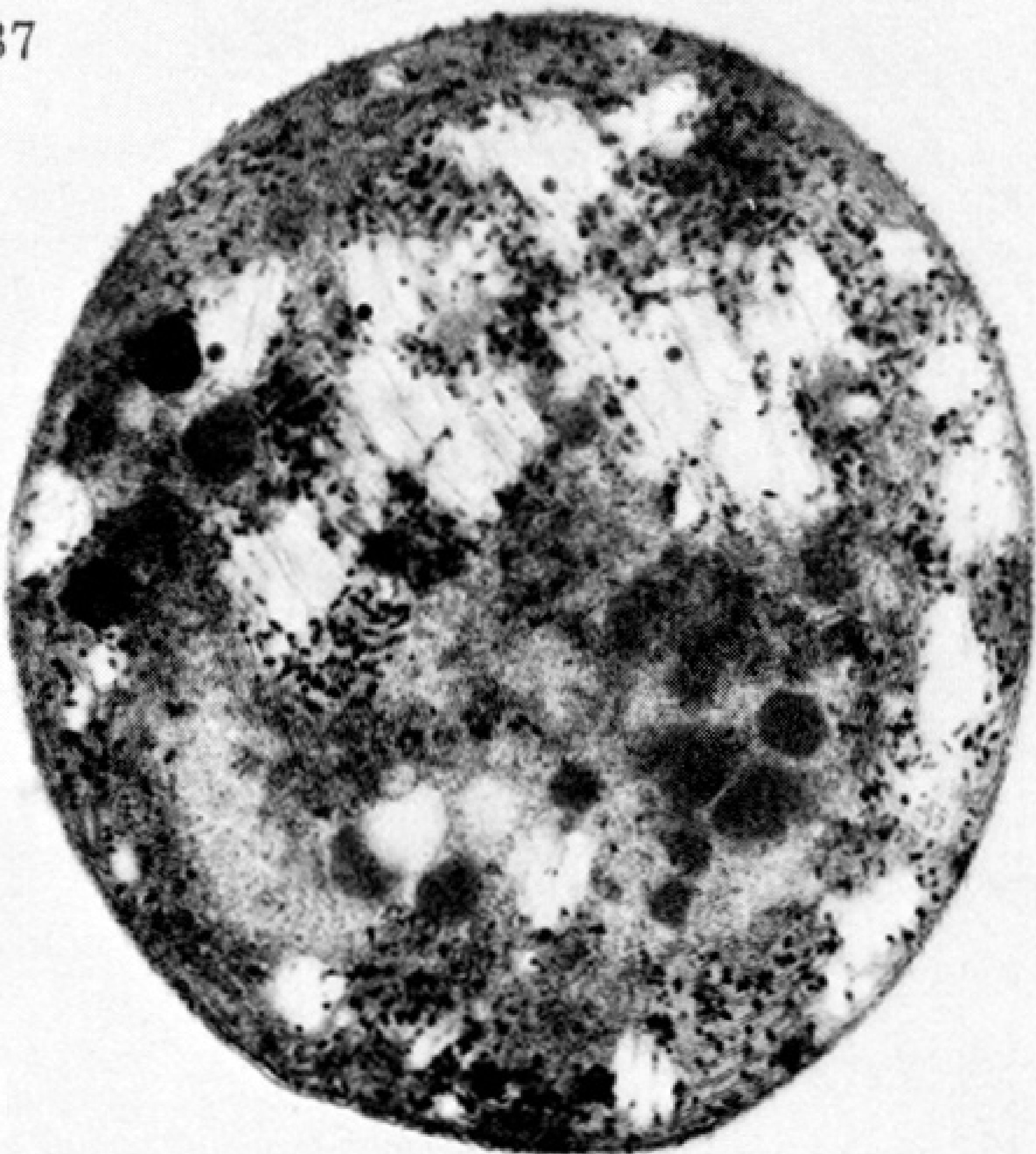
FIGURES 27–32. For description see p. 429.











FIGURES 33–38. For description see opposite.