Chapter 6

Freshwater blooms

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I. Introduction

The successful net growth of a species depends on its ability to optimise resource capture, to utilise efficiently these resources and to minimise losses. It is unlikely that any one organism will have the flexibility to excel under all circumstances, but the appearance of a dominant suggests it has characteristics needed to maximise net growth under the prevailing environmental conditions. At times cyanobacteria may come to dominate the phytoplankton of lakes, reservoirs and rivers. The purpose of this chapter is to appraise the physiological and ecological characteristics of planktonic cyanobacteria which enable them to dominate the phytoplankton. When environmental conditions are appropriate for growth freshwater blooms occur, but which species dominates depends on the interaction between the organism and its habitat.

The term "bloom" is poorly defined, but generally it describes a phytoplankton biomass significantly higher than the lake's average. By this definition even oligotrophic waters may have blooms, although this stretches the concept beyond its general meaning. Blooms are usually comprised of only one or two species and identified by the dominant phytoplankton type eg. cyanobacterial bloom, diatom bloom, *Anabaena* bloom etc. In potable and recreational waters a bloom is frequently defined in terms of cell concentrations that cause a nuisance to humans and a lower limit may be set at ca. 10 mg m^{-3} of chlorophyll-a (ca. 20,000 cells mL^{-1}). The occurrence of a bloom is a function of the environmental conditions and the resource requirements of the organism and many phytoplankton can form blooms under suitable circumstances.

In contrast, surface blooms are restricted to those organisms that are buoyant or motile and on occasions accumulate at the water surface to form a scum. Usually surface blooms are comprised of cyanobacteria made buoyant by the presence of gas-filled cell inclusions called gas-vacuoles, and it is these (Plate 2f) that have historically been referred to as "water blooms" (Reynolds and Walsby, 1975). A few non-cyanobacterial species can also form surface blooms, notably the green alga *Botryococcus braunii* which becomes buoyant by producing and storing oils. Occasionally flagellates such as *Euglena* also form surface blooms.

Cyanobacterial blooms have been recorded from early history (Reynolds and Walsby, 1975) and for some decades those involved in water supply have been aware of their economic impacts due to impairment of water treatment processes including filter blockage, increased disinfection costs and taste and odour problems. Cyanobacterial blooms also degrade the recreational value of surface waters, particularly where thick surface scums reduce the use of amenities for contact sports or large concentrations cause deoxygenation of the water leading to fish kills. Concern about the detrimental effects of freshwater cyanobacteria on water quality was heightened during the 1980s and 1990s as information accumulated on the potency of their toxins (Gorham and Carmichael, 1988; Carmichael, 1994; Codd, 1994; Falconer, 1993). The presence of cyanobacterial toxins has long been known (Francis, 1878), but these were generally associated with the death of domestic animals. Recognition of the potentially harmful effects of these toxins on humans led to re-assessment of these organisms as a threat to water supplies. An apparent increase in the occurrence of cyanobacterial blooms over the last few decades, coupled with the heightened concern about toxins, has created the need for a better understanding of the environmental conditions supporting the growth of the gas-vacuolate cyanobacteria to provide a basis for improved control and management of their occurrence and abundance.

II. Bloom-Forming Cyanobacteria

The cyanobacteria principally responsible for forming blooms are gas-vacuolate species. They are distributed across a number of genera and vary in form and size from small filaments to large globular colonies (Table 1).

The filamentous forms occur as straight, spiral or twisted chains of cells, and sometimes take on a secondary morphology as a result of the aggregation or entanglement of many filaments (Reynolds and Walsby, 1975; Lewis, 1976). A consequence of this complex secondary structure is the size of the biomass unit. Filamentous forms differ significantly

Table 1. The major genera of gas-vacuolate, planktonic cyanobacteria

	N_2-fixer	Family	Order
Filamentous			
Anabaena	+	Nostocaceae	Nostocales
Anabaenopsis	+	Nostocaceae	
Aphanizomenon	+	Nostocaceae	
Nodularia	+	Nostocaceae	
Cylindrospermopsis	+	Nostocaceae	
Gloeotrichia	+	Rivulariaceae	
Oscillatoria	??	Oscillatoriaceae	Oscillatoriales
Spirulina	-	Oscillatoriaceae	
Non-filamentous			
Microcystis	--	Chroococcaceae	Chroococcales
Gomphosphaeria	--	Chroococcaceae	
Coelosphaerium	--	Chroococcaceae	

in size, from small individual filaments such as *Anabaena minutissima* (4 µm wide and up to 104 µm long: Walsby et al., 1989) or *Oscillatoria agardhii var. isothrix* (3.5 µm wide and up to 125 µm long: Reynolds, 1984a) to spirally coiled filaments as in *Anabaena circinalis* that may be 220 µm long and can aggregate to form macroscopic colonies visible to the naked eye. *Aphanizomenon* filaments aggregate into rafts that are reminiscent of grass blades, while in *Gloeotrichia* the filaments are clustered around a central node to form large (1-2 mm) urchin-like balls.

The colonial cyanobacteria also vary in size and form. The globular colonies usually alter size as a result of growth and reduced colony disaggregation, although loose assemblages or aggregations of separate colonies can occur (Reynolds et al., 1981). *Microcystis aeruginosa* ranges in size from single cells of 5 - 6 µm diameter, the form most often found in culture, to large globular or semi-spherical colonies several millimetres in diameter containing tens of thousands of cells per colony (Reynolds et al., 1981). This represents a change in unit volume of more than three orders of magnitude and has important implications for a variety of processes including buoyancy regulation, nutrient uptake, gas exchange, light interception and susceptibility to grazing.

Surface blooms can appear rapidly, often within hours, and to the casual observer without prior warning of the presence of the organisms. Historically this imbued the blooms with a somewhat mystical character and a notion developed that the cyanobacteria could grow extremely rapidly. In fact the sudden appearance results from the upward migration of an existing dispersed population (Reynolds, 1971) and is not a consequence of rapid cell growth. Their sudden appearance is often associated with calm conditions and reduced turbulence that allows buoyant migration to the water surface. Consequently surface blooms occur only if there is an existing cyanobacterial population and its severity depends, in part, on the size of the pre-existing population. However, the notion that the pre-existing population must be of bloom proportions is mistaken because a dispersed population becomes greatly concentrated as it floats to the surface. Indeed the pre-existing population need not be particularly large at all. For example, if a population that is homogeneously dispersed through a 5 m water column with a cell concentration of 2×10^3 mL^{-1} were to float up into a 2 cm surface layer it would form a surface bloom with a concentration of 0.5×10^6 cells mL^{-1}.

III. Distribution

Cyanobacteria are a common feature of many aquatic systems including tropical and temperate lakes, rivers and estuaries but cell densities, species composition, vertical distribution, longevity and timing of the population maxima differ. To a large degree this may be explained by climatic and meteorological conditions which influence the degree of stratification and mixing as well as light and nutrient availability. It is this physical and chemical setting that provides the stage upon which competitive interactions between species are enacted.

In deep, monomictic, temperate lakes the strong seasonal climatic signal results in a progression of

phytoplankton from diatoms in early spring as thermal stratification commences through populations of green algae to culminate during summer in populations of cyanobacteria and dinoflagellates. The two major environmental variables stimulating the species progressions are changes in the stability of stratification and declining nutrient availability (Reynolds, 1984b). Over the growing season the intensity of stratification increases to a maximum in summer when apparently the mixing intensity is insufficient to help maintain heavy phytoplankton, such as diatoms, in suspension. The separation of the water column into an upper epilimnion where light is available for growth and a dark hypolimnion leads to nutrient depauperate conditions developing in the surface layers as a result of phytoplankton growth and sedimentation. It is during calm weather in summer and autumn that surface blooms of cyanobacteria frequently develop, often associated with minimum nutrient concentrations in the surface layer. A secondary peak of diatoms can be associated with the onset of meromixis in autumn before phytoplankton populations are reduced to low levels in winter. This simplified general over-view of the responses observed in deep temperate lakes will be strongly modified by local conditions so that seasonal progressions are altered (Round, 1971). Reynolds 1980 described periodic progressions from one dominant assemblage to another in lakes and enclosure of the English Lake District that are broadly characteristic of the chemical and physical environments that they inhabit. In the more eutrophic lakes the sequence was from diatoms through Volvocales, Nostocales to dinoflagellates or *Microcystis* and in mesotrophic lakes from diatoms through chrysophytes and *Sphaerocystis* to dinoflagellates. Gas-vacuolate cyanobacteria are more typical of eutrophic lakes.

In shallow, well-mixed eutrophic lakes of the temperate northern hemisphere the cyanobacteria that dominate during summer are commonly species of *Oscillatoria*. These lakes are typically very turbid, and when winters are not too cold dominance can persist throughout the year (Scheffer et al. 1997; Sas 1989).

In some clear water temperate lakes, where light penetrates beyond the depth of the epilimnion, species such as *Aphanizomenon flos-aquae* and *Oscillatoria agardhii* form metalimnetic populations of single filaments. In Crooked Lake, Indiana peak concentrations of 25-50 μg chlorophyll-a L^{-1} occurred at a depth of ca. 3 - 4 m, where there were opposing gradients of irradiance and nutrient availability (Konopka 1989). If conditions become unsuitable these filaments may aggregate together and move towards the surface as was observed in Lake Gjersjøen, Norway (Walsby et al. 1983).

In the tropics cyanobacterial blooms and surface scums can form at almost any time of the year, as the annual solar input and air temperature are relatively constant. However, despite this relative constancy, there are major seasonal hydrographic and meteorological changes that alter the phytoplankton community structure. Marked similarities occur between large tropical lakes such as Lake Victoria in East Africa (Talling, 1987) and Lake Lanao in the Philippines (Lewis, 1978). Diatom peaks coincide with marked depressions of the thermocline brought about by meteorological events, while both the chlorophytes and the cyanobacteria decline during these periods of diatom growth. As the thermocline re-establishes the phytoplankton follow a similar progression to that observed in temperate lakes but over a shorter time scale. The chlorophytes dominate first when the nutrient stress is less severe followed by the cyanobacteria during periods of severe nutrient depletion. Lewis (1978) divided the habitat of Lake Lanao through time on the basis of light and nutrient availability as deduced from the growth pulses of individual species. He concluded there was a predictable successional pathway from diatoms through to cyanobacteria and dinoflagellates which reflected adaptations to nutrient and light availability, and adaptations to minimise sinking rate. The diatoms and cryptomonads plus the small single-celled cyanobacterium *Dactylococcopsis* showed growth pulses in habitats characterised by high nutrients and mixing and consequently relatively low light availability. Turbulence was reduced with the onset of thermal stratification and the light climate improved while nutrient availability decreased, the community became dominated by green algae as well as the cyanobacterium, *Aphanothece*, which is characterised by many small cells embedded in a thick gelatinous matrix. As nutrients became less available and the light climate remained favourable cyanobacteria showed pulses of growth alongside the chlorophytes *Sphaerocystis* and *Selenastrum*. *Gymnodinium*, a dinoflagellate, was found in association with the larger cyanobacteria and occupies the habitat where light availability is high, but nutrients and turbulence are minimal. This successional sequence may be interrupted at any time by storm events or other

exceptional climatic conditions which either reset the successional clock or cause elements to be skipped.

At offshore stations in Lake Victoria during periods of thermal stratification the cyanobacteria *Anabaenopsis tanganyikae* and *Anabaena flos-aquae* dominate the community but give way to diatoms (eg *Melosira nyassensis* var. *victoriae*) as the lake becomes isothermal. The depth distribution during periods of thermal stratification also differs between species. During periods of relatively strong stratification *Lyngbya circumcreta* and *Aphanocapsa elachista* maxima are confined to the top 20 m (the depth of the euphotic zone in the offshore stations was ca. 13 - 18 m) and decline to very small numbers in the deeper water. In contrast diatom species are usually confined to or below the zone of thermal discontinuity. As thermal stratification breaks down both cyanobacteria become more evenly distributed with depth, although the larger colonial *Aphanocapsa elachista* is most abundant in the upper 20 m.

These temporal and spatial distribution patterns illustrate two important points. Firstly, the natural process of stratification and destratification influences the occurrence of cyanobacteria, but the response is not uniform between species. Secondly, the contrasting depth distribution of diatoms and cyanobacteria illustrate how the lower sinking rates of the cyanobacteria enhance their ability to remain in the epilimnion.

The behaviour and composition of the phytoplankton in Lake Victoria contrasts with that in nearby Lake George (Viner and Smith, 1973). Although Lake George is shallow (2.4 m), light does not penetrate to the sediments and the depth of the euphotic zone is usually less than 0.8 m. The lake stratifies diurnally: at dawn the water column is isothermal (25°C), but heats during the day so by late afternoon as much as 10°C may separate top and bottom waters. The surface water becomes supersaturated with oxygen (ca. 250%) due to the photosynthetic activity of the dense phytoplankton community (ca. 250 mg m^{-3} chlorophyll-a) by late afternoon and the pH rises to > 9. As nocturnal mixing occurs the thermal stratification breaks down, the pH returns to ca 7 and the oxygen concentration falls below 100%. The climatic constancy of Lake George superimposed on the over-riding diel pattern of stratification and destratification appears to be the ideal habitat for permanent, dense populations of the cyanobacteria *Microcystis aeruginosa* and *M. flos-aquae*, *Anabaenopsis* spp., *Aphanizomenon* sp. and *Lyngbya* spp. Surprisingly, the other major cyanobacterial component of the phytoplankton community, *Anabaena flos-aquae*, showed a pronounced but unexplained seasonal periodicity. *Microcystis* spp. appear well suited to the diel pattern of stratification and destratification, a thermal periodicity often found in the surface waters of deeper lakes overlying a summer thermocline. The rapid vertical migration of the large colonies of *Microcystis* provides them with the ability to exploit both the illuminated surface waters and the potentially nutrient rich shallow sediments.

Talling (1992) identified three cyclical environmental changes, each involving many variables, that structure the phytoplankton communities in these lakes. Systems like Lake George are primarily influenced by the diel cycle with relatively little annual change in water volume. The other two systems are influenced by annual cycles, but differ depending on whether or not there are major water volume changes. These comparisons emphasise the importance of both hydrological and hydrographic features as principal determinants of the species composition of phytoplankton communities, and in general diatoms and cyanobacteria occupy complementary positions related to nutrient availability, vertical mixing and water retention.

Many studies have addressed the question of why cyanobacteria should be so successful in such a wide range of environmental conditions. Explanations proposed for cyanobacterial dominance (Steinburg and Hartmann, 1988; Shapiro, 1990; Blomqvist, 1994) include traits to take advantage of warmer water temperatures, to capture reduced photosynthetic photon flux densities (PPFD), to utilise low TN:TP ratios or to access low dissolved carbon dioxide concentrations. Other characteristics suggested as being advantageous include buoyancy regulation, reduced zooplankton grazing and a capacity to store phosphorus. Occasionally one or other of these characteristics has been offered as the sole or principal reason for cyanobacterial dominance, but the distinct morphological, physiological and ecological characteristics of individual species suggests that the factors which promote one will not necessarily promote another. For example, although *Microcystis aeruginosa* may co-exist with *Anabaena flos-aquae*, it does not co-exist with *Oscillatoria rubescens*. Nevertheless, a unique characteristic of all bloom- forming species is the presence of gas vesicles, and there can be little doubt that the buoyancy provided by these structures is a significant attribute.

IV. Gas Vacuoles, Gas Vesicles, Buoyancy and its Regulation

The success of gas vacuolate cyanobacteria is often attributed to their buoyancy and to their ability to regulate buoyancy in response to changing environmental conditions (Reynolds and Walsby 1975; Ganf and Oliver 1982; van Rijn and Shilo 1985; Walsby 1987; Reynolds et al. 1987; Walsby 1994). Advantages associated with buoyancy and its regulation include a reduction in sedimentation losses (Reynolds 1984b), an improvement in the supply of light as buoyant cells move into the well illuminated surface layers (Humphries and Lyne 1988; Walsby et al. 1997) and access to improved nutrient supplies that are available at depth, particularly in waters that are thermally stratified with vertically separated sources of nutrients and light (Ganf and Oliver 1982).

A. Gas Vacuole Structure

Gas vacuoles were discovered by the German microbiologist Klebahn in 1895. Seventy years later Bowen and Jensen (1965) showed that the gas vacuoles were made up of numerous, cylindrical vesicles which were called gas vesicles. The molecular structure, morphology and physical properties of gas vesicles have been reviewed by Walsby (1994). They are hollow, but rigid, proteinacous, cylinders capped at either end by a cone and synthesised under the direction of specific gas vacuole genes which encode for the various proteins required (Walsby, 1994; Oliver, 1994). The gas vesicle wall allows the free passage of gases, but is impermeable to water due to the presence of amino acids with hydrophobic aliphatic side-chains exposed at the inner gas-facing surface of the protein wall.

Since gas vesicles are small, many are needed to provide buoyancy and estimates of 10000 per cell have been made. Gas vesicles are not randomly dispersed throughout the cytoplasm, but are ordered into gas vacuoles to occupy minimal space and provide maximum buoyancy. To achieve this the cylindrical gas vesicles are stacked in hexagonal arrays with the cones interdigitating. In *Anabaena* where the gas vesicle density is 120 kg m^{-3}, if the intervening space (15%) is filled with water at a density of 1000 kg m^{-3}, then the overall gas vacuole density will be 252 kg m^{-3}, one-fourth the density of water and an efficient mechanism to provide lift (Walsby 1994).

The common perception that gas vesicles are equivalent to balloons or bubbles in the cytoplasm is incorrect. Walsby (1994) has suggested a more appropriate analogy would be a pair of old fashioned earthenware flower pots, coated with oil on the inner surface to represent the hydrophobic inner layer of the gas vesicle, and glued together at the rims. Gas vesicles, as with the flower pots, are permeable to gas and therefore contain air at atmospheric pressure, but the air is not required to maintain the hollow space as the gas vesicle walls are rigid. If the gas vesicle "analogy" is submerged, water will seep into the wall, but will be prevented from entering the vesicle cavity by the hydrophobic layer. Air will diffuse back and forth between the cavity and the surrounding medium and the vesicle will be filled with air of composition similar to that in the surrounding liquid.

B. Pressures Acting on Gas Vesicles

The net pressure (p_n) experienced by a vesicle contained within a submerged cell will be the sum of the hydrostatic pressure (p_h), plus the turgor pressure (p_t) plus the pressure of the overlying atmosphere (p_f), minus the gas pressure (p_g) inside the vesicle which is determined by the concentration of gases dissolved in the surrounding liquid and is usually in balance with p_f (Fig. 1). It is important to recognise that the vesicle wall has a finite strength upon which hydrostatic and turgor pressure act, and if the combination of these exceed the strength of the wall the vesicle will collapse and the cell will lose buoyancy (Walsby, 1971).

Hydrostatic pressure increases with water depth at a rate of 0.1 MPa every 10m and for cells to remain buoyant their gas vesicles must be strong enough to withstand the pressures generated during episodes of deep mixing. Increases in turgor pressure result from enhanced rates of photosynthesis producing increased levels of soluble organic intermediates (Grant and Walsby, 1977; Konopka, 1984), coupled with an associated light-dependent uptake of potassium salts (Allison and Walsby, 1981). Turgor pressure can be measured as the difference in the applied pressure (p_c) required to collapse vesicles of cells suspended in 0.5 M sucrose which removes the turgor pressure, compared with the pressure (p_a) required for turgid cells suspended in filtered lake water or culture medium: $p_t = p_c - p_a$. Turgor pressures can vary from 0 to 0.5 MPa in different organisms (Walsby 1994) and increases can lead to gas vesicle collapse.

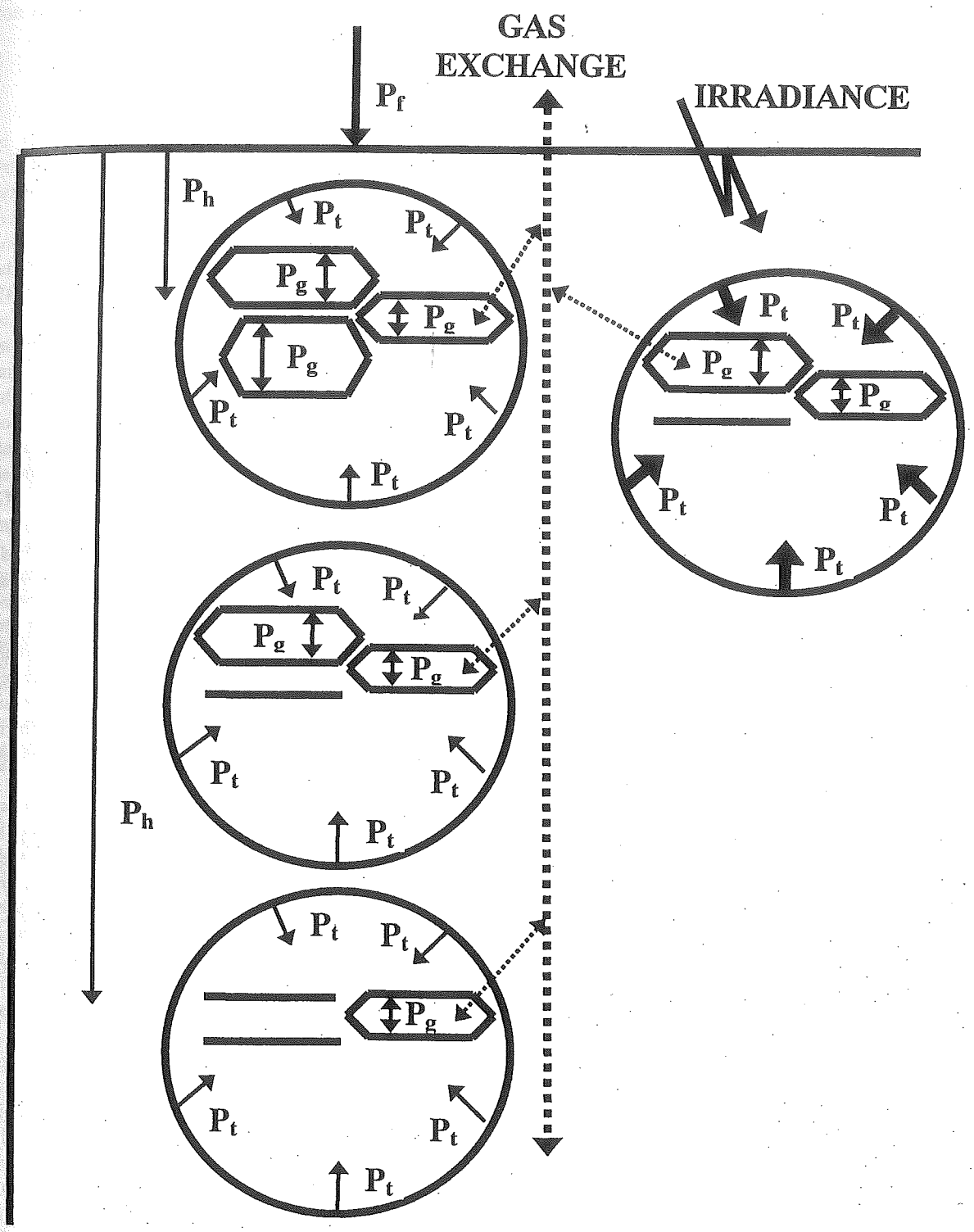

Fig. 1 Pressures acting on gas vesicles. Gas vesicles may collapse in response to increased hydrostatic pressure generated by depth and increased turgor pressure in response to light. Wider, weaker vesicles collapse more readily than thinner, weaker ones.
Key: p_h hydrostatic pressure, p_t turgor pressure, p_f pressure due to overlying atmosphere, p_g pressure of gas in vesicle.

C. Gas Vesicle Collapse

The critical pressure (p_c) at which 50 % of gas vesicles are collapsed varies widely between gas-vacuolate organisms within the range 0.1 to 3.5 MPa. In the bloom-forming freshwater cyanobacteria the range is less (ca. 0.35 to 0.95 MPa), but different species still have particular limits. In gas vesicles isolated from *Anabaena flos-aquae* the critical collapse pressures ranged from 0.45 to 0.85 MPa (Walsby 1980), while for natural populations of *Microcystis aeruginosa* fo. *aeruginosa* the range was 0.5 to 1.2 MPa (Brookes et al., 1994). The critical collapse pressure depends on gas vesicle strength, which is independent of the vesicle length but varies inversely with the cylinder radius (r in nanometres), as described approximately by the expression; $p_c = 275(r)^{-1.67}$ MPa (Walsby, 1994). The width of gas vesicles in various strains of cyanobacteria appears to be determined by the balance between efficient provision of buoyancy and the strength required to withstand hydrostatic pressures (Hayes and Walsby, 1986; Walsby and Bleything ,1988). While buoyancy is provided most efficiently by wider gas vesicles because they have a smaller surface area to volume ratio and provide more buoyancy per unit protein, the gas vesicle strength is reduced as radius increases and vesicles are more susceptible to hydrostatic pressure. *Trichodesmium thiebautii* inhabits the deep oceans, where hydrostatic pressure can be considerable, and has a gas vesicle width of only 45 nm (Gantt et al., 1984), while *Oscillatoria agardhii*, which inhabits deep lakes has a gas vesicle width of 62 nm, and *Microcystis aeruginosa*, *Anabaena flos-aquae* and *Aphanizomenon flos-aquae*, which are found principally in shallow lakes or in lakes where the mixing depth is reduced by thermal stratification have gas vesicle diameters of 67, 84 and 78 nm, respectively (Walsby and Bleything, 1988). *Dactylococcopsis salina* has the broadest gas vesicles (109 nm) and inhabits shallow saline pools where hydrostatic pressures are minimal.

Although gas vesicle strength is not affected by its length, few are longer than 1000 nm and the average length measured in eight species was 470 nm. To answer the question of why on average gas vesicles are not longer, Walsby used geometrical analysis to show that for a gas vesicle of given width the increase in the ratio of the gas vesicle volume to wall volume approaches a plateau at a length of ca. 5 times the width (see Fig 25 of Walsby 1994). He concluded that there would be little point in investing the protein required for a longer gas vesicle, since it would not much improve the provision of buoyancy.

D. Buoyancy Regulation

The buoyancy of gas-vacuolate organisms is dependent on the extent to which the lift provided by gas vesicles counteracts cellular density. Cyanobacteria have the ability to regulate their buoyancy in response to environmental conditions (Reynolds and Walsby, 1975; Walsby and Reynolds, 1980; Reynolds, 1987; Oliver, 1994; Walsby, 1994), either by modifying the degree of gas vacuolation or by altering the extent to which dense components such as carbohydrate (density ca. 1600 kg m^{-3}) and protein (density ca. 1300 kg m^{-3}) accumulate in the cell (Fig. 2). Three mechanisms have been described by which gas-vacuolate cyanobacteria regulate their buoyancy. One is where cell density (p') is changed through alterations in cellular composition. Of particular importance to this mechanism is the accumulation of carbohydrate reserves through photosynthesis and their reduction either through respiration or by conversion to less dense protein. Differences in the relative rates of accumulation and processing cause cell density to increase or decrease in response to environmental conditions (Oliver, 1994; Walsby, 1994). Changes in other cell components, including storage materials such as polyphosphate granules, can also alter cell density and affect buoyancy.

In the two other buoyancy regulating mechanisms the degree of gas vacuolation is altered by either the collapse of gas vesicles due to increased turgor pressure or a reduction in gas vesicle synthesis and their subsequent dilution by growth (Oliver 1994; Walsby 1994). Turgor pressure is generated within the organism as a result of photosynthesis, the magnitude of the increase depending upon the previous light history. The reliance on photosynthesis to generate turgor pressure forms a connection between this mechanism and buoyancy regulation brought about by alterations in cell composition. Whether the accumulation of polysaccharide exerts its influence on cell density through a change in cellular composition, or through gas vesicle collapse due to a rise in turgor pressure, depends on the fate of recently fixed photosynthate and this is a function of the physiological condition of the cells (Gibson, 1978; Kromkamp et al., 1986; Konopka et al., 1987; Klemer et al., 1988). For example, carbohydrate is more efficiently accumulated and stored in response

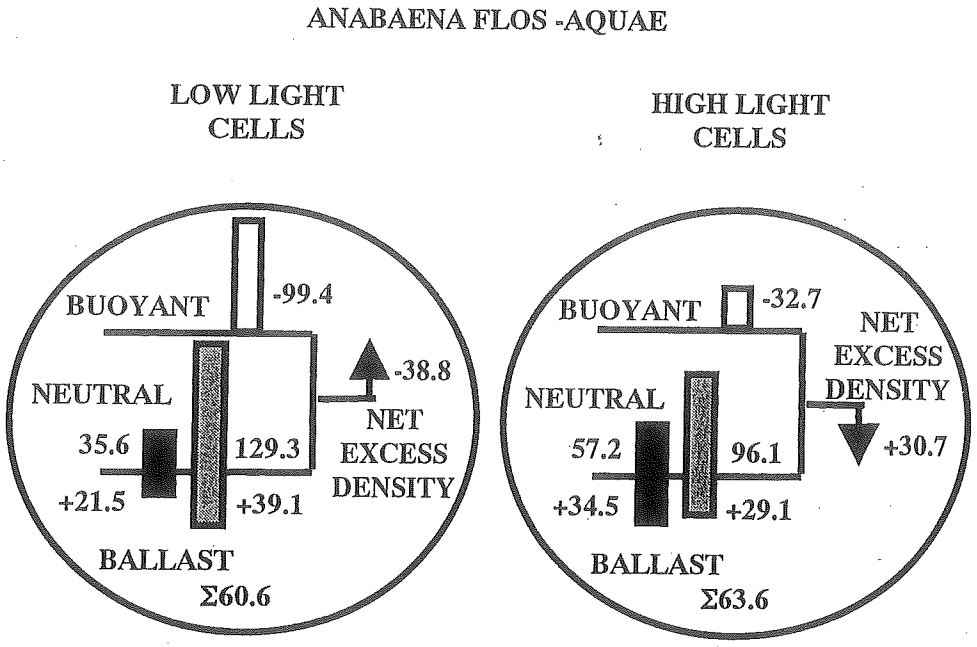

Fig. 2 Diagrammatic balance sheet demonstrating changes in cell density of *Anabaena flos-aquae* in response to increased illumination. Contributions to cell density made by carbohydrate (solid bar) and protein (hatched bar) are calculated from the total weight of each component per unit of cell volume (indicated by length of the bar and in $\mu g\ \mu L^{-1}$). The proportion that is offset by displacement of water is shown as having a neutral effect leaving the excess weight to contribute to cell ballast (21.5 + 39.1 $\mu g\ \mu L^{-1}$, and 34.5 + 29.1 $\mu g\ \mu L^{-1}$, low and high light cells, respectively). The buoyant lift provided by gas vesicles (open bar) is similarly calculated from the weight of water displaced by the total gas vesicle space per unit cell volume (indicated by the length of the bar and in $\mu g\ \mu L^{-1}$). The sum of the ballast and the buoyant lift gives the net excess density of the cell due to these components (-38.8 and +30.7 $\mu g\ \mu L^{-1}$). The excess density is the difference between the density of the cell and that of water. Increased illumination caused an increase in the ballast due to carbohydrate, but this was offset by a decrease in protein ballast and the cells changed from floating to sinking because of a decrease in gas vesicle volume. (Data from Oliver and Walsby, 1984).

to increases in irradiance in cells acclimatised to shorter light periods (Foy and Smith, 1980), whereas in cells grown under continuous light an increase in irradiance results in a more significant production of soluble intermediates and a greater turgor rise (Kromkamp et al., 1986).

The synthesis of gas vesicles is regulated at two levels, the molecular level through control of gene expression, and the physiological level by the availability of energy and structural components required for gas vesicle assembly. Evidence for molecular control has come from several sources. In light-limited cultures of *Aphanizomenon flos-aquae* the gas vesicle content per unit protein was regulated by the supply of energy so that cells were non-buoyant at all growth rates exceeding twenty percent of the maximum growth rate (Konopka et al., 1987; Kromkamp et al., 1988). Similarly, when *Oscillatoria agardhii* was shifted from low to higher light intensities the resulting buoyancy loss was explained by a dilution of gas vesicles as cell volume increased but without a matching increase in gas vesicle synthesis (Utkilen et al., 1985). In *Pseudanabaena* sp. the expression of a gene for one of the gas vesicle proteins was found to be regulated at the transcriptional level, with the abundance of mRNA inversely correlated to irradiance intensity (Damerval et al., 1991). In each of these cases photo-regulation of gas-vesicle genes may explain the altered rate of production of gas vesicles and the resulting change in buoyancy. However, much more information is needed on these molecular mechanisms before their relative importance can be ascertained, as not all cyanobacteria react in the same way. For example, cultures of *Microcystis aeruginosa* responded to an increase in the limiting energy supply by increasing gas vesicle protein synthesis in

proportion to total protein synthesis (Thomas and Walsby, 1985; Kromkamp et al., 1988).

Physiologically induced alterations in gas vesicle synthesis include the effects of nitrogen limitation on the production of proteins for gas vesicle assembly. Sustained limitation results in reduced gas vacuolation and a loss of buoyancy (Klemer et al., 1982). Sustained carbon limitation can also result in reduced cell buoyancy due to a restriction in the energy available for synthesis of gas vesicles (Klemer, 1991).

The relative importance of these various responses differs not only between strains of cyanobacteria, but also within strains, depending on both their physiological status and the prevailing environmental conditions. Of key importance is the relative availability of irradiance and the major nutrients, carbon, nitrogen and phosphorus. Although this complexity makes it difficult to provide a functional description appropriate for all gas-vacuolate cyanobacteria under all conditions, the interplay of factors can be described to demonstrate the nuances of buoyancy regulation (Fig. 3). A useful starting point is the observation that the accumulation of carbohydrate as polysaccharide is the result of excess photosynthate production over its incorporation into other compounds (Gibson, 1978; Konopka, 1984). The balance between accumulation or incorporation is altered by light intensity and nutrient deficiency (Healey, 1978).

Short-term, periodic carbon limitation leads to increased buoyancy as cells utilise the carbohydrate reserves accumulated during prior periods of nutrient limitation or excess energy supply (Fig. 3). These cells either already have sufficient gas vesicles for buoyancy, or use the energy reserves to synthesise the extra gas vesicles required (Klemer, 1991). As noted earlier, sustained carbon limitation can result in reduced gas vesicle synthesis and reduced buoyancy through a reduction in gas vesicle synthesis (Fig. 3).

Under nutrient limited conditions the level of excess energy that is accumulated as photosynthate is a function of the irradiance captured relative to the energy requirements of the cells at their nutrient-restricted growth rate. The relative growth rate (RGR) provides a means of comparing the effects of subsaturating levels of two essential growth factors on cell physiology (Konopka, 1989). The specific growth rate μ is limited by one factor, while the potential maximum growth rate under the environmental conditions μ_{max} is set by the second factor. When light and nutrient supply rates are balanced, then the relative growth rate given by μ/μ_{max} is high, even if the specific growth rate is low.

If growth is restricted by a limiting nutrient then energy capture exceeds that utilised in the nutrient restricted growth. As light supply is greater than that required to maintain the RGR_{max} carbohydrate is stored (Fig. 3). Konopka and Schnur (1980) obtained carbohydrate to protein ratios four to seven times higher in cultures limited by nitrogen, phosphorus or sulphur, than in non-limited cultures or those limited by carbon. In general, buoyancy decreases when major nutrients such as phosphorus or nitrogen limit cell growth because carbohydrate accumulates, but associated turgor pressure increases can also collapse gas vesicles especially if there is an accompanying rise in hydrostatic pressure due to cell sedimentation (Reynolds and Walsby, 1975; Klemer, 1978, 1991; Konopka, 1984; Walsby, 1987). The degree of gas vacuolation may also be reduced through molecular controls on gas vesicle production but this is species specific (SS) as shown in Fig. 3. If nutrient limitation greatly depresses growth rate, then carbohydrate accumulation and buoyancy loss can occur even at low light intensities. Under severe and sustained nitrogen limitation reduced gas vesicle synthesis results in reduced buoyancy (Fig. 3).

When all nutrients, including carbon, are present in abundance, then the buoyancy of the organisms is largely a function of the irradiance intensity relative to the growth requirements of the cells (Fig. 3). If the irradiance captured is less than that required to achieve the maximum growth rate under the prevailing environmental conditions, then energy supply will be low relative to what could be utilised and carbohydrate reserves will be reduced and cell buoyancy increased. In organisms like *Aphanizomenon flos-aquae*, where the degree of gas vacuolation is a function of the limiting energy supply, molecular processes will increase gas vesicle synthesis and enhance the positive buoyancy response (Utkilen et al., 1985; Konopka et al., 1987; Kromkamp et al., 1988; Damerval et al., 1991). As discussed earlier for *Microcystis aeruginosa*, not all organisms necessarily show this molecular response to light changes and in Fig. 3 it is depicted as being species specific (SS).

If irradiances are sufficient to support growth rates close to maximum under nutrient sufficient conditions, then cellular composition will approach a mean elemental ratio similar to the Redfield ratio (106 C:16 N:1 P by atoms) indicative of cells growing without nutrient limitation (Hecky and

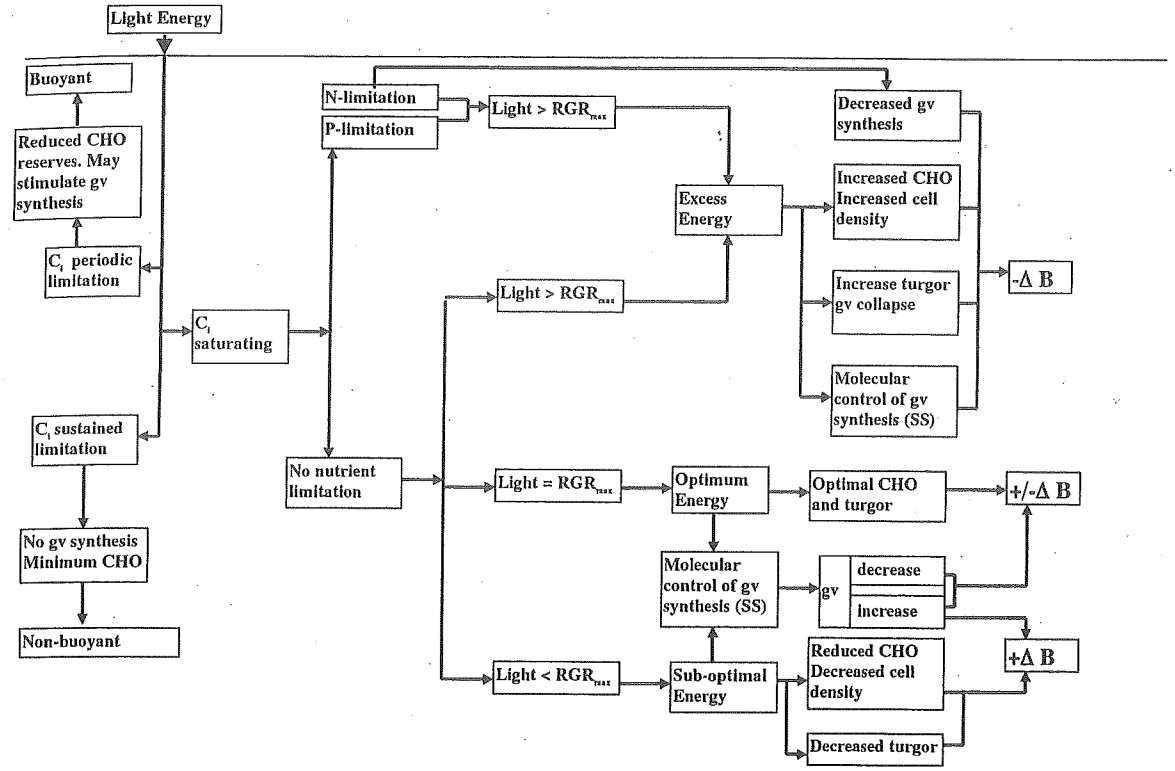

Fig. 3 Flow chart of the major factors influencing gas vesicle production and buoyancy regulation.
Key: C_i inorganic carbon, CHO carbohydrate, gv gas vesicle, N nitrogen, P phosphorus, *RGR_{max} maximum relative growth rate, SS species specific indicating the response is not universal, ΔB change in buoyancy which can be positive (+), negative (-) or either (+/-) depending on species.

Kilham, 1988; Hecky et al., 1993). The buoyancy status of cells under these balanced growth conditions is species-specific (Fig. 3), with *Aphanizomenon flos-aquae* being non-buoyant (Konopka et al., 1987; Kromkamp et al., 1988) and *Microcystis aeruginosa* buoyant (Kromkamp et al., 1988). Presumably the buoyancy status is set by molecular controls on cellular structure.

At irradiances above those saturating nutrient sufficient cell growth the responses are similar to those found under nutrient limitation. The carbohydrate store increases with irradiance up to a maximum when photosynthesis is saturated. This results in an enlarged carbohydrate store which increases cell density, while molecular controls in some organisms reduce the rate of gas vesicle synthesis (Fig. 3). If turgor pressure increases sufficiently then gas vesicle collapse can occur, although this may not be sufficient on its own to reduce buoyancy in cells acclimatised to normal light:dark cycles (Oliver and Walsby, 1984; Kromkamp et al., 1986).

The relationships between nutrients, energy, growth and buoyancy described in Fig. 3, show that gas vesicle regulation is not just related to cell growth, but also depends on the factors controlling growth (Konopka et al., 1987). At times the interaction between these factors is complicated, particularly under natural conditions where there can be large changes in nutrient and irradiance conditions over short periods that affect the utilisation and re-supply of cellular stores of nutrients and energy.

V. Mixing Regimes and Cyanobacteria

Buoyancy maintains cyanobacterial cells in suspension, while its regulation enables them to move vertically through the water in response to changing

growth conditions. However the extent to which gas-vacuolate cyanobacteria can control their vertical distribution is also a function of the turbulent mixing regime. Steinberg and Hartmann (1988) analysed cyanobacterial distributions across a number of water bodies and came to the conclusion that turbulence in lakes and rivers should be regarded as a special quasi-resource that can be differentially exploited by various phytoplankton in a manner analogous to nutrients or light (Reynolds and Walsby, 1975; Harris, 1986). Over the last two decades research on mixing processes has led to significant advances in understanding of the effects of turbulent mixing on the growth and distribution of phytoplankton, and particularly in selecting for gas-vacuolate cyanobacteria.

A. Floating and Sinking under Quiescent and Turbulent Mixing Conditions

In quiescent waters the sinking or floating rate of phytoplankton can be calculated from the Stokes equation, modified if necessary by the inclusion of a "form factor" that adjusts for the non-spherical shape of some organisms:

$$v = 2gr^2(\rho' - \rho) / 9\eta\theta \qquad [1]$$

where:

the terminal velocity of the organism (v), is dependent on gravitational acceleration (g), the size of the organism represented as the radius (r) of a sphere of equal volume, the density of the organism (ρ') and the density (ρ) and the viscosity (η) of the medium. The form resistance factor ϕ is defined as v/v_S where v_S is the terminal velocity of a sphere of equal volume and density to that of the organism.

For some shapes the form factor is known from empirical relationships (McNown and Malaika, 1950; Davey and Walsby, 1985), but generally it has to be determined after measuring all other variables (Oliver et al., 1981). The Stokes equation is suitable for calculating sinking and floating velocities if the assumption of laminar flow is not violated, and this will be the case provided the particle-Reynolds number ($Re=2rv\rho/\eta$) does not exceed 0.5 (Walsby and Reynolds, 1980; Reynolds, 1987). As a consequence, the equation will not be reliable for large phytoplankton colonies where $r > 300\ \mu m$ (Reynolds, 1987).

The Stokes function explicitly identifies major features that influence the floating and sinking rate of phytoplankton. Velocity is greatly enhanced by a large size as it is related to the square of the particle radius. The direction of movement, as well as the velocity, is a function of the density difference between particle and surrounding medium, while the shape of the particle may enhance or retard its motion. The large size range of the cyanobacteria, coupled with their ability to alter density, is reflected in a wide range of floating and sinking velocities (Fig. 4). In contrast most freshwater eukaryotic micro-algae have a cell density greater than that of water and only the motile, flagellate species have any means of negating their propensity to sink. A notable exception to this is *Botryococcus* that can become buoyant after producing and accumulating oils.

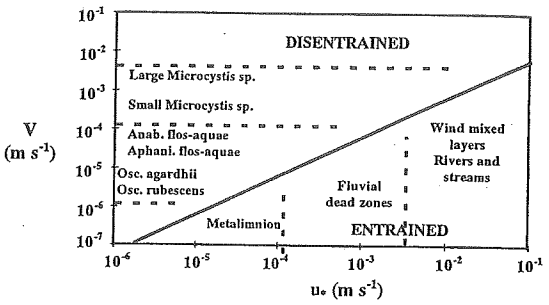

Fig. 4 A comparison of floating and sinking velocities (v) of selected cyanobacteria with the characteristic velocity of turbulence (u). The solid line separates regions of particle entrainment and disentrainment as defined by Eqn. 5. Values of u in different habitats provide perspective. (after Reynolds, 1994a).

B. Turbulence Intensity and the Mixed Layer Depth

Rarely will natural waters be so quiescent that the observed vertical rate of phytoplankton movement matches that expected from Equation 1. In open waters, where edge effects are minimal, vertical mixing is attributed to turbulence generated either by wind stress on the water surface, or to convective cooling due to fluctuations of temperature in the surface water (Spigel and Imberger 1987). Turbulent mixing in the water column acts to homogenise the vertical distribution of the phytoplankton by entraining slowly moving particles within the motion of the turbulence. If mixing is sufficiently intense it may negate advantages derived by populations using buoyancy regulation to make controlled vertical movements.

The characteristic velocity of turbulence can be envisaged either as the root mean square of the time-averaged vertical velocity fluctuations, $u = (\langle u'^2 \rangle)^{1/2}$ where angle brackets denote averaging, or as the rate of turbulent energy dissipation. Direct measurements of u' rely upon instruments of exceptionally high resolution such as laser or accoustic Doppler velocimeters and few direct measurements have been made in lakes. However, when wind speeds are moderate to low and the lake is not loosing heat the characteristic velocity (u) within the diurnal surface layer (Monismith et al. 1990) can be equated to the water friction velocity u_* estimated from the wind speed (Denman and Gargett 1983):

$$u_*^2 = \rho_a c U_{10}^2 / \rho \qquad [2]$$

where:

ρ_a is the air density (ca. 1.2 kg m^{-3}), ρ is the water density, c is the dimensionless drag coefficient (1.3 x 10^{-3}) and U_{10} is the wind speed at a height 10 m above the surface.

For typical densities, equation 2 simplifies to $u_* = 0.001U_{10}$. It must be emphasised that this estimate only holds when the lake is not losing heat and when wind speeds are low to moderate (<6 to 8 m s^{-1}). MacIntyre and her collaborators (pers. Com.), working with data from Lake Victoria (East Africa) and Lake Calado (Brazil), have shown that u will be 2 to 5 times higher than predicted by u, if a lake is losing heat or if windy conditions prevail.

The alternative approach is to take advantage of the relationship between the energy dissipation rate (ε) and u, $\varepsilon = u^3 / l$ where l is the scale of the overturning eddy (MacIntyre 1993). The rate of energy dissipation is routinely measured using temperature gradient or shear microstructure profilers, a technique more common in oceanography but gaining acceptance in limnology. The variability of turbulence in the upper mixed layer and thermocline over diurnal cycles is described in Brainerd and Gregg (1993), Imberger (1985), and MacIntyre (1993, 1996, 1998). Microstructure profiling allows discrimmination of the parts of the upper mixed layer that are mixing from those that are not and allows assessment of the variability of u as a function of depth.

The vertical penetration of mixing energy is resisted by the formation of a heated, buoyant surface layer (Spigel and Imberger, 1987; Reynolds et al., 1987; Reynolds 1989a, b, 1990). The depth of this layer is a function of the extent of solar energy penetration, heat capture and the degree of mixing. The depth of the mixed layer will tend to a point where the buoyant energy of the surface layer and the kinetic energy of the surface wind stress are balanced. The instantaneous balance between these two processes is described by the Wedderburn number (Imberger and Hamblin, 1982), which is the ratio of the energy per surface area from buoyancy to that generated by the shear forces;

$$w = gh^2 \Delta\rho_w / \rho u_*^2 L \qquad [3]$$

In this expression $\Delta\rho_W$ is the density difference between the water below the mixed layer and the water in the mixed layer, L is the length of the lake at the base of the mixed layer in the direction of the wind, and h is the depth of the diurnal thermocline (Spigel and Imberger, 1987).

When W>1 the structure is robust and resistant to further deepening unless there is a substantial change in $\Delta\rho_W$ or u_*, whereas if W<1 then the mixed layer deepens rapidly until h and $\Delta\rho_W$ are large enough to make W>1 (Spigel and Imberger, 1987; Reynolds, 1994a). Estimates of the depth of mixing (z_m) have been obtained from this function by setting W = 1 (Reynolds, 1989a); Ibelings et al., 1991a). More recently the suitability of equation 3 to predict the depth of the mixed layer has been questioned since it more correctly estimates whether or not the pycnocline will undergo full or partial upwelling and whether boundary mixing is likely. MacIntyre (pers. Com.) has suggested that the regions where the water column is mixing and the mixing intensity can be obtained more appropriately from modelling or from microstructure profiling.

The ratio between the depth to which 1% of light penetrates (euphotic depth, z_{eu}) and the depth of mixing is an important indicator of the light regime encountered by cells captured in the water motion. As the z_{eu}/z_m ratio declines below a value of one the proportion of time that the cells spend in the light decreases until eventually cells encounter insufficient light to grow.Consequently, in shallow turbid waters that are uniformly and continually mixed light may limit population growth as at any one time only a small proportion of the population can gain access to the euphotic zone. Conversely, in clear deep lakes the onset of thermal stratification increases the mean irradiance as the mixing depth decreases. If the z_{eu}/z_m ratio is greater than one in a thermally stratified lake then cells moving with the water are always in the

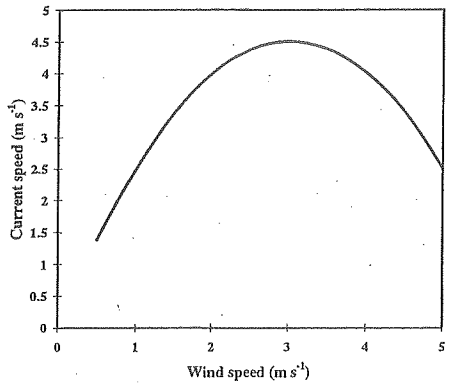

Fig. 5 Changes in the surface current speed (c_S) as a function of wind speed (U_a).

illuminated zone and light penetrates below the mixed layer into the thermocline.

C. Sinking Organisms

In considering the effect of turbulent mixing on the distribution of sinking organisms, Reynolds (1979) carried out field experiments measuring the settling of Lycopodium spores in large lake enclosures. The results demonstrated that the measured *effective* sinking rate of the spores under turbulent conditions was significantly less than the *intrinsic* sinking rate measured in quiescent water columns in the laboratory. Reynolds proposed a mixing model that envisaged a water column that was periodically fully mixed, but with a trapping zone at the bottom from which cells could not be resuspended. Smith (1982) developed a simple model to formalise these concepts and derived a general equation to calculate the number of particles remaining in suspension (N_t) after time t for conditions where organisms are fully entrained in the turbulence:

$$N_t = N_0 e^{-t/t_1} \qquad [4]$$

Here N_0 is the original number of particles in suspension and t' is the column clearance time, which for particles with a sinking velocity V in a water column of height H is t' = H/V.

Under turbulent conditions an infinite time is required to completely clear the water column of particles and a small number of cells may remain in suspension to seed subsequent growth phases (Smith 1982). Replacing t' with H/V in Eqn. 4 provides a means for measuring the intrinsic sinking or floating velocity of particles in a fully turbulent system (Smith, 1982; Reynolds, 1984a). Hutchinson and Webster (1994) have described and tested an apparatus for making these measurements.

The influence of a reduction in sinking rate on the number of cells retained within a mixed column of 5 m depth is illustrated in Fig. 6 for sinking velocities of 0.0036, 0.072 and 0.167 m h^{-1}. After two days at the slowest sinking rate 96% of the cells still remain in the water column, while at the fastest sinking rate only 20% remain. This example illustrates that gas vacuoles can provide a substantial ecological advantage at the population level, even if they do not provide sufficient lift to make cells positively buoyant but simply reduce the sinking rate. This may provide an explanation for the presence of gas-vacuoles in *Oscillatoria redekei* that are restricted in occurrence to the ends of the cells and do not appear to confer positive buoyancy.

The loss of sedimenting particles due to the presence of trapping zones at the lower boundary of a mixed layer occurs at all intensities of mixing, and represents a continual loss to non-buoyant or non-motile phytoplankton populations. In natural waters, particles will be removed from the mixing zone if they sediment into the thermocline where turbulence

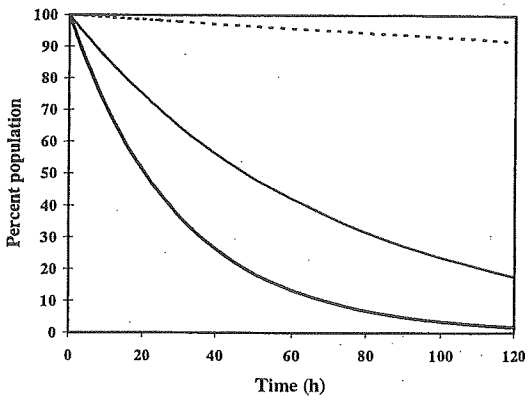

Fig. 6 Percent of the population retained within a 5 m, fully mixed water column. The three curves represent three sinking velocities; 0.0036 (upper), 0.072 (middle) and 0.167 m h^{-1} (bottom), over 120 h.

is small, or settle onto the substratum where water velocities approach zero. Buoyant cyanobacteria can avoid this loss entirely and so buoyancy alone, without the need for its precise regulation, can provide the cyanobacteria with a significant advantage over sedimenting phytoplankton. Furthermore, if a deep mixing episode moves part of the phytoplankton community to a depth below the level of the thermocline, then once stratification

reforms buoyant cells can move back into the surface mixed layer while sedimenting cells cannot (Humphries and Lyne, 1988).

D. Entrainment and Vertical Distributions

Mixing that fully entrains a phytoplankton population will homogenise its vertical distribution, but the degree of entrainment varies depending on the intrinsic velocity of the phytoplankton relative to the velocity of the turbulent motion. A function describing this interaction, and used to distinguish the extent of entrainment (ψ), was proposed by Humphries and Imberger (1982);

$$\psi = 15|v| / u \qquad [5]$$

where:

$|v|$ is the modulus (ie. the directional sign has been removed) of the sinking or floating velocity of the phytoplankton unit and u is the velocity of the turbulent eddies that transport the cells through the mixed layer (Humphries and Lyne, 1988).

This formulation differs from some published versions where the equation has been incorrectly transcribed (Humphries and Imberger, 1982; Reynolds, 1994a).

The size of u can be estimated by the shear velocity of u_* (see previous section). When $\psi < 1$, then water velocities are sufficiently large relative to the intrinsic sinking or floating velocities of the phytoplankton to entrain the cells within the water motion so that turbulence homogenises the vertical distribution of the population as envisaged in Eqn. 4 (Humphries and Imberger, 1982; Humphries and Lyne, 1988). When $\psi>1$, then the intrinsic velocity of the phytoplankton plays an increasing role in the population distribution. This begins to occur when the turbulent velocity falls below 15 times the phytoplankton velocity. In Fig. 5 the floating or sinking speed of selected organisms is compared with the mixing velocity using equation 5 to distinguish the degree of entrainment Estimated values of u_* in different habitats are indicated for comparison (Reynolds, 1994a). Single filaments of *Oscillatoria* with slow floating rates are entrained at all u values greater than those found in the metalimnion. In contrast large colonies of *Microcystis* can continue to utilise buoyancy to alter their vertical distribution within the mixing zone up to u values typical of reasonably well mixed layers. At u values in excess of 10^{-2} m s^{-1} all forms of cyanobacteria are entrained in the water motion and will have uniform vertical distributions.

Field data to test critically this relationship are scarce, but in Lake Vinkeveen in The Netherlands, Ibelings et al. (1991a) found 30% less floating colonies of the gas-vacuolate cyanobacterium *Microcystis* near the surface than at the bottom of the mixed layer, when the shear velocity u was only five times the maximum floating velocity of the *Microcystis*. Presumably the poor entrainment of colonies in the water motion had resulted in a separation between the top and bottom populations.

When $\psi > 1$ floating or sinking rate controls cell distribution, while if $\psi < 1$ diffusive transport becomes increasingly important in determining cell distribution (Reynolds et al., 1987). Buoyant colonies of the gas vacuolate cyanobacterium *Microcystis* sp. can have rapid floating rates (up to 250 m d^{-1}) compared to the generally slow sinking rates of many non-buoyant micro-algae (ca. 1 m d^{-1}). Simulation studies predicted the occurrence of top-heavy vertical profiles of buoyant *Microcystis* for which $\psi>1$, while under the same conditions the diffusion dominated distribution of sinking cells ($\psi < 1$) resulted in an even distribution with depth (Humphries and Imberger 1982; Humphries and Lyne 1988). Assuming an equivalent growth response to changes in light intensity the floating populations had higher production rates than sinking populations since a larger proportion encountered increased irradiance in the upper layers. In addition the simulations showed that the depth integrated growth rate of floating species closely followed the changes in mixing depth, while sinking cells were dispersed by deep mixing episodes and suffered a significant population loss when the water column re-stratified. The model predicted that diurnal cycles of mixing and stratification would provide rapidly floating cells with a significant growth advantage. Walsby et al (1997) carried out a quantitative analysis of the benefit of flotation to the primary production of *Aphanizomenon flos-aquae* over a 9-day period in the Baltic Sea. The analysis demonstrated that, averaged over the alternating periods of calm and mixing, buoyancy provided by gas vacuoles increased the daily net areal photosynthesis of the cyanobacterial population by nearly two-fold.

Currents generated by wind mixing not only influence the vertical distribution of phytoplankton, but also advect particles from one location to another causing horizontal and vertical heterogeneity of algal populations on both a local and basin scale (George

and Edwards, 1976; Reynolds, 1984a; Stauffer 1988). The influence of wind-induced currents on particle distributions is a function of particle buoyancy and water velocity. Neutrally buoyant particles will be randomly distributed, while positively buoyant particles tend to become concentrated in regions of downwelling water, and negatively buoyant particles tend to concentrate in upwelling regions (Stommel, 1949; George and Edwards, 1976; Webster, 1990). This horizontal heterogeneity has been described empirically by George and Edwards (1976) for Eglwys Nynydd, a shallow eutrophic lake in Wales, and modelled by Webster (1990) and Webster and Hutchinson (1994). The model describes the dependence of the horizontal and vertical distribution of particles on their floating or sinking rate, the wind speed, and the depth and fetch of the lake. This heterogeneity can be a major problem to monitoring programs aimed at describing seasonal population changes in phytoplankton and quantifying biomass dependent measurements such as phytoplankton production (Horne and Commins, 1989).

E. Surface Accumulations and Turbulence

The concepts describing particle sedimentation losses from mixed layers into low turbulence trapping-zones can also apply to floating particles provided there is a surface "trapping" layer of reduced vertical dissipation, such that buoyant cells are not re-entrained into underlying turbulent layers (Webster and Hutchinson, 1994). It has long been recognised that wind speeds of ca. 3 m s^{-1} mark a significant transition in the near-surface mixing regime that can be observed as a change in surface roughness (George and Edwards, 1976), the formation of waves (Reynolds, 1989a, b; Webster and Hutchinson, 1994), and the development of Langmuir circulations (Reynolds 1987).

George and Edwards (1976) observed in Eglwys Nynydd that at high wind velocities mixing was sufficient to suppress the development of vertical patchiness in cyanobacterial populations and they were homogeneously distributed throughout the water column. Local surface concentrations of cyanobacteria only appeared at wind speeds below 4 m s^{-1}, and increased in occurrence as wind velocity declined. Webster and Hutchinson (1994) modelled mathematically the distribution of phytoplankton under different wind stress conditions, and estimated that a wind speed of >2-3 m s^{-1} was required to mix floating phytoplankton cells away from the water surface. They calculated that the surface viscous boundary layer at a wind speed of 2 m s^{-1} was 4 mm, sufficient to contain large colonies of buoyant cyanobacteria.

Although wind speeds less than ca. 3 m s^{-1} can lead to trapping of buoyant cells within the surface layer, accumulations will not necessarily become obvious. Apart from the capacity of the cells to increase their density and sink out of the surface layer, they can also be dispersed horizontally. At wind speeds below ca. 3 m s^{-1} the surface is hydrodynamically smooth and the vertical turbulent transfer of momentum is weak, so a larger proportion of the wind stress is converted to horizontal surface water velocities. Above ca. 3 m s^{-1} the vertical transfer of turbulence into the water column increases and surface current speeds are a smaller fraction of the wind speed (George and Edwards, 1976). As a result a wind speed of 2 m s^{-1} generates a surface current of 6 cm s^{-1}, while a wind speed of 5 m s^{-1} generates a surface current of only 3 cm s^{-1} (Webster and Hutchinson, 1994). The ratio of the surface current speed to the wind speed increases approximately linearly as wind speeds decline from 5 m s^{-1} to 0.5 m s^{-1} and this relationship was described empirically by George and Edwards (1976). Recasting their equation gives:

$$c_s = 3.002U_a - 0.5U_a^2$$

For

$$0.5 < U_a < 5$$

where:

c_S is the surface current speed (cm s^{-1}) and U_a is the wind speed (m s^{-1}).

The current speeds given by this empirical equation are similar to those calculated by Webster and Hutchinson (1994). Within its range the equation indicates that a maximum surface current speed of 4.5 cm s^{-1} (162 m h^{-1}) occurs at a wind speed of 3 m s^{-1}, with surface current velocities decreasing either side of this peak (Fig. 4). However, even a wind speed of 1 m s^{-1} generates a surface current of 2.5 cm s^{-1} (90 m h^{-1}) so that surface accumulations are quickly transported horizontally. Depending on lake size and the time required for buoyancy regulation, organisms may be transported to shallow waters before they can sink out of the surface layer. Consequently surface blooms are frequently seen at the down wind edges of lakes, and only under very calm conditions will they be widely spread over the lake surface (Plate 11b). Accumulations at the lake edge (Plate 11a) create the

highest risk for livestock poisoning and wind speeds of 0.5 to 3 m s^{-1} are critical to their formation.

F. Surface Blooms

The gas-vacuolate cyanobacteria that commonly produce surface blooms are those species that form large biomass units, either colonies as in *Microcystis*, or aggregates of filaments as in *Anabaena* and *Aphanizomenon*. These units are often more than 0.2 mm across and at times greater than 1 mm, so that their sinking or floating velocities are enhanced, and under calm conditions intense surface concentrations appear rapidly. Short-term, periodic surface blooms can occur as a result of responses to daily meteorological events or cyclical changes in cell density. For example, under calm conditions surface blooms frequently occur in the early morning as the respiratory demands during the hours of darkness consume the carbohydrate which acts as ballast against the upward lift provided by the gas vesicles. As the colonies encounter favourable light conditions, carbohydrate is accumulated and the units steadily gain weight until the gas vesicles can no longer provide the lift required for positive buoyancy and the colonies descend out of the surface scum. This explains why blooms sometimes tend to "disappear" in the afternoon and reappear in the morning as observed by Ganf (1974) in Lake George, Walsby and McAllister (1987) in Lake Okaro and Walsby et al (1983) in Lake Gjersjøen. Under certain conditions the continuous exchange of colonies at the water surface may give the mistaken impression of a persistent bloom, a scenario described in the computer model of Kromkamp and Walsby (1990). However, on occasions surface blooms do persist for several weeks and in some cases, such as the infamous hyperscums of Hartbeespoort Dam in South Africa, they can last for many months (Robarts and Zohary 1984; Zohary and Robarts 1989). In between these extremes surface blooms occur at various intervals depending on the interplay between buoyancy regulation and environmental conditions.

The occurrence of persistent surface blooms has been variously interpreted as a failure of buoyancy regulation due to physiological damage and senescence (Walsby 1994), a mechanism by which cyanobacteria can dominate surface waters (Paerl and Ustach, 1982; Paerl 1988a, b; Ganf et al. 1989), or the result of physical obstruction where the sinking colonies that have lost their buoyancy in the surface layers are impeded by the presence of underlying buoyant colonies (Ibelings and Mur 1992, Walsby 1994). This physical restraint on vertical movement has been demonstrated by Ibelings and Mur (1992) in very dense surface scums of *Microcystis* where surface colonies in the upper most millimetres lost buoyancy but buoyant colonies below acted as a barrier to their downward movement.

Surface blooms frequently occur in calm conditions following a period of deep mixing when the cells have become highly buoyant as a result of the low mean irradiance (Reynolds 1984a). Because of their excess buoyancy it takes longer to negate the lift provided by the gas vesicles and the cells may spend sufficient time at the surface to be physiologically damaged by the high light intensities. Such damage includes photoinhibition, photo-oxidation (Abeliovich and Shilo 1972) and dehydration (Zohary and Pais-Madeira 1990) and leads to cell senescence. This reduces population growth and photosynthesis (Ibelings and Mur 1992) and disables buoyancy regulation. The reduced ability to regulate buoyancy at the surface may also be related to inorganic carbon limitation in the thick scums. In this model surface blooms are due to an inability of organisms to adapt their buoyancy regulation to a sudden change in hydrological conditions.

The formation of surface blooms has also been attributed to inorganic carbon limitation. Klemer et al. (1996) using continuous cultures of *Microcystis aeruginosa* showed that CO_2 limitation can promote buoyancy in the short term by preventing both gas vesicle collapse and the accumulation of carbohydrate ballast. This supports the results of Walsby and Booker (1980), who found that carbon limitation caused *Anabaena flos-aquae* to float up and form a surface bloom in a stratified laboratory water column. An extension of this scenario is that cells in the centre of large colonies may be deprived of an adequate supply of inorganic carbon (Paerl, 1983) providing sufficient gas vesicle lift to overwhelm the ballast accumulated by cells on the periphery of the colony. Once carbon-limited cells are at the surface, physiological damage as a result of the high light intensities could then disable buoyancy regulation resulting in a persistent surface bloom.

Paerl and Ustach (1982) proposed that surface blooms were not just a consequence of poorly acclimated cells suffering from excess buoyancy, but were part of an ecological strategy aimed at making optimal use of photosynthetically active radiation and atmospheric carbon dioxide. From investigations on *Aphanizomenon flos-aquae* and *Anabaena*

oscillarioides they concluded that CO_2 was the preferred inorganic carbon source for sustaining high rates of photosynthesis and that the formation of surface blooms would provide access to the preferred form at the air-water interface. In poorly buffered waters, where pH values are frequently in excess of 9 indicating diminishing supplies of CO_2 due to sustained periods of intense photosynthesis, this would provide an ecological advantage over non-buoyant species. Paerl et al. (1985) showed that natural populations of *Microcystis* had optimal photosynthetic rates and resistance to photoinhibition at surface irradiances. However, Ibelings and Mur (1992) working at a very fine scales (0 - 3000 μm) suggest that the case is not yet proven and provided evidence to show that photosynthesis in *Microcystis* scums can be inhibited at the surface.

Surface blooms markedly influence the depth to which light penetrates the water column, largely as a result of scattering by gas vesicles (Walsby 1994). Scattering from the vertical increases the path length of photons through horizontal water layers thereby enhancing the probability of absorption within the near surface layers. Those organisms which have taken up preferential residence within the euphotic zone, such as buoyant cyanobacteria, therefore have a greater probability of intercepting the light. For example, Ganf et al (1989) showed that 80% of the light scattered in Mt. Bold Reservoir was due to *Microcystis aeruginosa* colonies and concluded that this provided *Microcystis* with a distinct advantage when in competition with non-buoyant species.

G. Metalimnetic Populations

Some species of gas-vacuolate cyanobacteria (eg *Oscillatoria* spp.) form deep water concentrations often at or near the thermocline. The size of the biomass unit forming these metalimnetic populations is small so that even though they regulate their buoyancy the rate at which they move is slow and the amplitude of the daily vertical movement is often restricted to a few centimetres. A general requirement for the formation of metalimnetic populations is that the depth of the illuminated zone exceeds the depth to which the water column is mixed by wind or convective cooling ($z_{eu} > z_m$). It has been proposed that the cyanobacteria stratify at a depth where the opposing gradients of light and nutrient availability result in neutrally buoyant cells (Klemer, 1976, 1978; Konopka, 1984, 1989). Species that form metalimnetic populations are low-light adapted and sensitive to variations in light intensity. Konopka et al (1993) found that at intensities of > 15 μmol m^{-2} s^{-1} buoyant filaments of *O. agardhii* in Deming Lake, Minnesota lost buoyancy within a few hours. Buoyancy also responds to nutrient conditions. If nutrient limitation becomes more severe then the depth at which the cells stratify increases because the light level provides an excess of energy for the reduced growth rate (Fig. 3) resulting in an increased carbohydrate accumulation and loss of buoyancy (Klemer 1976, 1978; Konopka 1984). Conversely, in metalimnetic populations artificially enriched with either nitrogen or phosphorus, the increased availability of a limiting nutrient caused the population to stratify at a shallower depth (Klemer 1976, 1978; Konopka 1989).

Metalimnetic populations do sometimes form into colonies by aggregation of filaments and this increases their sinking or floating velocities enabling them to perform vertical migrations comparable with the colonial species. This was observed for *Oscillatoria agardhii* in Lake Gjersjøen (Walsby et al. 1983). The variable responsible for inducing the filament aggregation has not been identified.

H. Major Habitats Structured by Turbulent Mixing

Reynolds and Walsby (1975) suggested that different forms of gas-vacuolate cyanobacteria are adapted to occupy water masses with different mixing regimes. Reynolds et al (1987) reviewed the occurrence of these cyanobacteria and suggested that interactions between the size of the morphological unit, its shape and its density were selected for by habitats with the hydraulic conditions which optimised for these features. Subsequently, evidence supporting this notion has accumulated (Steinberg & Hartmann, 1988) permitting some further refinement of the concept (Walsby et al., 1989).

On the basis of the z_{eu}/z_m ratio water bodies can be divided into those where $z_{eu} \leq z_m$ and those where $z_{eu} > z_m$.

A. In waters where the euphotic depth is approximately equal to, or less than, the mixing depth but the z_{eu}/z_m ratio is not so small that light is insufficient to support growth, two principal patterns of mixing support different cyanobacterial populations:

1(a). Shallow, productive waters that are frequently fully mixed support populations of non-aggregated, filamentous cyanobacteria (Plate 11d) such as

Oscillatoria spp. (Gibson et al., 1988; Scheffer et al., 1997).

1(b). An analogous mixing regime occurs in deeper lakes that are thermally stratified and have an intense seasonal thermocline, but where the surface layer is consistently well mixed. These too are frequently inhabited by cyanobacteria consisting of small solitary filaments, a unique example being *Anabaena minutissima* in Lake Rotongaio, New Zealand (Walsby et al., 1989).

2(a). In shallow, productive waters, where thermal stratification of the water column occurs diurnally or occasionally persists for longer periods, the larger morphological forms of cyanobacteria occur, including colonial forms such as *Microcystis aeruginosa* (Ganf, 1974; Ibelings et al., 1991b), and aggregated or contorted filamentous forms such as *Aphanizomenon* and *Anabaena* (Plate 11f).

2(b). An analogous mixing regime occurs in those deeper lakes where intense thermal stratification of the upper mixed layer is common, often occurring on a diurnal or even longer time period, and where mixing episodes are contained within the depth of the seasonal thermocline. These environments also contain the larger morphological forms of cyanobacteria.

B. In less productive lakes where the euphotic depth exceeds the extent of the surface mixed layer ($z_{eu} > z_m$) and a strong seasonal thermocline develops within the euphotic depth, a stable, illuminated zone is formed below the mixed layer. Under these conditions metalimnetic populations of cyanobacteria can develop that are concentrated over a narrow depth range. Usually these are comprised of solitary filamentous forms eg. species of *Oscillatoria*, *Anabaena* or *Aphanizomenon* (Edmondson, 1970; Klemer, 1976; Walsby et al., 1983; Konopka et al., 1993), but metalimnetic populations of other forms including *Microcystis*, have been recorded (Ward and Wetzel, 1980).

These patterns suggest that the smaller forms of cyanobacteria occur in conditions where the importance of buoyancy regulation is reduced, either in situations where buoyancy regulation is overwhelmed by mixing or when the organisms are holding station below the depth of the mixed layer. In contrast, when the duration of thermal stratification is prolonged and the utility of buoyancy regulation is enhanced, then the larger forms of cyanobacteria become more prevalent. In essence morphology reflects the advantage provided by buoyancy regulation and this is dependent on the mixing regime.

The genera of gas-vacuolate cyanobacteria do not separate out strictly on the basis of these three habitat categories as variations in form and function occur within and between taxa. In particular some of the filamentous species, such as *Oscillatoria agardhii* (Walsby et al., 1983) and *Aphanizomenon flos-aquae* (Lynch, 1980; Ganf, 1983), can occur either as single filaments in metalimnetic populations or as large aggregates in epilimnetic populations (Walsby et al., 1983). Even within a morphological type there is a mix of species with quite different physiological characteristics. For example, some filamentous forms can fix molecular nitrogen, while others cannot. Differences between the three species *Oscillatoria rubescens*, *O. agardhii* and *O. redekei* appear to separate their occurrence between mixing regimes. *O. rubescens* occurs more commonly as metalimnetic populations, while *O. agardhii* can form metalimnetic populations (Edmondson 1970, Klemer 1976, Konopka et al. 1993), but also occurs in well mixed layers (Brook et al. 1971, Gibson et al. 1988). *O. redekei* appears to be restricted to shallow, unstratified lakes, probably because it has too few gas vesicles for positive buoyancy (Whitton and Peat 1969, Meffert and Krambeck 1977). *O. agardhii* and *O. redekei* may co-occur as in the shallow, completely mixed Lough Neagh (Gibson et al. 1988).

VI. Physical Control of Cyanobacteria

The interaction between mixing processes, buoyancy regulation and species composition, suggests that lake restoration via manipulation of the turbulent environment is a viable lake management strategy.

A. Collapsing Gas Vesicles in Lakes

If cyanobacterial cells are artificially or naturally circulated to a sufficient depth then hydrostatic pressure will cause gas vesicle collapse. A cell circulating to 40 m would experience a hydrostatic pressure of 0.4 MPa which could be sufficient to collapse a significant proportion of the gas vacuoles if cells were highly turgid. Cells with lower turgor would require a proportionately greater depth of mixing. If a lake is continuously mixed the cells without gas vacuoles will be entrained in the motion and the effective sinking rate will be reduced. The time to eliminate particles from a mixed column is ca. 4.6 times longer than predicted from still water

settling (Reynolds 1984). If mixing is artificially induced then intermittent circulation maybe more appropriate than continuous circulation (Steinberg and Gruhl 1992; Visser et al. 1996). This would allow non-buoyant cells to sediment more rapidly below the zone of circulation and into darkness where the synthesis of vesicles is much slower being dependent upon stored energy reserves. Deacon and Walsby (1990) found that at an optimal irradiance of 30 µmol m^{-2} s^{-1} it took *Microcystis* three days before buoyancy recovered. Intermittent circulation would also provide an opportunity for buoyant cells to move towards the illuminated surface layers where turgor pressure is likely to increase prior to the next mixing event. Kinsman et al. (1991) found that turgor pressure increased from 0.3 to 0.54 in *Anabaena flos-aquae* after 16 h at a photon irradiance of 135 µmol m^{-2} s^{-1}.

An alternative strategy to lake mixing and adopted by the former Suffolk Water Board in England is to abstract water from the lake and circulate it through a 86 m deep pipe where the hydrostatic pressure at the bottom is sufficient to collapse all gas vacuoles in *Microcystis aeruginosa* (Clarke and Walsby 1988). Alternatively Walsby (1992) noted that Menday and Buck (1972) had successfully used explosions to create shockwaves to collapse gas vacuoles, but the side-effects of this treatment were not always acceptable, particularly due to their effect on other organisms.

B. Artificial Mixing and Cyanobacterial Growth

Steinberg and Hartmann (1988) concluded from an analysis of a number of water bodies that above a threshold total-phosphorus concentration of 10 mg m^{-3} the occurrence of cyanobacteria was dependent largely on physical factors, particularly the degree of water column stability. On the basis of their investigations they supported the widely held view that physical manipulation of the mixing regime is likely to be a quicker and more effective means of managing cyanobacterial populations than either nutrient reductions or biomanipulation. In particular manipulation of the turbulent environment in an appropriate manner should promote a species shift away from cyanobacteria towards other species.

A number of papers have reported a reduction in the growth of *Microcystis aeruginosa* in response to mixing (Toetz, 1981; Reynolds et al., 1984; Visser et al., 1996). Lake Nieuwe Meer is hypertrophic and dominated by *Microcystis* during the summer. To overcome this problem artificial mixing was installed (1992 & 1993) and the results compared with the previous two years (1990 and 1991) without artificial mixing (Visser et al., 1996). The concentration of chlorophyll fell from an average of 23.3 to 8.9 mg m^{-3}, but this was principally due to a dilution effect as the mixed depth increased. When the results were expressed as mg m^{-2} the artificially mixed years had an average chlorophyll of 208 mg m^{-2} compared with 110 mg m^{-2}. The increased overall biomass was accompanied by a major shift in species composition. In the two years without artificial mixing cyanobacteria dominated from July to September but with the introduction of mixing *Scenedesmus*, centric diatoms and flagellates dominated.

In 1993 artificial mixing had been continuous from March to September, whereas during 1994, to reduce energy costs, mixing was applied intermittently and controlled by the surface and bottom temperatures and oxygen concentrations. If greater than 2°C or 2 g m^{-3}, respectively, the compressor was switched on, and if the difference was <1°C or 1 g m^{-3}, it was switched off. This gave an annual saving of 27%. *Microcystis* failed to develop in either of the years when artificial mixing took place and *Anabaena* numbers per m^{-2} decreased but the numbers of *Aphanizomenon* and to a less extent *Aphanocapsa* were greater. This observation suggests that uncritical adoption of artificial destratification to manage cyanobacteria is unwise, although it can cause major shifts in species composition (Steinberg and Zimmermann 1988). In a review of the effectiveness of artificial destratification in 52 Australian reservoirs McAuliffe and Rosich (1989) found that it failed to satisfactorily control phytoplankton in over 60% of cases.

Steinberg and Gruhl (1992) reported on a series of experiments investigating physical measures to inhibit planktonic cyanobacteria in Fischkaltersee a small Bavarian lake (3.4 ha and 5.7 m mean depth). Permanent destratification initially resulted in positive responses with almost complete removal of the cyanobacteria but nearly a doubling in the biomass of chlorophytes and diatoms. However, *Limnothrix* reappeared in the third year and reached a peak biomass in the fourth year of the treatment that was higher than had occurred prior to destratification being used. It was concluded that although the continuous mixing produced relatively constant mixing patterns with a low mean irradiance the lake was not sufficiently deep to reduce the mean

irradiance below that required by the Oscillatoriaceae. Intermittent destratification was instituted on the basis that it should elevate biomass losses via sedimentation and improve the light climate as a consequence of diminished phytoplankton biomass and this would curtail the low light adapted cyanobacteria. Under this regime *Limnothrix* peaked only for a few days and was outcompeted by the chlorophytes and diatoms as had occurred initially with continuous destratification. In contrast to permanent mixing the cyanobacteria did not reappear and the biomass of algae remained low.

C. Mixing in Regulated Rivers

Manipulation of mixing regimes is not confined to still water bodies but can also be used in regulated rivers. Burch et al (1994) and Webster et al (1996) have shown that the occurrence of *Anabaena circinalis* is a function of discharge rates in regulated rivers. At Maude Weir on the Murrumbidgee River, Australia, flows below 500 to 1000 M L d^{-1} caused a decline in the population density of the diatom *Aulacoseira* (*Melosira*) *granulata*, while *Anabaena circinalis* increased (Sherman et al.,1998). This shift in species composition was explained by changes in thermal stratification and mixing. Discharges < 1000 M L d^{-1} permitted the development of persistent stratification (6°C over 5m), and the cooler river water at the upstream end of the pool plunged beneath the stratified epilimnion discharging via the underflow weir. These conditions resulted in epilimnetic water velocities close to zero providing a retention time sufficient to permit *A. circinalis* growing at a net rate of 0.37 d^{-1} to develop a significant biomass over a period of one to two weeks. With the persistent stratification the buoyant *Anabaena* accumulated in the top 1-2 m, while negatively buoyant *Aulacoseira* sank and accumulated in the bottom waters. As the river has a euphotic zone of only 1.5 m and a maximum depth of 6 m ($z_{eu}/z_m = 0.25$), this provided a distinct advantage to the cyanobacterium.

The emergence of *Anabaena* as the dominant genus was attributed to nitrogen limitation which had been demonstrated in the *Aulacoseira* population prior to its demise using a physiological assay based on chlorophyll-a fluorescence (Wood and Oliver 1995). It was suggested that nitrogen limitation provided conditions suitable for *Anabaena* to dominate rather than a non-nitrogen fixing cyanobacterium. During a similar low flow period the *A. circinalis* population reached a plateau as the total reactive and filtrable reactive phosphorus fell below <10 mg m^{-3} suggesting that phosphorus availability may have controlled the population maximum (Webster et al. 1996).

Burch et al (1994) in the South Australian section of the River Murray demonstrated that populations of *A. circinalis* occurred in the river when discharge was small enough to permit thermal stratification. More importantly the drop in river levels (due to irrigation demand at low flows) allowed water from the neighbouring wetlands (billabongs) to flow back into the main channel carrying with it significant numbers of *A. circinalis* (P. Baker, pers. comm.). In this system it appears as though the main inoculum of *A. circinalis* comes from the calm, adjacent wetlands.

VII. Cell Size, Growth Rate and Temperature

In searching for clues to explain the occurrence and abundance of gas-vacuolate cyanobacteria efforts have been made to identify adaptive advantages that they might have over competing eukaryotic micro-algae. This has led to a number of generalisations which more recent studies now suggest are inaccurate or inappropriately applied.

The generalisation that cyanobacteria are smaller than their eukaryotic counterparts is not supported by comparative measurements of planktonic units (cells, filaments or colonies) since they fall within the same size range (Foy 1980; Reynolds 1989a, 1993). This is an important similarity as the rates of many cellular functions are strongly governed by the surface area to volume ratio.

The maximum growth rates of cyanobacteria and micro-algae, standardised for their surface area:volume ratio and measured in cultures at 20°C are similar (Reynolds 1989a, 1993), ranging from ca. 0.4 to 2.0 d^{-1}. The changes in specific growth rate between 10 and 20°C for light and nutrient saturated cultures of cyanobacteria and micro-algae (q_{10} values) are also similar (Reynolds, 1989a), although for any given surface area to volume ratio the q_{10} for cyanobacteria was on average lower than that of green algae, but overlapped that of diatoms. A marked exception to this was *Microcystis* which had a large q_{10} value of ca. 9, and also a higher minimum temperature for growth (Thomas and Walsby, 1986; Robarts and Zohary, 1987).

The belief that cyanobacteria prefer higher temperatures is based mainly on field studies and seasonal correlations, but these may be spurious as

high temperatures are also associated with thermal stratification and changes in turbulent mixing that provide an environment conducive to the gas-vacuolate cyanobacteria. Foy et al. (1976) concluded that the temperature optima of cultures of *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* fo. *gracile*, *Oscillatoria agardhii* and *Oscillatoria redekei* were similar to those of other planktonic autotrophs.

It is not surprising that there are similarities between cyanobacteria and micro-algae, as both groups are adapted to planktonic growth. However, within each group, individual species have developed attributes suitable for particular environmental niches and the task is to distinguish the significant adaptations that differentiate between competitors. This necessarily requires an understanding of the environmental conditions pertinent to the growth of the organisms. The observation that cyanobacteria can at times dominate waters to the virtual exclusion of micro-algae suggests that the search for characteristics of the cyanobacteria that set them apart is still a valid pursuit.

VIII. Light Capture

In addition to its crucial role in controlling buoyancy regulation in cyanobacteria, direct competition for light energy will also influence their likely success. Cyanobacteria can be distinguished from all other eubacteria by their ability to carry out oxygenic photosynthesis in a manner closely resembling that of eukaryotic photoautotrophs. As in the eukaryotic micro-algae, cyanobacteria have two connected photosystems that provide energy and reductant largely for fixation of CO_2 by the Calvin cycle. Although the chlorophyll-a containing reaction centres of photosystem I (PSI) and photosystem II (PSII) are similar in cyanobacteria and micro-algae, the major antennae, or light harvesting complexes (LHC), that capture the incident PAR are quite different (Ormerod, 1992; Grossman et al., 1995). In the micro-algae the antenna is integral to the thylakoid membrane and comprised largely of accessory chlorophylls (b and c), whereas the major LHC in the cyanobacteria is the phycobilisome (PBS), a hemispherical structure attached to the periphery of the thylakoid membranes. The PBS has markedly different attributes compared to the antennae of micro-algae (Grossman et al., 1995).

The blue-green colour typical of many freshwater cyanobacteria is due to the presence of pigments called phycobilins. These pigments are associated with proteins and arranged in the phycobilisome in a distinctive order (Glazer, 1982; Ormerod, 1992; Grossman et al., 1995). The three major phycobilins, and their absorption maxima, are allophycocyanin (AP, A_{max}= 650 nm), phycocyanin (PC, A_{max}= 620 nm) and phycoerythrin (PE, A_{max}= 565 nm). All cyanobacterial phycobilisomes contain allophycocyanin and phycocyanin. In some cyanobacteria, especially the red coloured phycoerythrin containing species, the proportions of these pigments can be altered to increase the absorption of light of specific wavelengths (Tandeau de Marsac and Houmard, 1993).

The phycobiliproteins absorb PAR over a much wider range of wavelengths than the antennae of the micro-algae (Glazer et al., 1994), particularly in the region between the absorption bands of the accessory chlorophylls b and c, and the carotenoids (Fig. 7). This is a fundamental difference between cyanobacteria and the eukaryotic micro-algae and is likely to be of distinct advantage in situations where either the spectral quality of the underwater light is

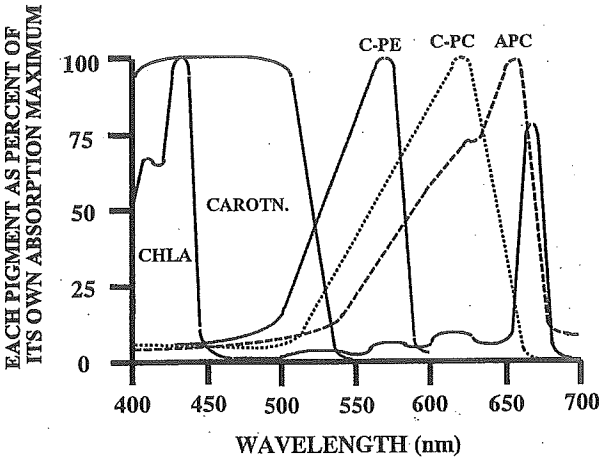

Fig. 7 Absorption spectra of chlorophyll-a (chla), carotenoids (carotn.), and the phycobiliproteins, c-phycoerythrin (C-PE), c-phycocyanin (C-PC) and allophycocyanin (APC) compiled from a number of sources. The absorption spectrum for each pigment is shown relative to its absorption peak.

concentrated in these wavebands, or when there are substantial fluctuations in light quality over time.

The underwater light climate changes both in quantity and in quality with depth. The intensity decreases exponentially as a result of absorption and scattering by particles and coloured compounds, and the selective removal of wavelengths causes shifts in the spectral distribution (Kirk, 1983; Oliver and Ganf, 1988; Oliver, 1990; Kirk and Oliver, 1995). Water

absorbs strongly in the red so that in marine systems and very clear inland waters where the majority of light attenuation is due to water, the irradiance becomes dominated at depth by shorter wavelengths. In contrast, many inland and coastal waters contain dissolved organic compounds and suspended inorganic particles that absorb strongly in the blue, causing a shift towards longer wavelengths with depth (Fig. 8). Phytoplankton also modify the spectral distribution of light. For example, high concentrations of green algae leave an orange-green window of irradiance where their pigments absorb poorly (Kirk, 1983). These wavelengths are suitable for absorption by the phycobiliproteins and as a result microalgae may modify the spectral distribution of light at depth to the advantage of cyanobacteria. The converse argument is not so forceful. Cyanobacteria will not modify the spectral distribution of light to the distinct advantage of micro-algae because they absorb over a wide range of wavelengths including those utilised by the major chlorophyll pigments of the micro-algae.

As a result of these depth-dependent variations in irradiance, phytoplankton cells that move vertically, either as a result of turbulent mixing or through buoyancy regulation or motility, will encounter changes in the intensity and spectral distribution of photosynthetically active radiation (PAR). The vertical extent, and duration of the movement will also affect the periodicity of the PAR supply. If cells remain within the euphotic zone during mixing (ie. $z_{eu} \circ z_m$), then the periodicity of the irradiance supply is determined by day length, whereas if mixing is sufficient to move the cells below the euphotic depth ($z_{eu} < z_m$), then shorter light-dark cycles will be superimposed on a daily light cycle.

The problem of quantifying the light field experienced by individual calls is complicated by the depth dependent nature of both the light field and the characteristic velocity of turbulence (u) as well as the intrinsic sinking or floating velocities of cyanobacteria. Although not always appreciated, the upper mixed layer often does not circulate fully. On calm days, only the phytoplankton near the very surface will circulate due to slight wind mixing and will experience fluctuating irradiances (MacIntyre 1996, 1998). MacIntyre (1993) showed that in a shallow, turbid, productive lake in Western Australia the part of the water column that was mixing could be sub-divided into two zones each with different characteristics. An upper layer 0.3 to 1.5 m deep was activley mixing and a deeper layer where turbulence

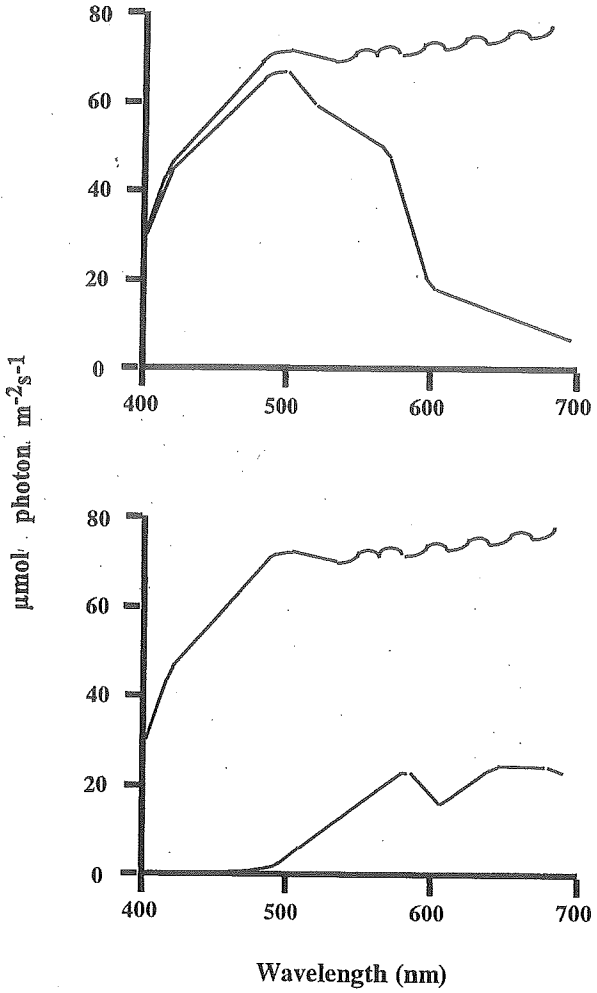

Fig. 8 Spectral distribution of quanta calculated for clear natural water at the surface and at 5m depth (top figure) and for organically coloured water from Mt Bold Reservoir, South Australia, at the surface and at 2m depth (bottom figure).

was constrained by buoyancy. In the upper layer *Microcystis* could experience a light gradient from 90 to 5% of surface irradiance in 3 to 4 min. Whether or not cyanobacteria adapt to rapidly fluctuating irradiances will depend upon the ratio of the time scale for photoadaptation to that of turbulent mixing (Lewis et al., 1984a,b).

To maintain their light-harvesting efficiency, phytoplankton have developed mechanisms for adjusting to alterations in the intensity, spectral distribution and periodicity of the PAR supply (Falkowski and LaRoche, 1991). Two major strategies are employed for adjusting to irradiance intensity. The first involves alterations in the size of the light harvesting antennae that serve the photosystems, and the second is a change in the total

number of photosynthetic units (Falkowski and LaRoche, 1991). If photosynthesis becomes limited by the rate of delivery of light energy to the photosystems, as under low irradiance, then an increase in antenna size provides one means of increasing the photon supply. If the supply of photons from the antenna approaches the maximum turnover rate of the photosystem, then an increase in the number of photosynthetic units will increase the total supply of energy to the cell for photosynthesis and growth (Falkowski and LaRoche, 1991). This adaptation could be particularly important if cells were regularly exposed to saturating light intensities for short periods of time, for example, if mixed rapidly between the surface and aphotic zone, as the increase in the number of photosynthetic units would help maximise the capture of energy during the brief light period (Tilzer, 1987). When cells are exposed to high irradiance intensities for extended periods their is an increased risk of photodamage to the photosystems. This risk can be curtailed by a reduction in antenna size, and further, if the rate of energy capture exceeds the capacity of the cell machinery then the number of photosynthetic units can be reduced. Changes in antenna size and/or changes in the number of photosynthetic units are photo-acclimation strategies employed by both cyanobacteria and micro-algae under various conditions.

It might be expected from comparisons of antenna structure and spectral absorption characteristics that the cyanobacteria would respond quite differently to changes in PAR compared to the micro-algae. To date there is little experimental data to assess this expectation as the majority of laboratory studies have used white light and investigated responses to changing irradiance intensity. The different characteristics of the photosynthetic units suggests that increased effort should be given to describing the effects of spectral changes on photosynthesis and growth. Perhaps an indication of the importance of this is the common observation that cyanobacteria are frequently associated with organically-rich waters. Although often interpreted as indicating a role for (photo)heterotrophy, it might also reflect a more conducive spectral distribution of PAR for cyanobacteria.

A. Light Intensity

Cyanobacteria show an inverse correlation between pigment content and irradiance intensity, a response commonly found in phototrophic organisms (Konopka and Schnur, 1980; Foy and Gibson, 1982; Richardson et al., 1983, Wyman and Fay, 1986a, Tandeau de Marsac and Houmard, 1993). This response occurs in cyanobacteria whether they are growing under a short light:dark cycle (L:D) (Foy, 1993), a long L:D cycle (Post et al., 1985a), or continuous illumination (Van Liere and Mur, 1980; Post et al., 1986).

In eight strains of cyanobacteria (*Anabaena flos-aquae*, *Anabaena solitaria*, *Anabaena circinalis* (x 2), *Oscillatoria redekei*, *Oscillatoria agardhii*, *Microcystis aeruginosa*, *Gloeotrichia echinulata*) studied by Wyman and Fay (1986a) the ratio of chlorophyll-a to phycobiliproteins (chl:PBP) remained constant during light limited growth, despite the reduction in pigments as irradiance intensity increased. This indicated that the number of photosynthetic units per cell declined in response to increasing irradiance (Wyman and Fay, 1986a, Post et al., 1986; Tandeau de Marsac and Houmard, 1993). Once maximum growth rate was attained, the chl:PBP ratio increased with further increases of irradiance in all species except the phycoerythrin-rich strains (*Oscillatoria agardhii* and *Gloeotrichia echinulata*). This change in ratio indicated a decline in the size of the PBS (Wyman and Fay, 1986a), presumably reducing exciton delivery to PSII and helping postpone the onset of photoinhibition and damage to the reaction centre. Although the two phycoerythrin-rich strains did not increase their chl:PBP ratio at irradiances in excess of that required for maximum growth rate, the phycoerythrin content of the PBS declined, reducing its effective spectral size. These same adjustments were observed on shifting *Synechococcus* PCC 6301 from high to low light, with an initial increase in the PBS antenna size followed by an increase in PBS per area of thylakoid (Tandeau de Marsac and Houmard, 1993).

Although similar changes in pigment content occur in the eukaryotic algae in response to alterations in light intensity, there are preliminary indications that the acclimation to photoperiod is different (Kromkamp, 1987; Flameling and Kromkamp, 1997). In *Scenedesmus protuberans* the pigment content increased as expected when the growth intensity was decreased under a 16/8 L:D cycle (Gons and Mur, 1979; Post et al., 1985b). However, the response was modified when the incident irradiance on a culture was increased but the total light dose kept constant by introducing a light:dark cycle. The cellular chlorophyll content and the size of photosynthetic

units declined as expected but the number of photosynthetic units increased (Flameling and Kromkamp, 1997). This increased the maximum photosynthetic capacity and enabled the organism to take better advantage of the periodic high light intensities. Ibelings (1992) also suggested that the ability of *Scenedesmus* to acclimatise more readily to high photon densities and to take advantage of short duration peaks in irradiance by increasing its maximum rate of photosynthesis was responsible for its competitive advantage over *Microcystis* in a highly turbulent environment. It was found that the time for adaptation to high light intensities was longer for *Microcystis* so that if the two species were rapidly circulating through a strong vertical light gradient *Scenedesmus* would out-compete *Microcystis*.

In the hypertrophic Bautzen Reservoir, *M. aeruginosa* contributes > 70% of the total phytoplankton biomass from June to September (Kohler, 1992). The growth of *Microcystis* was restricted to periods when the water column was stratified. Under mixing conditions primary production fell, although the biomass remained high. Surface photosynthesis was inhibited at intensities of > 1100 µmol m^{-2} s^{-1} during and immediately after periods of mixing but inhibition was not observed after two or more calm days. Kohler (1992) suggests that *Microcystis* takes two days to adapt to the high irradiances characteristic of surface blooms which is in agreement with the observations of Ward and Wetzel (1980)

Photoinhibition occurs when phytoplankton are shifted to irradiances substantially above those to which they have been acclimatised. High light intensities can reduce the functionality of reaction centres and may be of particular significance to buoyant cyanobacteria, especially during surface blooms when extreme intensities may have to be endured for extended periods. Although photoinhibition of photosynthesis has been demonstrated in the field and the laboratory, its impact on natural populations is difficult to assess. Most field measurements involve enclosure of samples in bottles during extended incubations in surface layers and it is not apparent just how relevant these measurements are to unfettered cells constantly redistributed by turbulence, or moving of their own volition. Paerl et al. (1983; 1985) did not observe surface inhibition of photosynthesis in natural populations of *Microcystis aeruginosa* from the Neuse River when they were incubated in glass and quartz bottles. He related the lack of photoinhibition to the presence of carotenoids which protected the photosystems from photooxidation and served as accessory pigments. In contrast laboratory cultures of *Microcystis aeruginosa* with low levels of carotenoids displayed significant reductions in photosynthesis when exposed to sunlight in the same way. Although photoinhibition reduces the rate of photosynthesis its impact varies with light intensity and exposure period and may be quickly reversed on return to low light. Consequently the degree of photoinhibition will be influenced by water mixing and the vertical movement of organisms. Furthermore, as maximum rates of photosynthesis generally occur at irradiances exceeding that required to saturate growth, the impact of photoinhibition on growth rates is unlikely to be direct, although it is generally presumed that photoinhibition reduces growth rates.

Laboratory experiments using white light have generally indicated that at very low irradiance intensities cyanobacteria have lower light requirements for growth than green algae due to a lower maintenance energy (Mur, 1983; Richardson et al., 1983). It would be predicted from this that cyanobacteria would outcompete the micro-algae when light was in very limiting supply. However, because of spectral differences in energy capture and apparent changes in the maintenance energy requirement depending on light history, this might not always be the case (Gibson 1985) and further investigation is required.

Within the cyanobacteria there are differences between species in the light requirements for growth. Foy et al. (1976) compared the growth of *Anabaena flos-aquae*, *Aphanizomenon flos-aquae*, *Oscillatoria agardhii* and *Oscillatoria redekei* under different light intensities on a 6h:18h light:dark cycle. The results showed that *Oscillatoria redekei* is adapted to use low light intensities and would be expected to dominate the other species only at light intensities below ca.12-18 µmol photon m^{-2} s^{-1} (assuming 1 lux ≈ 0.018 µmol photon m^{-2} s^{-1}: van Liere and Walsby, 1982). At higher intensities the cell growth of *Oscillatoria redekei* was photoinhibited. These results are in accord with field observations from Lough Neagh where *Oscillatoria redekei* has its maximum population in early spring and declines during the summer months. In contrast, *Oscillatoria agardhii* reaches its population maximum in mid-summer (Foy et al., 1976). Across the species there was a decreasing efficiency of light-limited growth in the order *O. redekei*, *O. agardhii*, *Anabaena flos-*

aquae, *M. aeruginosa*, with *Oscillatoria* spp. being regarded as particularly low-light adapted organisms.

Scheffer et al. (1997) analysed 55 shallow turbid lakes < 3 m deep to assess the importance to the presence of Oscillatoriaceae of TN, TP, TN:TP ratio, lake depth, secchi depth and a shade factor defined as the product of the vertical attenuation coefficient and average lake depth. The shade factor is inversely proportional to the ratio of euphotic depth to mixed depth (z_{eu}/z_m). Their analysis showed no correlation between the relative abundance of Oscillatoriaceae and nutrient concentrations, nutrient ratios or lake depth, but there was a significant correlation with secchi depth, and an even more significant correlation between the relative abundance of *Oscillatoria* and the shade factor. This suggests that the Oscillatoriaceae respond more to the changing light climate than to changes in nutrient availability and is favoured by low levels of irradiance, supporting the findings of Foy et al. (1976). The detailed analysis of Mur et al. (1993 in Scheffer et al., 1997) further supports this view and demonstrates that the decline of *Oscillatoria* populations is more closely correlated with increases in the mean irradiance as Z_{eu}/Z_{mix} rises above a critical value of ca. 0.4 - 0.5 than with declining TP concentrations. As Sas (1989) has noted, a characteristic of *O. agardhii* populations is that they retreat to the metalimnion as the light climate improves in response to nutrient and turbidity reduction and may disappear altogether as water clarity improves further. Scheffer et al. (1997) used a simple graphical model based upon field observations to demonstrate that with increasing TP concentrations, turbidity increases due to both the presence of other algae and suspended particulate matter. At a critical attenuation coefficient (high shade) *Oscillatoria agardhii* is favoured and will suddenly dominate the community. If the nutrient load is then decreased *O. agardhii* will continue to dominate until the biomass is reduced to a point where the critical attenuation coefficient is reached, but because of self-shading this will be at a lower TP than that which was present when the bloom was initiated. These critical break points offer a possible explanation of why *Oscillatoria* lakes switch so rapidly from one state to another and why in some lakes with a high background turbidity *O. agardhii* may not disappear in response to nutrient reductions.

B. Spectral Changes

Wyman and Fay (1986b) grew eight strains of cyanobacteria under equivalent photon fluxes of red, green, blue and white light and found large differences in the cell concentrations of photosynthetic pigments. In red light there was a decline in chlorophyll and phycobiliprotein content, but all strains grew at a significantly faster growth rate than under an equivalent photon flux of white light. For example, *Anabaena solitaria* grew 2.9 times faster in red light than in white light. Under green light the pigment compositions were similar to those in white light, but only the two phycoerythrin-rich strains (*Oscillatoria agardhii* and *Gloeotrichia echinulata*) grew significantly faster than in white light, all other strains growing at 60 to 75% of their white light rate. In blue light the pigment compositions were again similar to those in white light although a majority of the phycocyanin-rich strains showed a reduction in chlorophyll content. The phycocyanin rich forms had growth rates<50% of their white light rate, while the phycoerythrin rich strains, *O. agardhii* and *G. echinulata*, were able to maintain growth rates of 65% and 100% of the white light growth rate respectively. Direct comparisons with micro-algae were not made, but, as they contain chlorophylls b and c, they are expected to capture and utilise blue light with greater efficiency (Richardson et al., 1983).

The substantial changes in cyanobacterial growth rates in response to the spectral distribution of incident light would suggest that spectral changes in the underwater light field could play a major role in structuring community composition. Field data to assess this hypothesis do not appear to be available.

IX. Nutrients

General responses of phytoplankton to nutrient limitation include, carbohydrate accumulation (discussed further for cyanobacteria under buoyancy regulation), a reduction in the cell-specific quantum yield of photosynthesis (Turpin, 1985, 1991), a reduction in the cellular content of the limiting nutrient (Droop, 1973; Riegman and Mur, 1984) and an increase in the nutrient specific uptake rate of the limiting nutrient (Gotham and Rhee, 1981; Riegman and Mur, 1984; Kromkamp, 1987). Nutrient limitation stimulates the storage of non-limiting nutrients as a result of their relative excess compared to the reduced requirements of the cell. Nutrient

storage is a valuable attribute, enabling cells to utilise pools of nutrients that are spatially and temporally separated so that growth is maintained during periods of nutrient scarcity.

X. Phosphorus

Under phosphorus limiting conditions cellular phosphorus concentrations decline as phosphorus limited growth rate declines, while the phosphorus uptake potential increases. As a consequence, a pulse of phosphorus delivered to phosphorus limited cells results in substantial formation of polyphosphate reserves, (the polyphosphate "overplus" phenomenon), with cellular phosphorus levels able to exceed those occurring under steady state maximum growth rates (Allen, 1984; Riegmann and Mur, 1984). Most phytoplankton are able to store surplus phosphorus, usually in the form of polyphosphate (PP), and these reserves can be sufficient for several cell doublings. It has been suggested that phosphorus storage in cyanobacteria may be larger than in micro-algae providing them with a competitive advantage (Sommer 1985), but the storage capacity of some micro-algae seems equally large (Lund, 1965). There do not seem to be any consistent phylogenetic differences between micro-algae and cyanobacteria in the range of values for phosphorus uptake and the kinetics appear to be species specific (Healey, 1982; Tilman et al., 1982; Kromkamp, 1987; Reynolds, 1993). Riegman and Mur (1984) for example found the half saturation constant for growth under P-limitation to be similar for *Oscillatoria agardhii* and the two diatoms *Cyclotella meneghiniana* and *Asterionella formosa*..

Rather than a consistent phylogenetic difference in phosphorus uptake and accumulation characteristics, the gas-vacuolate cyanobacteria appear to be advantaged by behavioural features of their life-history that enable them to utilise specific phosphorus conditions more effectively. For example, *Microcystis* has a high V_{max} for phosphorus uptake, a low minimum P content and a large capacity to accumulate phosphorus (Kromkamp et al., 1989). These attributes suggest it is a storage specialist and this is in accord with its ability to regulate buoyancy to gain access to phosphorus in deeper water layers when epilimnetic concentrations are low (Ganf and Oliver, 1982). In contrast *Oscillatoria agardhii* had a similar phosphate uptake rate, but a larger minimum P content, a lower V_{max} and was less adept at accumulating phosphorus. In competition experiments with a pulsed supply of phosphorus at saturating concentrations *Microcystis* outcompeted *Oscillatoria* because of its larger V_{max} and more efficient use of phosphorus (Kromkamp et al., 1989).

A more extreme example of behavioural adaptation is afforded by *Gloeotrichia echinulata* where often a large percentage of planktonic populations are comprised of colonies newly recruited from the sediments (Barbiero and Welch, 1992; Istvánovics et al., 1993; Perakis et al., 1996). *G. echinulata* is a nitrogen-fixing, heterocystous, filamentous cyanobacterium which forms spherical colonies up to 1 or 2 mm diameter. Large numbers of colonies and akinetes are found in the phosphorus enriched sediments of some shallow lakes that are mildly eutrophic, but with relatively low soluble reactive phosphorus concentrations in solution. Examples include Green Lake with soluble reactive phosphorus concentrations in the range $1 - 4\ \mu g\ L^{-1}$ (Barbiero and Welch, 1992) and Lake Erken with soluble reactive phosphorus concentrations usually less than $5\ \mu g\ L^{-1}$ P (Pettersson et al., 1993). In Lake Erken 5×10^5 colonies m^{-2} of *G. echinulata* were found in the top 4 cm of sediments in areas of the lake with a depth < 10m (Pettersson et al., 1993).

During *G. echinulata* blooms the direct contribution to increases in the planktonic population by newly recruited colonies from the sediments can be over 100% (Barbiero and Welch, 1992; Perakis et al., 1996) suggesting that they require large, sustained inputs of colonies from the sediments to persist. The massive recruitment of benthic *G. echinulata* colonies to the plankton is associated with a large transfer of phosphorus into the water column. Istvánovics et al. (1993) investigated the phosphorus uptake characteristics of *G. echinulata* in Lake Erken and showed that it was unable to utilise the low epilimnetic phosphorus concentrations present during the bloom. It did not seem to regulate buoyancy to access phosphorus rich sub-surface water like *Microcystis* but instead remained continuously buoyant and they concluded that the colonies assimilate phosphorus in the sediments prior to their migration into the plankton and use this internal store to support planktonic growth. On the basis of these results *G. echinulata* was described as an extreme storage-adapted species, with its P-assimilation and growth phases completely separated in time and space (Istvánovics et al., 1993).

Recruitment of populations from the sediments occurs in several other bloom-forming cyanobacteria, including *Microcystis*, *Anabaena*, *Aphanizomenon*

and *Coelosphaerium* and occasionally these are large enough to influence their population dynamics (Perakis et al. 1996). However, in general these genera increase from growth in the water column (Preston et al., 1980; Trimbee and Harris, 1984; Barbiero and Welch, 1992; Perakis et al., 1996) and are reliant on obtaining on-going supplies of phosphorus.

XI. Nitrogen

Nitrogen is of particular significance to the gas-vacuolate cyanobacteria, as it is an essential component in the synthesis of their gas-vesicles. Consequently nitrogen limitation may not only impact on cell growth, but also on cell buoyancy and the ability to regulate buoyancy. Nitrogen limitation will be particularly detrimental to the non-nitrogen fixing bloom-forming cyanobacteria and may be a critical factor in their replacement by other phytoplankton species.

It was previously thought that inorganic nitrogen metabolism differed between micro-algae and cyanobacteria (Gibson and Smith, 1982), but more recent studies have demonstrated that in many respects they are similar (Guerrero and Lara, 1987; Turpin, 1991; Garcia-Gonzalez et al., 1992; Coronil et al., 1993; Tapia et al., 1996). Nitrogen can be acquired as NO_3^-, NO_2^-, or NH_4^+, and also as N_2 in thosecapable of nitrogen fixation. The order of preference is $NH_4^+ > NO_3^- > N_2$ (Tandeau de Marsac and Houmard, 1993), and when NH_4^+ is available cyanobacteria and micro-algae do not assimilate alternative N sources (Turpin, 1991; Ochoa de Alda et al., 1996). Combined inorganic nitrogen is actively assimilated through a series of steps dependent on the N-source. Nitrate is reduced by nitrate reductase to nitrite and nitrite is reduced by nitrite reductase to ammonium (Guerrero and Lara, 1987). In both micro-algae and cyanobacteria the most important pathway for ammonium assimilation is via the glutamine synthetase-glutamine synthase (GS-GOGAT) enzyme systems.

Nitrogen metabolism is closely connected with carbon fixation, as both processes compete for energy and reductant generated by the light reactions of photosynthesis. The assimilation of carbon dioxide to carbohydrate requires four electrons while the formation of amino-N from nitrate requires 10 electrons (Guerrero and Lara, 1987; Turpin, 1991). At a cellular C/N ratio of ca. 5, up to 50% of the reductant generated through the light reactions will be used to assimilate nitrogen (Guerrero and Lara, 1987).

The interaction with carbon metabolism is further enhanced by the need for carbon skeletons to incorporate nitrogen into protein. In nutrient replete cells carbohydrate stores are small and assimilation of combined inorganic nitrogen is strongly dependent on recent CO_2 fixation (Guerrero and Lara, 1987; Turpin, 1991). Under these conditions reductions in photosynthesis, for example due to darkness or CO_2 deprivation, will reduce nitrogen assimilation. In contrast, N-limited cells accumulate carbohydrate reserves that can be utilised as a source of energy and carbon skeletons for nitrogen assimilation both in the dark and the light (Guerrero and Lara, 1987; Turpin, 1991; Garcia-Gonzalez et al., 1992; Tapia et al., 1996). However, cells growing under N-limited conditions increase their capacity for nitrogen uptake so that the re-supply of inorganic nitrogen causes a large demand for reductant and carbon skeletons. If this demand cannot be met by photosynthesis, then carbon skeletons are supplied through glycolysis of the existing carbohydrate reserves (Turpin 1991; Tapia et al., 1996). The assimilation of nitrate and nitrite into amino-N is limited by the availability of reductant to form ammonium and this reduces their requirement for carbon skeletons compared with ammonium assimilation. In comparison, the large demand for carbon skeletons generated by the assimilation of ammonium can significantly reduce carbohydrate reserves (Turpin, 1991; Garcia-Gonzalez et al., 1992; Tapia et al., 1996). As a result of these interactions nitrogen assimilation influences the rate of CO_2 fixation, the fate of newly fixed carbon, and the level of carbohydrate reserves (Guerrero and Lara, 1987; Turpin, 1991; Garcia-Gonzalez et al., 1992; Tapia et al., 1996) with major effects expected on cell growth, cell turgor pressure and cell density (see section on buoyancy regulation).

Metabolic processes that alter rapidly the size of carbohydrate reserves will be of major significance to buoyancy regulating cyanobacteria as these reserves provide ballast to offset the lift due to gas vesicles. The results of laboratory studies (Turpin, 1991; Garcia-Gonzalez et al., 1992; Tapia et al., 1996) suggest that where cyanobacteria move between the well illuminated, nutrient-poor surface layers and nutrient-rich aphotic zones, the source of available nitrogen at depth could have a significant effect on rates of buoyancy regulation. For example, the increased availability of ammonium common in the hypolimnion of stratified lakes may cause a reduction

in the carbohydrate reserves of sedimenting cyanobacteria leading to a quicker reversal of cell buoyancy and a reduction in the extent of vertical migration. Detailed studies of this interaction are required.

Blomqvist et al. (1994) noted from measurements on the oligotrophic, low-alkaline, clear water Lake Njupfatet and the mesotrophic, alkaline Lake Erken, that the development of cyanobacterial populations did not commence until nitrate was almost depleted. Based on results from a series of enclosure experiments enriched with either ammonium or nitrate, they postulated that non-nitrogen fixing cyanobacteria are favoured by ammonium, eukaryotic algae by nitrate-nitrogen and nitrogen fixing cyanobacteria by nitrogen deficiency. The suggestion of nitrate and ammonium being favoured by different organisms has not been well substantiated although laboratory studies provide some support.

In cultures of micro-algae kept under light limiting conditions growth on nitrate is equivalent to, or better than, growth on ammonium (Syrett, 1981; Thompson et al., 1989; Levasseur et al., 1993). In the cyanobacterium *Anacystis nidulans* assimilation and growth on nitrate or ammonia is comparable if light is saturating (Guerrero and Lara, 1987), but at light intensities half saturating to photosynthesis nitrate assimilation is reduced while ammonium assimilation remains unchanged (Garcia-Gonzalez et al., 1992). Ward and Wetzel (1980) showed that the lowest light intensity at which growth of *Aphanizomenon flos-aquae*, *Microcystis aeruginosa* and *Anabaena flos-aquae* would occur was determined by the nitrogen source. Of the three tested (ammonia, nitrate and nitrogen gas) ammonia supported the highest growth rate under all light regimes. These comparisons indicate a preference for NH_4^+ by cyanobacteria particularly at low light intensities, but they do not support the notion that this enables them to dominate the micro-algae when ammonium is the major source of nitrogen. Similarly the results do not support the contention that the micro-algae have a particular preference for nitrate. However they do indicate that under suboptimal light conditions cyanobacterial growth is reduced when the nitrogen source is nitrate, whereas growth rates of micro-algae are not affected. So rather than non-nitrogen fixing cyanobacteria being favoured by ammonium and eukaryotic algae by nitrate nitrogen (Blomqvist et al., 1994), it would seem that the cyanobacteria may be disadvantaged by using nitrate-nitrogen under low light conditions.

A. Nitrogen Fixation

Some cyanobacteria are able to utilise N_2 when sources of combined inorganic-N are depleted. Numerous reviews have described the biochemistry, physiology and molecular biology of cyanobacterial nitrogen fixation (see Bothe, 1982; Van Baalen, 1987; Howarth et al., 1988; Tandeau de Marsac and Houmard, 1993; Flores and Herrero, 1994). No known micro-algae that can fix molecular nitrogen, so the nitrogen-fixing cyanobacteria have a major advantage at times when sources of combined inorganic nitrogen have been depleted from the water.

The common bloom-forming cyanobacteria that are nitrogen fixers are the heterocystous, filamentous members of the Nostocales, including *Anabaena*, *Aphanizomenon* and *Gloeotrichia*. When combined nitrogen is absent, these organisms differentiate thick walled cells called heterocysts that isolate the nitrogen-fixing enzyme system, nitrogenase, from inactivation by oxygen. The heterocyst provides this protection by enhanced respiration, and by the barrier of the heterocyst envelope (Wolk et al., 1994). Some non-heterocystous species are also able to fix N_2, including species of *Oscillatoria*, but the extent of this in natural systems has not been quantitatively estimated.

Howarth et al. (1988), who summarised field data on nitrogen fixation in both marine and freshwater environments, concluded that cyanobacteria are responsible for most planktonic nitrogen fixation in freshwaters and that rates are only high when these organisms are present in large numbers. A comparison of seven eutrophic lakes showed that nitrogen fixation accounted for 6 - 82% of the nitrogen load, differing markedly between lakes. Even when N_2-fixation contributes only a small percentage of the total load to a lake, the direct supply of nitrogen to the bloom-forming cyanobacteria still is of major importance to their success. In particular nitrogen fixation enables continued production when nitrogen supplies are depleted, and the observation that phosphorus is the nutrient frequently found to control the development of phytoplankton biomass even in lakes where nitrogen is initially limiting is a result of nitrogen deficits being compensated for through nitrogen fixation (Schindler, 1977; Howarth et al., 1988). However, nitrogen deficits that commonly occur in waters where nutrient loadings have low N : P ratios are not always counteracted by nitrogen fixation. In tropical Lake Valencia nitrogen fixation rates were

high, but insufficient to restore the low N:P ratio to the Redfield ratio, suggesting that nitrogen limitation controlled the phytoplankton biomass (Levine and Lewis, 1987).

Frequently N:P ratios are used to assess the likelihood of nitrogen-fixing cyanobacteria occurring, but as Horne and Commins (1987) have stressed, nutrient ratios alone are not reliable indicators as the critical limiting level of total inorganic nitrogen required for the induction of nitrogen fixation must be reached regardless of the N:P ratio. They reviewed the literature on laboratory and field experiments and concluded that total inorganic nitrogen needs to fall below 50-100 mg m^{-3} to induce nitrogenase activity.

B. Nitrogen Storage

Unlike eukaryotic micro-algae, the cyanobacteria have a capacity to store significant amounts of nitrogen in excess of their immediate requirements. The two storage components are cyanophycin, a co-polymer of aspartate and arginine, and the phycobiliprotein, phycocyanin. Whereas the only function of cyanophycin is to store nitrogen and perhaps energy, phycocyanin is also a major pigment component of the light-harvesting antenna, but under conditions of nitrogen limitation it acts as a nitrogen reserve (Kromkamp, 1987).

Cyanophycin and phycocyanin are both at low concentrations in nitrogen-limited cells (Allen, 1984), but even in non-limited, rapidly growing cells, the amount of nitrogen stored as cyanophycin is comparatively low relative to when cell growth is limited by other requirements. Cyanophycin is accumulated when cells are starved of light, phosphorus or sulphur, and when grown at low temperatures (Allen, 1984). In a manner reminiscent of the phosphorus overplus phenomenon, cyanophycin accumulates on the addition of a utilisable nitrogen source to N-limited cells (Simon, 1987).

In response to nitrogen starvation the cyanophycin granules are first degraded, followed by cell bleaching due to degradation of components of the phycobilisome including phycocyanin (Tandeau de Marsac and Houmard, 1993). Nitrogen stores are also utilised when low light cells are shifted to high light, with cyanophycin and phycocyanin both decreasing.

XII. Responses of Cyanobacteria to N and P

A. Whole Lake Phosphorus Enrichment

The classical work of a number of authors in the 1960s (eg Sakomoto, 1966; Vollenweider, 1968) led to the recognition of the importance of increased phosphorus loadings in the process of eutrophication of lakes. These studies on phosphorus, and later on the interaction between nitrogen and phosphorus (eg Smith, 1983), led to ecological research focused on the manipulation of whole lakes or portions of them to explore the responses of phytoplankton abundance and community structure to nutrient conditions (Schindler, 1971; Lund and Reynolds, 1982).

An implication of phosphorus loading models is that discharging nutrient-rich waters into a water body will increase productivity, and if physical conditions permit, lead to the proliferation and eventual dominance of bloom-forming cyanobacteria. Pearsall (1932) suggested a relationship between increasing nutrients, dissolved organic matter, and the presence of bloom-forming cyanobacteria in English lakes. Pick and Lean (1987) recalculated data from Gorham et al. (1974) for lakes in northern England and came to the conclusion that there was a significant relationship between the mean annual phytoplankton biomass and that of the bloom-forming cyanobacteria, with the proportion of cyanobacteria increasing as the total phytoplankton biomass rose above 5 - 10 g m^{-3} fresh weight. Reynolds (1987), Steinberg and Hartmann (1988) and Steinberg and Gruhl (1992) suggest eutrophication, especially by phosphorus, often leads to significant shifts in phytoplankton species composition towards bloom-forming cyanobacteria.

Additional evidence to support this conclusion comes from Europe and North America (Harris, 1986; Cullen and Forsberg, 1988; Sas, 1989; Cooke et al., 1993) as well as from Australia and Africa. The Peel Harvey Estuary in Western Australia and the Hawkesbury River in New South Wales both illustrate that increases in nutrient loads from either agricultural run-off or sewage treatment plants results in extensive populations of bloom-forming cyanobacteria, in these examples *Nodularia spumigenea* (Plate 11e) and *Microcystis aeruginosa* / *Anabaena* sp., respectively (George & Bradby, 1993; Cullen, 1994; Humphries & Robinson, 1995).

The relationship between eutrophication and an increased biomass of gas-vacuolate cyanobacteria has been attributed to the requirement that sufficient nutrients be available, either in the water or from internal recycling, when physical conditions eventually become suitable to provide the cyanobacteria with a competitive advantage. In temperate systems if nutrients are depleted by phytoplankton growth during spring and early summer then the bloom-forming cyanobacteria are faced with depauperate nutrient conditions when the physical environment is most suitable for their growth. Similar arguments can be proffered for tropical waters but on cycles driven by both meteorological events as well as seasonal conditions (Lewis, 1978).

B. Biomass Response to P-Removal

Lake McIlwaine, near the city of Harare, Zimbabwe, experienced anthropogenic eutrophication as a result of sewage discharge into the lake (Thornton, 1982). As nitrogen and phosphorus concentrations rose 5 to 10 times their original levels, chlorophyll-a concentrations peaked at ca. 150 mg m^{-3} and did not fall below ca. 50 mg m^{-3} throughout 1968-69. During this period the lake was dominated almost exclusively by *Microcystis aeruginosa* and *Anabaena flos-aquae*. After diversion of the sewage to pasture irrigation between 1970 and 1975, the soluble reactive phosphate levels fell by an order of magnitude and chlorophyll-a concentrations fell to a mean of 15 mg m^{-3}. Although, the lake still supported populations of cyanobacteria, as well as *Melosira granulata*, there was a re-appearance of significant populations of other eukaryotic genera.

The Lake McIlwaine experience suggests that when nutrient loads are reduced phytoplankton biomass decreases, but the conclusion that cyanobacteria will also disappear is a convenient rather than a realistic one. Nevertheless, there are situations where this does occur. For example, as a result of the diversion of sewage from Lake Washington (Edmondson and Lehman, 1981; Seip et al., 1992) the percentage of total phosphorus from sewage was reduced from ca. 70% to zero and the total phosphorus content of the lake fell from 200 x 10^3 kg to 59 x 10^3 kg. This resulted in a decrease in maximum chlorophyll-a concentrations from ca. 45 mg m^{-3} (1962 - 1965) to less than 10 mg m^{-3} (1976 - 1978). Concurrently the proportion of the total phytoplankton biomass attributable to filamentous cyanobacteria (*Oscillatoria rubescens*) in surface samples fell from > 90% to < 20%.

In contrast, there are examples where a reduction in the phosphorus load has had no apparent influence on water quality. Talling and Heaney (1983) recommended the removal of sewage borne phosphorus which contributed 47 - 67% of the total phosphorus loading to Esthwaite Water. Heaney et al. (1992) reported the outcome of this remedial action and concluded that, although the impact of phosphorus removal had yet to be fully realised, it was questionable if water quality would ever improve with the current phosphorus loading. This was due in part to the internal loading from the P-rich sediments compensating for the reduction in the external load as the lake approached a new equilibrium state, and partly due to the continued input of phosphorus from an adjacent fish farm. One of the interesting aspects of Esthwaite Water is that the most significant changes in phytoplankton species composition occurred prior to the remedial action. In the early 1970s and 1980s the summer algal community was dominated by *Ceratium* spp., but due to intense parasitism by *Aphanomycopsis cryptica*, a biflagellate fungus, the *Ceratium* diminished from 1983 was were replaced by *Aphanizomenon flos-aquae* fo. *gracile*, *Anabaena flos-aquae* and *A. solitaria*. Subsequent changes in the timing and maxima of the cyanobacteria were attributable to the intense grazing by the protozoan ciliate *Nassula aurea* (Canter et al., 1990) and not to the reduction in P-load.

These examples, plus many others (Reynolds and Walsby, 1975; Reynolds, 1984b,1987, 1989a, b, 1992b), illustrate that relationships between nutrient concentrations and phytoplankton biomass and species composition are not simple. Indeed it may appear as though each lake should be considered as an individual water body and this reductionist view may be correct. However there is evidence to suggest general relationships between the physical and chemical characteristics of lakes and phytoplankton communities and in particular the conditions that promote the wax and wane of cyanobacteria (Round 1971).

To understand the complexity of the relationships Sas and his co-workers (Sas, 1989; Seip et al., 1992; Reynolds, 1992; Cooke et al., 1993) analysed data from 18 water bodies in Europe ranging in are (0.03 to 503 ha), depth (0.5 to 177m) and theoretical retention time (0.1 to 11 years). The total phosphorus (TP) concentration range was 6 to 1440 mg m^{-3} and inorganic nitrogen 2 to 2000 mg m^{-3}. All water

bodies had undergone a reduction in the P input principally, but not exclusively, via P-precipitation at sewage treatment plants. To analyse the data, criteria were set to identify lakes where nutrient limitation (N or P) occurred. If filtrable reactive phosphate (FRP) concentration was consistently >10 mg m^{-3} then P-limitation to growth was not considered likely, whereas if the lake-FRP concentration was < 10 mg m^{-3} either (1) on average over the entire growing season, or (2) absolutely during at least half of the period of the growing season, then phytoplankton growth was assumed to be P-limited during the growing season. Similarly, the threshold value below which N-limitation occurred was assumed to be 100 mg m^{-3} inorganic nitrogen.

Despite phosphorus removal, seven of the 18 water bodies studied by Sas (1989) did not show a significant decrease in phytoplankton biomass as measured by either chlorophyll-a concentration or biovolume. Four of these were deep lakes (z_M = >18 m) which were incompletely mixed and three were shallow lakes (z = < 5 m) where mixing was a regular event. The four deep lakes were characterised by relatively low initial chlorophyll concentrations and it appears that the principal aim was to reduce the likelihood of future increases in cyanobacteria rather than to prevent the bloom-forming species appearing.

Of the shallow lakes only Lake Hylke experienced periods of P-limitation with FRP < 10 mg m^{-3} for two weeks during the growing season, while inorganic nitrogen always exceeded 100 mg m^{-3}. Lake Sobygard, dominated by *Scenedesmus*, never experienced P- limitation and, although N-limitation was reported for 2 to 4 weeks, the growth season average inorganic nitrogen concentrations were well in excess of 100 mg m^{-3}. On the basis of the critical value for phosphorus, the third shallow lake (Lough Neagh) with a relatively low, mean, growing season FRP concentration (ca 25 mg m^{-3}) experienced P limitation for an average of six weeks of the growing season (1976-1986). However inorganic nitrogen fell below the critical level for 2 - 5.5 months in eight of the eleven years monitored, suggesting that nitrogen was more likely to be limiting the phytoplankton. Apparently the lack of response of these three shallow lakes to phosphorus reduction indicated that phosphorus had not been reduced sufficiently to decrease the phytoplankton biomass.

The responses of the remaining 11 lakes to P-removal were manifested by both a reduction in the mean growth-season chlorophyll concentration and less frequently by a shift in species composition. The time lag for a reduction in chlorophyll to occur varied between lakes. In Lake Wahnbach where the chlorophyll declined from ca 30 mg m^{-3} in 1969 to < 5 mg m^{-3} in 1985, the response was immediate and followed that predicted by the Vollenweider/OECD model. In Lake Veluwe the chlorophyll concentration pre-restoration (1975) was > 200 mg m^{-3} and fell to ca 50 mg m^{-3} by 1982, but with a two year lag time. In Lake Schlachten the average summer chlorophyll concentration remained high (64-85 mg m^{-3}) for the first four years after restoration but as phosphorus was further reduced chlorophyll fell to 14 mg m^{-3}. In each case the reduction in chlorophyll with phosphorus availability had a slope close to 1.0 and the time lags reflected the magnitude of the sediment release of phosphorus. In addition to the influence of nutrients, grazing was considered important to the overall reduction in the algal biomass for five of the lakes and light was implicated in nine of the lakes.

C. Four-Stage Response to P-Removal

The significance of the study by Sas (1989) was that quantitative comparisons were made between lakes, across years and validation was an integral part of the analysis. The conclusion was that the phytoplankton biomass and the cyanobacterial component responded to remedial action in four stages (Fig. 9)

- Stage 1 no biomass reduction as phosphorus in excess to requirements
- Stage 2 declining amount of unused phosphorus, small reduction in biomass
- Stage 3 phytoplankton biomass falls, minimal unused phosphorus
- Stage 4 further decline in biomass and changes in composition of the phytoplankton.

The first stage occurred in lakes with a nutrient rich pre-restoration phase where nutrients were never growth limiting and there was an unused fraction of the total phosphorus (Fig. 9). In these cases there was no immediate effect on phytoplankton biomass or species composition. In general the influence of P-limitation did not occur until the FRP in the trophogenic layer had fallen on average during the growth season to < 10 mg m^{-3}. Evidence from a number of the responsive lakes showed that as the chlorophyll concentration fell the difference between total phosphorus and the particulate fraction became small which indicated that FRP was virtually exhausted (Reynolds, 1992) and all the P was incorporated into algal cells (Fig. 9). This interpretation would need to be modified in turbid

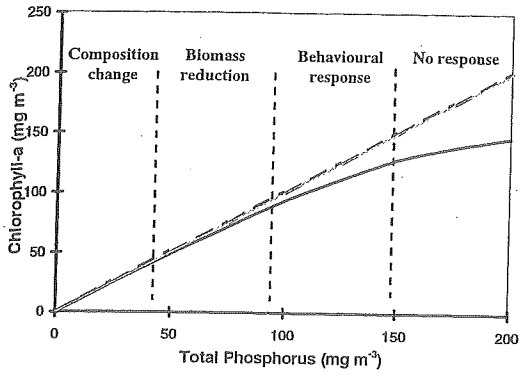

Fig. 9 Hypothetical example of the response of lake chlorophyll-a concentration to reductions in phosphorus concentration according to Sas (1989). The theoretical chlorophyll-phosphorus relationship is shown by the straight line (dashed) while the actual response follows the solid line. At high phosphorus concentrations the difference between the theoretical and actual chlorophyll concentrations is due to restriction of the algal biomass by some other factor and phosphorus is present in excess.

lakes where a large fraction of the bioavailable-P may be associated with the inorganic particulate fraction and not measured as FRP, and a proportion of the particulate total phosphorus may be unavailable for incorporation into phytoplankton (Oliver, 1993). The second stage of recovery depends upon the behaviour of the phytoplankton and the morphometry and mixing regime of the lake. In those lakes which have a persistent thermocline (usually the deep, calm lakes) the phytoplankton community is dominated by motile algae such as dinoflagellates and buoyant cyanobacteria.

As a consequence of the reduced nutrient load these phytoplankton disperse to greater depths as they seek additional nutrients.

This causes a marked increase in the water transparency even though the biomass per unit area may not fall. In contrast, shallow, vertically mixed lakes do not afford a deep refuge zone and consequently this behavioural aspect of phytoplankton is not realised. The recovery process is not buffered by access to previously unused phosphorus sources and it skips to the third phase.

In stage 3 the P-concentration continues to decline as a consequence of both the internal and external reduction of the P-loading (Fig. 9). The overall result was a significant decrease in the phytoplankton biomass as P-limitation began to take effect, described by the equation:

$$C_A/C_B = (P_A/P_B)^m$$

where:

C_B and C_A denote the growth-seasonal average chlorophyll-a concentrations pre- and post-restoration respectively, P_B and P_A the pre- and post-restoration values of the whole-lake annual mean total phosphorus concentration and m the empirical exponent derived from the correlation (Sas 1989).

The relationship between decreasing P-load and phytoplankton biomass reduction conformed closely with the Vollenweider/OECD model such that the ratio of the growth-season average chlorophyll post- to pre-restoration was equal to the ratio of the whole lake annual mean total phosphorus post- to pre-restoration raised to the exponent 1.0 compared with the Vollenweider/OECD model of 0.96 ± 0.12. This relationship was further subdivided into shallow lakes where the exponent was 1.4 ± 0.3 and deep lakes where the exponent was 0.7 ± 0.5. Although the small number of lakes involved in the study and the differences between the sampling procedures for phytoplankton biomass cause some inconsistencies it was nevertheless possible to discern a qualitative difference between deep and shallow lakes.

The fourth stage of recovery once the lake reaches its new equilibrium state entails a change in species composition. For the perennial species such as *Oscillatoria* spp the data suggest that the trend of increasing cell numbers with increasing nutrient supply is more or less reversible as the nutrient load decreases below threshold values. In shallow lakes this threshold appears to be 50 - 100 mg m^{-3} FRP, but is rather lower for deep lakes at 10-20 mg m^{-3} (Sas 1989).

D. Responses of Cyanobacteria to P-Removal

The slope of the linear log-log relationship between concentrations of chlorophyll-a and total phosphorus were used to assess whether or not cyanobacteria responded to changes in the TP concentration in a similar or different manner to micro-algae. The frequency of occurrence of particular slopes was used to compare the trajectory of the relationship between chlorophyll-a and TP for cyanobacteria and for micro-algae (Seip et al., 1992). Positive slopes of 45° indicated a 1:1 relationship between chlorophyll-a and TP. Slopes near 0° indicate small ratios that suggest changes in TP correspond with much smaller

changes in chlorophyll-a, such as occurs when there are significant time lags between action and response. Angles > 78°, equivalent to ratios greater than 4.7, indicate large changes in chlorophyll with small changes in TP, while higher ratios with angles close to 90° indicate that factors other than TP influence the trajectory. Negative angles suggest that chlorophyll increases with decreasing TP or vice versa. For deep lakes cyanobacteria had very similar responses to other algae. For shallow lakes the occurrence of low ratios with angles near to 0° is more pronounced for micro-algae than for cyanobacteria indicating there are fewer instances where cyanobacterial chlorophyll does not respond to an increase or decrease in TP. There were a significant number of angles < 0° or close to 90° which highlights the probability of factors other than nutrients influencing species composition.

Shifts in species composition were most noticeable for *Oscillatoria* spp. which either decreased significantly, disappeared, or retreated to the metalimnion as water transparency increased in response to reductions in biomass. Less obvious was the response of cyanobacterial species such as *Aphanizomenon* spp, *Anabaena* spp and *Microcystis* spp. that can form surface blooms. In a significant number of cases where temperatures were favourable, these tended to increase as *Oscillatoria* spp decreased in abundance, and this was correlated with a decline in the ratio of TP: z_{eu}/z_{mix} (ie. the ratio of phosphorus to available light).

This species response to lake restoration is a prime avenue for future research. It is evident that species changes are not simply a function of the relative growth rates of different species although this may be contributory, rather it is the interaction between a number of biological features and the stability of the water column as well as the quality of the underwater light field.

E. Bioassays, Lake Enrichment and Nitrogen Limitation

Elser et al. (1990) reviewed 62 North American lakes from which data were available on the response of algal biomass (chlorophyll, ^{14}C-fixation, cell counts, in vivo chlorophyll fluorescence) to nutrient enrichment of N, P or N+P versus a control (no addition) in enrichment bioassays and whole-lake experiments. The average concentrations added were 46.3 μM N and 2.63 μM P. Simultaneous N and P enrichment in bioassays nearly always elicited a positive growth response (86% of cases), whilst single nutrient additions of either N or P produced positive results in 40% and 47%, respectively. This suggests that in the 62 lakes studied nitrogen was just as frequently in limiting supply as phosphorus. Results obtained for lakes and rivers in south-eastern Australia using both growth assays and physiological assays (Wood and Oliver, 1995; Fink and Oliver, submitted) support this view.

Of the studies reviewed by Elser et al. (1990) whole-lake fertilisations provided a total of 80 lake-years of data. Of these, only 2 of 31 lake-years showed a positive response to single nutrient additions (+ P: Lake Maryjo and ELA 261). In agreement with the bioassay experiments, 39 lake-years showed a positive response to the simultaneous addition of N and P, while 10 lake-years showed no significant response to any of these additions (Elser et al., 1990). Interpretation of whole-lake data was hampered by a lack of factorial design for N and P additions and the authors concluded that even for this extensive data set the effects of N and P had not been separated adequately and urged scientists in future to implement designs which evaluate the interactive roles of N and P. In addition they suggested that the time scales of experiments may be inadequate to assess the extent to which N_2-fixing cyanobacteria impact on the nitrogen budget of a lake. The frequent requirement for combine N and P enrichment consistently to produce a substantial growth response confirmed the results of the bioassays and indicated a more important role for nitrogen limitation in freshwaters than previously recognised.

F. Lake and Laboratory Studies on the Influence of TN:TP Ratios

In recognition of the influence of nitrogen availability on phytoplankton growth the interpretation of log-log plots of P-loading and annual mean chlorophyll-a concentrations have been modified by the inclusion of TN:TP ratios to account for situations of nitrogen limitation and provide an indication of the probability of nitrogen fixing cyanobacteria appearing in the plankton (Smith 1982, 1983). From a study of 17 lakes world-wide Smith concluded that bloom-forming cyanobacteria tended to dominate in lakes where the TN:TP mass ratio was less than 29. This has led to claims that increasing the mass ratio above 30 will reduce the proportion of cyanobacteria as a fraction of the total algal biomass. Many substantial reviews that have discussed the impact of TN:TP

ratios on phytoplankton populations (Harris, 1986; Pick and Lean, 1987; Elser et al., 1990; Jensen et al., 1994; Scheffer et al., 1997) have found little evidence to support the contention that TN:TP ratios are an important determinant of cyanobacterial dominance. Pick and Lean (1987) suggest that neither laboratory nor whole-lake studies provide conclusive evidence that N:P ratios play a major role in cyanobacterial dominance. Others (Trimbee and Prepas, 1987; Scheffer et al., 1997) have suggested that even when a response is observed it maybe spurious and due to increasing P concentrations rather than a decrease in the N:P ratio.

The problem appears to be that the majority of experiments and lake manipulations are done without assessing whether major nutrients are limiting. Horne and Commins (1987) and Reynolds (1992) have pointed out that the ratio is immaterial if the nutrient concentrations are in excess of those limiting to growth. Any discussion on the influence of TN:TP ratios on the occurrence of cyanobacteria must start from the premise that the cells are, or will become limited by either nitrogen or phosphorus.

Two examples which support a central role for TN:TP in stimulating cyanobacterial growth are those by Schindler (1977) and Stockner and Shortreed (1988) both carried out in oligotrophic Canadian lakes. In the first experiment, Lake 227 was fertilised at a ratio of 14:1 (by weight). During the entire six year period the lake was dominated by *Scenedusmus* and other algae unable to fix atmospheric nitrogen. However, when the N:P ratio was cut back to 5:1, a bloom of the nitrogen-fixing *Aphanizomenon gracile* occurred, which fixed significant amounts of nitrogen. A similar response was noted in Lake 226 when fertilised with a TN:TP ratio of 5:1, althoughhere the dominant genus was *Anabaena*.

In the second example (Stockner and Shortreed, 1988), the Clayoquot Arm of oligotrophic Kennedy Lakee was enriched with solutions of NH_4NO_3 and $(NH_4)_2HPO_4$ (o to 7.6 mg P m^{-2} week $^{-1}$) to give N:P molar ratios that rose from 10 (ca. 5:1 by weight) in 1978, the first year of fertiliasation, to 15 in 1979-1981, and then further increased to 26 and 35 over the next three years. *Anabaeuna circinalis* concentrations were <20 cells ml^{-1} prior to fertilisation, but reached >30000 cells ml^{-1} in 1981 and subsequently fell to an average cell concentration of 25 ml^{-1} in 1983-85. Concurrently the growing season average secchi depths decreased frpm 8.1 down to 3.5 m in 1981, before deepening again to 9m in 1985. During this period the total phosphorus concentration increased to a maximum of only 6 mg m^{-3}. The authors concluded that the low initial N:P ratios in the presence of rising phosphorus concentrations promoted *A. circinalis* but, as the N:P ratio rose and nitrogen was no longer limiting, then other species, predominantly *Synechococcus* sp., became dominant.

G. Cyanobacteria, Water Column Stability, and TN:TP Ratios

Few attempts have been made to combine information on the degree of water mixing and nutrient conditions and to relate this with the occurrence of particular cyanobacteria. The correlative analysis of 435 US lakes by Harris (1986) attempted to distinguish between the distribution of species on a basis of TN:TP ratios and M, a measure of water column stability ($M = D_{th}/z$) where D_{th} is the mixed depth estimated from the location of the thermocline and z is the mean depth of the lake. $M < 1$ indicates stable conditions in summer as the mean depth of the lake exceeds the depth of the thermocline. $M >> 1$ indicates strong vertical mixing as the mixed depth greatly exceeds the mean depth of the lake. Table 2 gives the maximum percent probabilities of occurrence of four species of cyanobacteria under different mixing conditions and the prevailing TN:TP ratio within decadal intervals from <10 to >50 (Harris, 1986; US EPA data).

Table 2. The percent probabilities of occurrence of four cyanobacteria under different mixing conditions (M) and the prevailing TN:TP ratios (Harris 1986, US EPA data).

Species	M 0-1	TN:TP	M 1-2	TN:TP	M 2-3	TN:TP	M 3-4	TN:TP	M > 4	TN:TP
Anabaena	31	<20	24	<10	26	<10	35	<10	**38**	<10
Aphanizomenon	**67**	<10	49	<10	30	<10	40	<10	14	<10
Microcystis	45	>50	**50**	>50	21	<10	0		0	
Oscillatoria	15	<20	40	<20	50	>50	**100**	<40	36	<10

Anabaena's distribution spanned the complete range of M from stable to highly turbulent water columns but was favoured by TN:TP ratios of <10, suggesting that it competes best in habitats where the flux of N into the water maybe limiting. *Aphanizomenon* also occurred in habitats with low TN:TP ratios, consistent with the observation that when the loading was reduced from 15 to 5:1 in the Experimental Lakes Area a bloom of *Aphanizomenon* developed, whereas it had not been observed before (Schindler, 1977). *Aphanizomenon* had a clear preference for stable summer conditions, although it also occurred at $M > 2$. The growth of the two filamentous species over a wide range of mixing regimes may reflect their ability to occur both as single filaments and as large aggregated colonies and is crudely in accord with the classification discussed in the earlier section. Habitats structured by turbulent mixing. *Oscillatoria* appeared independent of TN:TP ratios, but preferred less stable water columns, suggesting that most samples were from well mixed lakes and that those with metalimnetic populations were not well represented in the data. In contrast, *Microcystis* occurred in stable habitats where there was little evidence for nitrogen limitation.

XIII. Inorganic Carbon

The common observation that cyanobacteria frequently dominate lake phytoplankton at times when the pH is high has led to the hypothesis that these organisms are able to outcompete the eukaryotic micro-algae in situations where carbon dioxide concentration is low and that this is fundamental to their dominance (King, 1970). Shapiro (1990) has reviewed the field and laboratory data supporting this notion and considers that it provides a more robust explanation than most other hypotheses accounting for cyanobacterial dominance. However, in a later publication he concludes low CO_2 concentrations or high pH do not intiate cyanobacterial blooms, but rather their abundance reduces CO_2 to levels that only they can utilise (Shapiro, 1997). The concentration and speciation of dissolved inorganic carbon is strongly linked to pH through equilibrium reactions between the species CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}. The proportion of CO_2 declines from a maximum at pH 4 to only 0.003% of the total inorganic carbon concentration at pH 9, with the actual CO_2 concentration dependent on the total inorganic carbon content of the water.

Phytoplankton photosynthesis removes CO_2 from solution resulting in disruption to the equilibrium and a rise in pH, the magnitude of which depends on the buffering intensity of the water (Shapiro, 1990). It has been shown by manipulating CO_2 concentration and pH in enclosure and lake experiments that cyanobacteria are better adapted to take advantage of low CO_2 concentrations than some, but not all microalgae (Talling, 1976; Shapiro, 1990). Some cyanobacteria have been shown to utilise HCO_3^- (Talling, 1976), but as they generally contain carbonic anhydrase that catalyses the dehydration of bicarbonate it is likely that CO_2 is used within the cell. However these features are not unique to the cyanobacteria and some eukaryotic algae are also able to utilise very low CO_2 concentrations (Talling, 1976) and some can access HCO_3^- as they also contain carbonic anhydrase. The debate over the importance of competition for inorganic carbon has been further complicated by the discovery of the carbon concentrating mechanism in phytoplankton. It is still unclear whether this mechanism is more beneficial in cyanobacteria or microalgae and so the role of inorganic carbon limitation on competition is difficult to assess.

Field experiments where manipulations of carbon and pH have resulted in shifts in species dominance to cyanobacteria under conditions of inorganic carbon limitation (Shapiro, 1990) suggest that a low availability of CO_2 can advantage the gas-vacuolate cyanobacteria. This advantage would be re-enforced during periods of large cyanobacterial blooms, assisting these organisms to maintain dominance. However, the bloom-forming cyanobacteria are not restricted to eutrophic waters and can dominate the communities of less productive lakes, even though the total phytoplankton biomass is low. Conversely, cyanobacteria can also be found to dominate in some well-mixed shallow waters where inorganic carbon limitation is considered unlikely (Steinberg and Gruhl, 1992; Scheffer et al., 1997). These observations would argue against CO_2 limitation being essential for the formation of cyanobacterial blooms, but whatever the final outcome regarding direct competition for inorganic carbon, any limitation will still have a major influence through its impact on buoyancy regulation. Undoubtedly more information is required on the inorganic carbon requirements of the phytoplankton.

XIV. Grazing

Grazing can be a major loss factor modifying the biomass and community composition of the phytoplankton. For example, Gliwicz (1968) and Haney (1973) estimated that the zooplankton community ingested 48 - 162% of their biomass per day, and daily community grazing rates could process 10 to > 100% of the volume they occupy.

The four major groups of animals represented in the zooplankton community; the rhizopods, ciliophorans, rotifers and crustaceans, have diverse means of selecting, obtaining and ingesting food organisms (Reynolds, 1994b). Although the specialist protozoans and rotifers impact significantly on their particular food sources, it is the generalist feeders that often make the greatest impact on the phytoplankton and particularly on the cyanobacteria. Above certain threshold concentrations of prey the grazing impact moves towards the less selective filter feeding rotifers and cladocera (Reynolds, 1994b). Of these it is populations of *Daphnia* species that frequently have the greatest impact.

In Lake Mendota, Wisconsin, the phytoplankton community was regulated by both grazing and nutrients but the influence of each varied seasonally (Vanni and Temte, 1990). In general grazing impacted most on the phytoplankton community during spring while nutrient limitation was more severe in summer. This change was in part due to the replacement of the dominant edible phytoplankton species that occurred in spring by more resistant species in summer. Presumably the appearance of the more resistant species was a result of selection during the earlier grazing period. The composition of the zooplankton community also altered during this period. In early spring the community was dominated by cyclopoids while the clear water period in late spring was dominated by *Daphnia galeata mendotae* which continued into early summer before becoming sub-dominant to calanoids and cylcopoids. *Daphnia* had the largest impact on the spring phytoplankton and was considered mainly responsible for the clear water phase. It was less effective on the large, inedible phytoplankton species that were dominant in summer, particularly cyanobacteria and the dinoflagellate *Ceratium*.

In Lakes Hume and Dartmouth, both man-made lakes located in south-eastern Australia, manipulations of the large cladocerans (*Daphnia* and *Diaphanosoma*) and the large copepod (*Boeckella*) had negative effects on the phytoplankton biomass, while smaller copepods (*Mesocyclops* and *Calamoecia*) had little impact or occasionally a positive effect (Matveev and Matveeva, 1997). In both reservoirs the variation in *Daphnia carinata* alone could account for 50% of the variance in total phytoplankton biovolume. Colonies of *Microcystis* < 50 μm diameter were grazed in feeding trials by both *Daphnia* and *Boeckella* without any suggestion of selectivity. When *Microcystis* dominated in the lakes enclosure experiments showed a negative response of biomass to *Daphnia* and *Boeckella* concentrations.

These studies are in general accord with many others that have found correlations between the abundance of zooplankton grazers and the timing of cyanobacterial blooms (Haney, 1987). In general the reduced grazing on cyanobacteria is associated with large size, high density, allelopathic compounds and poor assimilability. The maximum size of food particles (y) taken in by *Daphnia* species is a function of the animal's length (L) as described by Burns (1968),

$$y = 22L + 4.87$$

which indicates a maximum particle size of 49 μm for a 2 mm animal (Reynolds, 1994b). In general the optimum food sources are small planktonic algae whereas larger colonial and filamentous cyanobacteria have a depressive effect on filter feeding due to mechanical interference with the feeding apparatus (Reynolds, 1994b).

Enclosure experiments have been used to investigate these relationships and Burns (1987) summarised the experiments describing interactions between zooplankton and cyanobacteria by making the following points:

- There was only circumstantial evidence from field experiments for decreased fecundity and growth in response to food limitation effects or toxicity.
- In long term enclosure experiments dominated by large herbivores the more edible cyanobacteria showed an inverse relationship with grazer density such that there appeared to be a threshold density of ca. $5 \cdot \times 10^4$ grazers m^{-2}, above which filamentous cyanobacteria could not withstand grazing but large inedible colonies could (Lynch and Shapiro 1981). A similar result was reported by Ganf (1983) comparing the ungrazed *Aphanizomenon flos-aquae* which occurs as a large flake and dominates in the presence of *Daphnia pulex*, with filamentous *Aphanizomenon elenkinii* which dominated only where *D. pulex*

was absent. Reductions in the edible species required a threshold concentration of grazers of ca. 12 L^{-1}.

- Zooplankton grazing could suppress cyanobacterial growth by altering light and nutrient conditions, in particular by increasing transparency, reducing primary productivity and pH, and increasing nutrient re-supply (Schoenberg and Carlson 1984).

It seems from this that zooplankton grazing can reduce the biomass of cyanobacterial populations provided they are present before the cyanobacteria attain a size larger than the animals can manage. If the phytoplankton species can reach a large size prior to substantial increases in the most effective grazers then the likelihood of control by grazing will be diminished. The grazing impact will therefore depend on the dynamics of the phytoplankton and zooplankton communities.

A number of indices have been suggested for assessing zooplankton grazing effects at the whole lake level (Reynolds, 1994b) including zooplankton number (Lynch and Shapiro, 1981), total zooplankton biomass, and the ratio of biomass of zooplankton to phytoplankton. Matveev and Matveeva (1997) showed using enclosure experiments that grazing was significant when the Cladocera/Phytoplankton biomass ratio was greater than 0.1, and found in both Lakes Hume and Dartmouth that clear water phases occurred when this ratio was exceeded, even when cyanobacteria were present.

XV. Concluding remarks

We have examined many of the attributes of gas-vacuolate cyanobacteria that have been proposed to account for their success. These have been compared between species of cyanobacteria, and between the cyanobacteria and the eukaryotic micro-algae in an attempt to assess their relative importance. The following comments summarise these considerations:

- Buoyancy and its regulation provide gas-vacuolate cyanobacteria with a significant advantage over the micro-algae. In deep waters where turbulence intensity is low buoyancy regulation is an asset, as it enables the cyanobacteria to maximise their growth conditions, largely by circumventing the vertical separation in resources that develops in thermally stratified waters. When the velocity of turbulent eddies is more than 15 times the floating velocity then the population will be evenly distributed and the advantage of buoyancy for accessing the illuminated surface layers is negated. However, even in well mixed surface layers of stratified lakes and in moderately turbulent shallow waters the attribute of buoyancy can reduce losses by sedimentation and provide the cyanobacteria with an advantage over non-buoyant micro-algae. The benefit of buoyancy is intimately linked with the nature of the turbulent mixing regime.
- Within the cyanobacteria there are major differences between species which influence their sinking/floating velocity, in particular the size of the biomass unit varies from small, single filaments in *Oscillatoria* to complex, large colonies in *Microcystis*. The small size and slow sinking/floating velocities of *Oscillatoria* permits it to position itself at its preferred low light environment where it often forms deep water maxima in stratified lakes. The large colonial forms of *Microcystis* can move quickly enabling it to take advantage of habitats which oscillate between stratified and mixed conditions.
- There are marked differences in the light harvesting complexes and pigment composition of micro-algae and cyanobacteria, however, it is still unclear whether cyanobacteria respond differently to micro-algae to changing light intensity, day length and spectral composition. Laboratory studies suggest that micro-algae do not respond to changing photoperiod but to light intensity. Field studies suggest that certain cyanobacteria (eg *Microcystis*) may take two days to adapt to high irradiances, but green algae (eg *Scenedesmus*) respond more quickly which provides an explanation of why *Scenedesmus* may dominate in turbid, continuously mixed, shallow water bodies. However, the energy required to synthesise gas vesicles in the competing cyanobacteria may be sufficient to push the energy balance in favour of green algae under low light conditions. The phycoerythrin rich species such as *Oscillatoria agardhii* and *Gloeotrichia echinulata* are low light adapted having a wide spectral activity and have an advantage over both micro-algae and phycoerythrin poor cyanobacteria, especially in water bodies where the most penetrating waveband is 560-570 nm.
- There is no evidence to support the hypothesis that bloom-forming cyanobacteria prefer higher temperatures than micro-algae. However, in *Microcystis* carbohydrate metabolism is depressed

at temperatures below 8-12°C, which influences buoyancy regulation.

- There does not appear to be any consistent phylogenetic differences in phosphorus kinetics between the cyanobacteria and the micro-algae. Some cyanobacteria are able to store significant quantities of phosphorus, but in the case of *Microcystis* and *Gloeotrichia* their success appears just as dependent on behavioural features that enable them to utilise this attribute.
- The general response to phosphorus reduction is a lowering of the cyanobacterial biomass. However, the extent and nature of the response is dependent upon the internal phosphorus load and the magnitude of the persistent external load. Where initial phosphorus concentrations are in excess of requirements, the effects of P-removal may be minimal. If internal loads are significant then responses may take many years to eventuate. If the FRP in the trophogenic layer during the growing season falls below 10 mg m^{-3}, a significant reduction of biomass occurs as the community becomes P-limited.
- The source of inorganic nitrogen available to cyanobacteria may influence their success. It has been proposed that non-nitrogen fixing cyanobacteria are favoured by ammonium, micro-algae by nitrate and the nitrogen-fixing cyanobacteria by nitrogen deficiency. The evidence for this is not conclusive particularly for organisms using ammonia, but laboratory studies do support the suggestion that the cyanobacteria are disadvantaged when using nitrate under low light conditions.
- Significant nitrogen fixation in the plankton is restricted to the heterocystous cyanobacteria and provides them with a distinct advantage when inorganic nitrogen concentrations fall below ca. 100 mg m^{-3}; nevertheless, these populations will often be limited ultimately by the available phosphorus.
- The overwhelming evidence is that TN:TP ratios do not *per se* influence the occurrence of planktonic cyanobacteria. However, nitrogen fixers (eg *Anabaena* spp.) may occur if the TN:TP ratio is low and correctly predicts that inorganic nitrogen is limiting. On the other hand *Gloeotrichia echinulata* may dominate when TN:TP ratios are high, but the P-content of the water column is insufficient to support growth.
- Very few data are available to test critically the proposal that cyanobacteria are better able to utilise low inorganic carbon concentrations than the micro-algae. Again this does not appear to be a consistent phylogenetic difference, but more extensive measurements are required.
- Grazing can reduce cyanobacterial populations if they are comprised of sufficiently small units (filaments or colonies), but if they reach a large size before substantial increases in the most effective grazers then the likelihood of control is diminished. In general the reduced grazing on cyanobacteria is due to their large size and high density and this can provide an advantage over small microalgae.

In conclusion, we suggest that the occurrence and abundance of various types of gas-vacuolate cyanobacteria is not reliant on any one particular environmental stimulus, but depends on a complex interplay of factors. A flow chart has been used in an attempt to portray these interactions and to highlight the role of environmental conditions in supporting the growth of particular species of gas-vacuolate cyanobacteria (Fig. 10). The flow chart is not definitive and is unlikely to be a dependable tool for predicting either the likelihood of cyanobacterial blooms or their identity; it is presented simply for illustrative purposes. It consists of a series of questions assessing the major environmental conditions believed to influence the success of gas-vacuolate cyanobacteria and in this way it summarises what we view as key interactions. The critical values that are proposed at each level to discriminate between the success or not of gas-vacuolate cyanobacteria must be viewed as hypotheses only and are unlikely to be robust. Most of these are covered in more detail within the text.

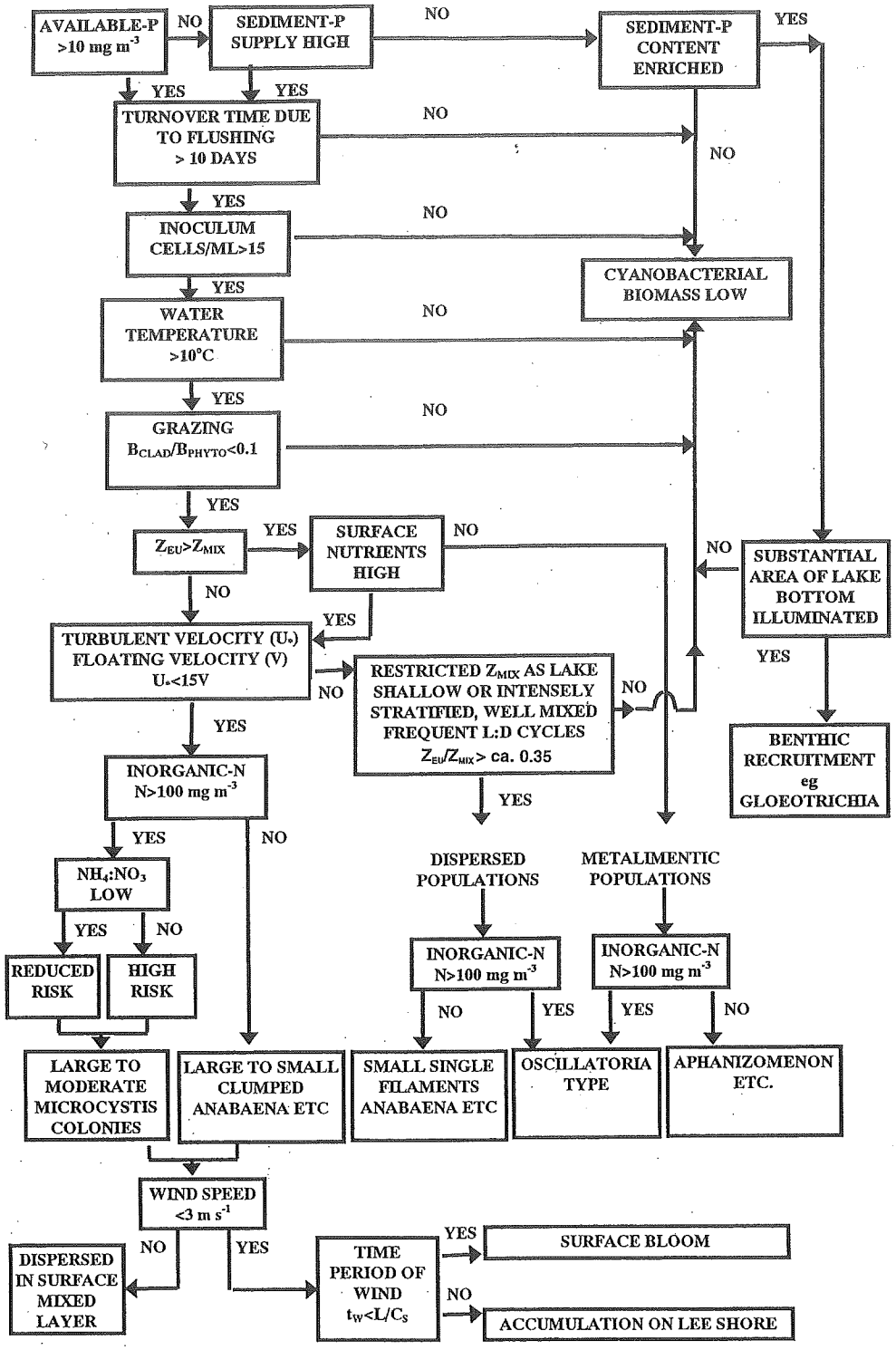

Fig. 10 Flow chart summarising prominent environmental characteristics supporting the development of cyanobacterial blooms and selecting for particular genera. The text provides further detail on some of these components. Key: B_{CLAD} biomass of cladocerans, B_{PHYTO} biomass of phytoplankton, z_{eu} euphotic depth, z_{mix} depth of mixing, u_* shear velocity, V floating or sinking velocity of cyanobacteria, t_w time that the wind blows, L lake fetch, c_s surface current speed.

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