CHAPTER 6

THE ECOPHYSIOLOGY OF THE HARMFUL CYANOBACTERIUM *MICROCYSTIS*

Features explaining its success and measures for its control

Petra M. VISSER, Bas W. IBELINGS, Luuc R. MUR
& Anthony E. WALSBY

1 Introduction

1.1 HARMFUL CYANOBACTERIA

Cyanobacteria (blue-green algae) are the scourge of water management. Several species are toxic and on forming scums generate dense cell accumulations in which the toxins concentrate many fold. Reynolds (1991) has calculated that as little as 5 μL of a dense scum could contain a lethal dose of cyanotoxin. Many types of harmful cyanobacteria have been described, mostly from the freshwater phytoplankton community. We focus here on *Microcystis*, which is cosmopolitan, usually harmful (it produces toxins and surface blooms), and one of the best studied genera of the cyanobacteria. In selecting *Microcystis* for a case study, the intention is to find explanations that apply to toxic cyanobacteria in general. We first compare the main forms of cyanobacteria that inhabit lakes and reservoirs, in order to determine the common denominators.

The principal genera of planktonic cyanobacteria in lakes are listed in Table 1. There are three basic morphological forms: picoplanktonic unicells (e.g., *Synechococcus* sp.); separate filamentous forms (e.g., *Planktothrix rubescens*); and colonies, either of unicells (e.g., *Microcystis aeruginosa*) or of filaments (*Aphanizomenon flos-aquae*). Against this morphological diversity, what do these organisms have in common? All of these cyanobacteria grow by photoautotrophy, which is possible only within the euphotic zone of lakes. Most of the filamentous forms and all of the colonial forms produce gas vesicles, which confer buoyancy. In many other respects, however, they differ. Even the toxins they produce vary: they include cyclic peptides, heterocyclic compounds and lipids.

J. Huisman, H.C.P. Matthijs and P.M. Visser (eds.), Harmful Cyanobacteria, 109–142.

1.2 VARIATION IN *MICROCYSTIS* SPP.

The taxonomy of the genus *Microcystis* was originally based on the morphology of cyanobacteria present in natural waters to include coccoid, unicellular cyanobacteria that form colonies. Bourrelly (1970) restricted *Microcystis* to spp. with spherical cells, suggesting that those with ellipsoidal cells (like *M. elabens*) are put in *Aphanothece*. The genus *Microcystis* (first described by Kützing in 1833) was made synonymous with *Aphanocapsa* on the grounds that the distinguishing character that separated them (cells scarcely or densely packed) was difficult to define, but Komàrek and Anagnostidis (1986) kept the genera separate on the grounds that *Aphanocapsa* cells divide in only two successive planes whereas *Microcystis* cells appear to divide in three. *Microcystis* is distinguished from the colonial *Gomphosphaeria*, in which the cells are localised radially in a surface layer and joined by stalks radiating from the centre (Komárek and Hindák, 1988), and from various solitary unicellular forms, such as *Synechocystis*. Examples isolated into culture, however, usually lose their ability to form colonies. It is possible that the strains that grow in culture are not the principal components of the natural colonies but other minor components of the mixed population. The majority of *Microcystis* spp. listed by Geitler (1932) contains gas vacuoles (though some, e.g., *M. pallida*, do not). It is now often assumed that gas vacuoles are diagnostic; in the Pasteur Culture Collection of cyanobacteria (PCC) a putative *Microcystis* isolate that did not retain its gas vesicles in culture was re-classified as *Synechocystis* sp. (Rippka et al., 1979).

Table 1. Examples of planktonic cyanobacterial genera and species, grouped by morphology. The presence of gas vacuoles is noted with +.

Morphology *Genus or species*	***Gas vacuoles***	***Colony form***
Picoplanktic unicells		
Synechococcus sp.	–	–
Single filaments		
Aphanizomenon ovalisporum	+	–
Planktothrix (Oscillatoria)	+	–
Tychonema (O. borrellyi)	–	–
Limnothrix	+	
Colonies of unicells		
Microcystis spp.	+	pleomorphic
Coelosphaerium	+	oval
Gomphosphaerium	+	oval
Colonies of filaments		
Gloeotrichia echinulata	+	radial colony
Nodularia spumigena	+	tangle; some single
Aphanizomenon flos-aquae	+	trichomes in rafts

Microcystis belongs to the order Chroococcales and to the family *Microcystaceae* Elenkin 1933, together with *Eucapsis* spp., *Gloeocapsa* spp. and *Chondrocystis* spp. *Microcystis* is the only genus in this family without gelatinous envelopes around individual cells or small groups of cells. The coccal *Microcystis* cells always grow in colonies in natural populations. Cells are spherical and cell division occurs regularly in three planes perpendicular one to another in successive generations. Consequently, daughter cells are hemispherical but they grow into the original spherical form and size before the next division.

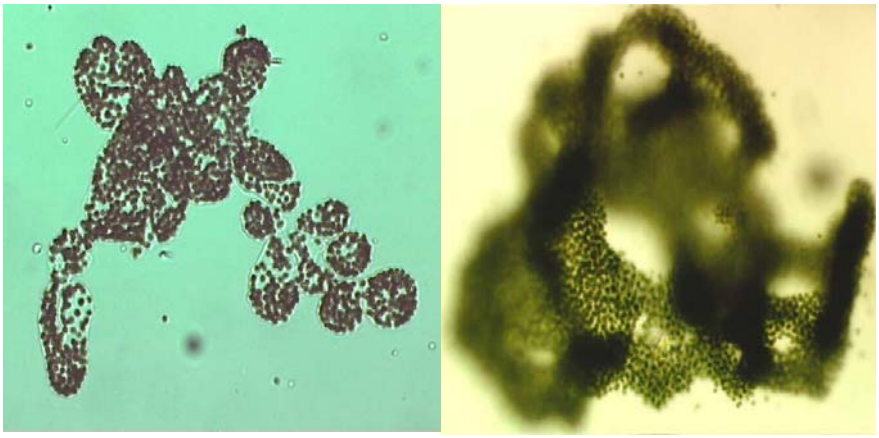

Figure 1. Two Microcystis *species:* M. wesenbergii *(left) and* M. aeruginosa *(right). Photographs by P.M. Visser.*

Natural populations of *Microcystis* spp. are often referred to as *Microcystis aeruginosa* though taxonomists distinguish many more species separated by characters such as the size and shape of the cells and the colonies (Fig. 1; see Komárek and Anagnostidis, 1986, 1999; Komárek and Hindák, 1988). The non-taxonomist is daunted by the plasticity of the salient characters and by reports of seasonal changes in cell size and colony type (Reynolds et al., 1981). The observations made in this article may refer to a rather diverse group of unicellular colonial cyanobacteria.

1.3 THE OCCURRENCE OF *MICROCYSTIS*

What is the niche that *Microcystis* occupies and what are the factors that explain its dominance in the phytoplankton community of so many lakes? Figure 2 shows an example of a surface bloom of *Microcystis*. Surface blooms of *Microcystis* have been described in water bodies ranging in size from small ponds to the largest lakes, distributed geographically from cold-temperate regions to the tropics. Examples that

have been studied include the following: Lake Limmaren in Sweden (Brunberg and Blomqvist, 2003), Crose Mere in England (Reynolds, 1976); Lake Vinkeveen (Ibelings et al., 1991a, 1991b), Lake Nieuwe Meer (Visser et al., 1996a) and Lake IJsselmeer (Ibelings et al., 2003) in the Netherlands; fish ponds in Israel (Van Rijn and Shilo, 1985); a hypertrophic shallow lake in Cameroon (Kemka et al., 2003); Lake George, on the equator in Uganda (Ganf, 1974); Hartbeespoort Dam in South Africa (Robarts and Zohary, 1984); lakes in China (Xie et al., 2003; Chen et al., 2003); Lake Biwa in Japan (Tsujimura et al., 2000); the lower Great Lakes in USA (Murphy et al., 2003); Mount Bold Reservoir in South Australia (Ganf et al., 1989), and Lake Okaro in New Zealand (Walsby and Macallister, 1987). In tropical lakes, *Microcystis* can persist throughout the year but in temperate lakes blooms occur mainly in summer. In this chapter, the emphasis is on the occurrence of *Microcystis* in lakes in the temperate zone.

Reynolds et al. (1981) suggested various explanations for the occurrence of *Microcystis* water blooms in such diverse water bodies: i) high nutrient loadings; ii) raised pH and decreased CO_2; iii) the interaction between light attenuation and stability of the water column; iv) tolerance of low O_2-concentrations and low redox potential affecting the availability of sulphur, iron and other metals; v) resistance of colonies to grazing, either through size and or toxicity. Others have added vi) a low N:P ratio as a step in the pathway leading to noxious cyanobacterial blooms (Smith, 1983; Elser, 1999).

Figure 2. A surface bloom of Microcystis *(Lake Nieuwe Meer, Amsterdam, The Netherlands). Photograph by P.M. Visser.*

1.4 SCOPE OF THIS CHAPTER

In this chapter the basic features of *Microcystis* are related to the conditions under which mass developments of *Microcystis* occur. We attempt to explain the adaptations that make *Microcystis* such a conspicuous member of the phytoplankton of many lakes. We conclude by reviewing the ways in which knowledge of its ecology has been applied to the development of measures for preventing *Microcystis* blooms.

2 Features of *Microcystis*

2.1 BUOYANCY OF *MICROCYSTIS* COLONIES

During the summer, *Microcystis* often develops in the epilimnion of lakes that are subject to frequent mixing. When a lake is strongly mixed by wind, all suspended phytoplankton are stirred through the epilimnion. In eutrophic lakes, the light will be steeply attenuated by the circulating phytoplankton and their growth will become light-limited. When calm conditions return, phytoplankton left in the surface layers will receive enough light for growth but those becalmed at depth may be below the photosynthetic compensation point. Small-celled phytoplankton will be incapable of moving up the water column but buoyant *Microcystis* colonies will float up into the higher irradiance near the water surface. The buoyancy of these cyanobacteria is provided by gas vesicles. To understand the mechanisms (and costs) of buoyancy regulation it is useful first to summarise some of the properties of the gas vesicle and the way in which it is formed.

2.1.1 Gas vesicles

Gas vesicles have the form of minute hollow cylinders closed with conical end caps. The structure is rigid and waterproof. Gases dissolved in the surrounding solution diffuse freely through the wall, so that the gas inside is usually air at atmospheric pressure (Walsby, 1969). Surface tension at the hydrophobic inner surface prevents liquid water from entering. The gas vesicle wall is made entirely of protein that self-assembles to form first a biconical structure, which, when it has reached a certain diameter, then continues to grow by the extension of the cylindrical middle section. The wall of both the cylinder and the cones resembles a coil pot. The coil is formed by a small hydrophobic protein, GvpA, the main constituent. On the outside surface is another protein, GvpC, with a repeating structure that binds to GvpA and stabilises the structure (Walsby, 1994).

The rigid wall of the gas vesicle will withstand moderate pressures but at a certain critical pressure (p_c) it collapses flat: the wall is broken on collapse and cannot be reinflated. For mechanical reasons, the mean critical pressure depends mainly on the cylinder diameter, which varies in different cyanobacteria. Strains of *Planktothrix rubescens* from deep lakes have narrow gas vesicles (d = 50 nm) with a p_c of about 1.1 MPa (11 bar, equivalent to a water column about 110 m deep); in shallower lakes are found cyanobacteria with progressively wider and weaker gas

vesicles; in the shallowest freshwater lakes strains of *Anabaena, Aphanizomenon* and *Gloeotrichia* are found with gas vesicles of width 85 nm and of p_c 0.55 MPa. There has evidently been natural selection for gas vesicles of increasing p_c in lakes of increasing depth. This production of narrower gas vesicles carries a penalty, however, because the wider gas vesicles have a lower buoyant density (only 120 kg m^{-3} in *Anabaena* compared with 190 kg m^{-3} in *Planktothrix*): in shallower lakes the efficient provision of buoyancy provides counter selection for the widest gas vesicles allowed by the depth requirement. The widths of the gas vesicles in different strains of cyanobacteria are determined at least in part by differences in the length and sequence of GvpC (Beard et al., 1999, 2000). These esoteric considerations are central to the understanding of buoyancy regulation and the design of devices for gas vesicle collapse used in the control of cyanobacteria.

Isolates of *Microcystis* from different lakes have gas vesicles whose mean p_c ranges from 0.6 to 0.9 MPa. One strain investigated in detail, *Microcystis* BC 8501, has gas vesicles with a diameter of 65 nm, a p_c of 0.85 MPa and a buoyant density of 158 kg m^{-3}.

2.1.2 Provision of buoyancy

Most of the principal constituents of cyanobacteria have densities (protein 1330 kg m^{-3}, carbohydrate 1600 kg m^{-3}, glycolipid 1050 kg m^{-3}) that are greater than that of water (998 kg m^{-3} at 20 °C). The overall density of the cells depends on the relative proportions of these constituents and the cell water, 70 – 80% of wet mass. The buoyant density of cyanobacteria, without gas vesicles, is usually in the region of 1060 kg m^{-3}. To reduce this density to that of water (neutral buoyancy) the cells must accumulate sufficient gas vesicles: the amount required depends on the width-dependent density of the gas vesicles: for this neutral buoyancy, the *Microcystis* cell needs to accumulate a volume of gas equivalent to 6.2% of the cell volume, which requires gas vesicle protein equivalent to 6.6% of the cell protein (4.9% of the dry mass). These are the irreducible costs of buoyancy. With the stronger, narrower gas vesicles in *Planktothrix* the costs rise to 8.2% of the cell protein, whereas with the weaker, wider *Anabaena* gas vesicles they fall to 6.2%. When *Microcystis* cells become over-buoyant in response to low irradiance they may devote as much as 10% of cell protein to gas vesicle production (Walsby, 1994).

2.1.3 Buoyancy changes in response to irradiance and nutrients

All gas-vacuolate cyanobacteria vary their buoyancy in response to irradiance: at low irradiance they become buoyant and on transfer to high irradiance they lose their buoyancy (Dinsdale and Walsby, 1972; Oliver, 1994). The proportion of buoyant cyanobacteria in natural populations has been shown to increase with depth and at night: both are explicable by the irradiance response (Walsby et al., 1983; Visser et al., 1996b; Porat et al., 2001). In laboratory cultures kept on light-dark (L:D) cycles there is a similar rise and fall in buoyancy state through the dark and light phases (Thomas and Walsby, 1986; Konopka et al., 1987b). Cultures of *P. rubescens* are on average neutrally buoyant over the L:D cycle when they receive a photon irradiance of between 7 and 11 μmol m^{-2} s^{-1} during the 12-h light phase, equivalent to a *neutral buoyancy insolation* (Q_n) of 0.3 – 0.5 mol m^{-2} over the 24-h

cycle (Walsby et al., 1983; Walsby et al., 2004). In *Anabaena flos-aquae* Q_n is higher, 1.1 mol m^{-2} (Walsby and Booker, 1980), a consequence of the lower photosynthetic affinity coefficient for light (α) for this cyanobacterium, which lacks phycoerythrin. There has been no measurement of Q_n in *Microcystis* spp. but it is likely to be closer to the *Anabaena* value.

Irradiance is not the only factor that affects buoyancy; it is also affected by nutrient availability. The addition of combined nitrogen to a transparent plastic column lowered in a stratified lake caused a population of *Planktothrix* (*Oscillatoria*) *agardhii*, neutrally buoyant in the metalimnion, to become more buoyant and rise to the water surface (Klemer, 1978; Klemer et al., 1982). Cyanobacteria in culture also become more buoyant at higher nutrient concentrations. Cultures of *Microcystis aeruginosa*, which increased in buoyancy at low irradiance were less buoyant when phosphate-limited and increased in buoyancy when the limitation was relieved (Konopka et al., 1987a). These interactions of irradiance and nutrient availability can be understood with knowledge of the mechanisms of buoyancy change (see next section).

There are two main consequences of light-driven buoyancy regulation: stratification of cyanobacteria and vertical migration. They are the outcome of the same physiological response performed by cyanobacteria of different size (Kromkamp and Walsby, 1990). According to Stokes's Law, large colonies move faster through the water column than small colonies or single filaments. Filamentous cyanobacteria capable of buoyancy regulation may therefore stratify at the metalimnion of oligotrophic lakes. The water of the epilimnion must be sufficiently transparent to allow enough light for buoyancy regulation at the depths of the stable thermocline region. During the daytime the cyanobacteria lose buoyancy and slowly sink; at night they regain buoyancy and slowly float up. Only single filaments can do this: large colonies move too quickly and are lost from the stable density gradient. In eutrophic lakes insufficient light reaches the metalimnion for buoyancy regulation and filaments rising into the epilimnion become entrained in the surface mixed layer.

Vertical migration is performed by colonial cyanobacteria in the epilimnion of lakes. They also sink down during the day and float up at night, but, with their inherently greater velocity, they move over much greater depths. They may perform daily excursions to and from the lake surface, as observed with a population of *Microcystis* in Lake Okaro, New Zealand (Walsby and McAllister, 1987). It has been suggested that by performing buoyancy regulation, the colonies may benefit by avoiding prolonged exposure to potentially damaging high irradiance at the surface and perhaps also by gaining access to nutrient-rich waters below the thermocline at night (Fogg and Walsby, 1971), but a more recent study (Bormans et al., 1999) addressing this last question could not demonstrate this.

Models provide important tools for understanding the distribution of buoyant cyanobacteria. Vertical migration by *Planktothrix agardhii* has been modelled by Kromkamp and Walsby (1990). Visser et al. (1997) adapted this model to simulate migration of *Microcystis* in a quiescent water column and validated the model with the use of cultures. This *Microcystis*-model has been used in other publications with further modifications (Wallace and Hamilton, 1999; Rabouille et al., 2003).

2.1.4 Mechanisms of buoyancy regulation

Changes in the buoyant density of a cell must be brought about by changes in the relative proportions of gas vesicles, which are less dense, and the other cell components, which are denser than water. The loss of buoyancy in high irradiance may have three causes.

Gas vacuole collapse. In *Anabaena flos-aquae* the loss of buoyancy on exposure to high irradiance is correlated with an increase in cell turgor pressure sufficient to cause loss of the weaker gas vesicles present (Dinsdale and Walsby, 1972). Oliver and Walsby (1984) measured the volume of gas vesicles lost and showed that it could explain the buoyancy loss (but see below). The maximum turgor pressure is about 0.5 MPa, however, and in other cyanobacteria with stronger gas vesicles, turgor pressure alone is insufficient to collapse gas vesicles.

Regulation of gas vesicle production. In a red-coloured *Planktothrix* whose gas vesicles are too strong to be collapsed by turgor pressure, Utkilen et al. (1985) found that the relative gas vesicle content of the cells decreased at high irradiance. There was no loss of gas vesicles but the cells stopped making new gas vesicles: gas vesicles were diluted out by growth.

Carbohydrate production. The high rates of photosynthesis at (moderately) high irradiances produce an excess of carbohydrate, which is stored as polyglucose granules. This accumulates at high irradiances and counteracts the buoyancy provided by gas vesicles. In the dark, the store decreases as carbohydrate is respired and converted into less dense protein. These changes are universal and in gas-vacuolate strains this is the main cause of buoyancy loss. In *Anabaena flos-aquae* the buoyancy loss due to carbohydrate synthesis at high irradiance occurs even faster than that due to collapse of gas vesicles by turgor rise (Kinsman et al., 1991). In *Microcystis* sp., there is no down-regulation of gas vesicle production or collapse by turgor pressure at high irradiance and carbohydrate accumulation is the principal cause of buoyancy loss (Thomas and Walsby, 1985).

2.1.5 Benefits of buoyancy in lakes

The obvious benefit of buoyancy is that it lifts phototrophic cyanobacteria closer to the water surface where the higher irradiance supports a higher rate of photosynthesis. Humphries and Lyne (1988) used a mathematical model to investigate the effect of positive buoyancy on the vertical distribution of *Microcystis* colonies circulating in a sporadically mixed epilimnion, and analysed its effect on growth. They concluded that colonies that floated would receive more light and would always be more productive than those that sank but that the magnitude of the advantage depended on the ratio of the euphotic depth, z_{eu}, to the mixed depth, z_m. Humphries and Lyne (1988) described the behaviour of *Microcystis* in water columns of varying stability as "tracking" the near-surface mixed layer, though this can be misunderstood as suggesting a behavioural response rather than the outcome of passive buoyancy.

Walsby (1997) used a computer spreadsheet to calculate, from measured values of the varying surface irradiance and the vertical profiles of light attenuation and temperature, the potential photosynthesis of a cyanobacterial population at each

1-m depth through a 30-m water column and 5-min time interval over 24 h. In a population of *Aphanizomenon* followed for 9 days in the Baltic Sea, the potential photosynthesis of colonies that were mixed down during windy periods but floated up during calm periods, was twice that of a population uniformly distributed through the water column (Walsby et al., 1997). This doubling of production was contrasted with the cost of providing buoyancy, only 10% of protein production. A similar analysis has been made on a population of *Anabaena circinalis* in the turbid, slowly-flowing Darling River in Australia, in which the potential photosynthesis of the colonies that floated up exceeded five-fold that of a population evenly dispersed through the depths (Mitrovic et al., 2001).

For cyanobacteria that float up in calm periods following episodes of mixing, the advantage they obtain depends on their floating velocity. For example, in the Baltic Sea the *Aphanizomenon* population was uniformly mixed down to a depth of 16 m after a short storm, giving a mean depth for the population of 8 m: over the next few days the mean depth decreased to 4 m as the population was telescoped into a shallower surface layer. The accumulation depended on a high floating velocity of the colonies, about 20 m day^{-1} (Walsby et al., 1997). This is important not only in relation to the distance that the colonies must move but also in relation to upward movement within the surface mixed layer (see Oliver and Ganf, 2000).

The high floating velocity of cyanobacterial colonies is strongly dependent on size. According to the Stokes Equation, the floating velocity (U_s) of a sphere is proportional to the square of its radius (r): a tenfold increase in r results in a 100-fold increase in U_s. Except under very calm conditions, only cyanobacteria that form large colonies, such as *Anabaena*, *Aphanizomenon* and *Microcystis,* are able to produce surface blooms (Reynolds and Walsby, 1975; Reynolds, 1987). An example of surface bloom formation by *Planktothrix* was explained by the unusual aggregation of the separate filaments into urchin-like colonies (Walsby et al., 1983).

2.1.6 Buoyancy and surface scums

Consequences of being colonial. *Microcystis* species grow as colonies in their natural habitats. Colony formation obviously increases the size of these cyanobacteria. This has some important consequences for their ecology. First, the larger size (through a reduction in the ratio of surface area to volume) apparently has negative effects on nutrient uptake, on light harvesting, on photosynthesis, and on overall growth rate (Reynolds, 1997). In fact, Reynolds (1997) ranks *Microcystis* amongst the poorest resource competitors and slowest-growing species in the phytoplankton. This is probably due not only to the colony size, since they are also bad competitors in single cell cultures (Huisman et al., 1999). Second, larger size may offer protection against grazing, one of the main loss factors in phytoplankton populations. And third, large size enables *Microcystis* to translate buoyancy into rapid upward movement in the absence of mixing. This rapid flotation may even result in the formation of surface blooms with potentially enhanced access to light and CO_2 (Paerl and Ustach, 1982), but also with the risk of photodamage by the exposure to full sunlight (Ibelings, 1996). Is surface bloom formation a strategy of buoyant species aimed at intercepting light and CO_2 at the lake surface, as

suggested by Paerl and Ustach (1982), or should scum formation be considered as a fortuitous consequence of being buoyant, the price *Microcystis* has to pay for the benefits of floating into the surface mixed layer?

The phenomenon of noxious surface blooms of decaying cyanobacteria has suggested there are disadvantages in buoyancy and has prompted explanations that they represented failure of the buoyancy regulating mechanism that might prevent their occurrence (Reynolds and Walsby, 1975). There are, however, mitigating circumstances near the water surface: the high irradiance will support a higher photosynthetic rate and the supply of CO_2 for the process is ultimately dependent for its replenishment on diffusion from the overlying atmosphere (Walsby, 1970; Ibelings and Maberly, 1998).

Scum thickness: flotation and skimming. The total population of cyanobacteria integrated through the water column of a lake does not usually much exceed an areal cell biovolume concentration of 200 cm^3 m^{-3}. If all of the population floats to the water surface it will form a continuous layer of cells only 0.2 mm thick (equivalent to 40 cells deep): even at this areal concentration there is severe self-shading. In practice, the surface layer may appear thicker because the cells are embedded in mucilage whose volume may be 5-fold greater (giving a 1-mm layer) and interstitial spaces must occur between even closely packed colonies, but by growth and flotation alone, the layer thickness should not much exceed 2 mm. See Figure 2 for an example of such a surface scum.

What explains the formation of scums several centimetres thick is an additional lateral concentration by wind-induced movements in the surface layer. Paradoxically, the highest surface current velocities (U_s) velocities occur at moderate wind speeds (U_a). Within the wind speed range of 0.5 to 5.0 m s^{-1}, the surface current velocity is given by the empirically determined relationship: $U_s = 0.03U_a - 0.005U_a^2$ (George and Edwards, 1976); the maximum U_s of 0.045 m s^{-1} (equivalent to 3.9 km day^{-1}) is given by a wind speed of 3 m s^{-1} (Oliver and Ganf, 2000). A skimming of colonies at the surface can cause the substantial build up of thick cyanobacterial scums along leeward shores. Scums found at the lee shores of lakes can be considered as an important part of the production in a lake and in very large lakes with a high state of eutrophication they may constitute a huge biomass. Extreme examples are the hyperscums formed in the South African Hartbeespoort Dam Reservoir (Zohary and Robarts, 1989) where scums of *Microcystis* persist over a period of ten months of the year. In moderate climate zones, blooms and scums are found only in summer time (but see Howard, 2001).

2.1.7 Conditions in scums

Cyanobacteria like *Microcystis* can take up HCO_3^- to provide CO_2 for photosynthesis and they are thought to possess particularly efficient carbon concentrating mechanisms (CCM) that elevate the concentration of CO_2 around the photosynthetic carboxylating enzyme Rubisco (Talling, 1976; Kaplan and Reinhold, 1999). In the absence of a CCM, Rubisco would mainly work as an oxygenase resulting in photorespiration – one of the processes that result in the uptake of oxygen in the presence of light. The buoyancy of *Microcystis* may help in assuring its access to carbon. A surface bloom positions cells close to re-supply of CO_2 from

the air. Yet, because of the high biomass in a scum, ensuring strong local demand for CO_2, carbon may actually become depleted in scums (Ibelings and Maberly, 1998). Inorganic carbon is present in water as dissolved CO_2, HCO_3^-, and CO_3^{2-}, in proportions controlled by the master variable, pH. When the demand for carbon outstrips the re-supply, pH will rise shifting the equilibrium away from CO_2 towards HCO_3^-, and even CO_3^{2-}. Ibelings and Maberly (1998) studied scums of floating cyanobacteria in water with high and low alkalinity and a varying CO_2 concentration (between 0 and 3500 ppm) in the headspace above the bloom. Recovery of cells from exposure to high irradiance at noon was impeded when CO_2 was absent in the headspace, indicating that the combinations of limiting inorganic carbon and high irradiance overwhelmed the photoprotective mechanisms of the cyanobacteria in the bloom. Earlier, Whitelam and Codd (1983) also demonstrated experimentally that photoinhibition of photosynthesis in *Microcystis* is augmented under CO_2 depletion.

The main consequence for *Microcystis* colonies of transition from mixed conditions to static conditions at the lake surface is the exposure to full sunlight. In addition, the temperature may increase by several degrees inside a scum. Remote sensing from NOAA satellites shows that temperatures of the surface water in areas of scums may be up to 5°C higher than areas outside the scum (Ibelings et al., 2003). Ibelings (1996) showed that this combination of high irradiance and high temperature is more damaging to the cells in a scum than that of either factor on its own. The higher temperature explained why *Microcystis* cells in a scum do not recover from photoinhibition when irradiance decreased later in the afternoon (Ibelings, 1996). In the absence of such stresses, cells should recover from photoinhibition in a matter of minutes.

The combined effects of extreme irradiance and temperature suggest scum formation constitutes a potential source of loss for buoyant species. Loss factors are especially costly for slow growing species like *Microcystis*. The fate of *Microcystis* cells in a scum will be highly dependent on its recent (light) history. If the cells prior to bloom formation were already acclimated to high irradiance, they may survive. Cells acclimated to low irradiance, however, lack the necessary level of photoprotection (see next section). Zohary and Robarts (1990) found long-term survival of *Microcystis* in the extreme hyperscums they studied. In these systems the upper crust of the scum protected cells in deeper layers against photooxidation and desiccation.

2.2 PHOTOACCLIMATION AND PHOTOINHIBITION

Photoacclimation is the phenotypic adjustment to changes in the availability of light, most notably the up- or down-regulation of cellular pigment content, but also in the components of the electron transport or enzymes of the Calvin cycle (Falkowski and Laroche, 1991; MacIntyre et al., 2002). The role of photoacclimation is not just to maximize the rate of photosynthetic carbon assimilation, but also to protect the cells against damage by an excess of energy. Photoacclimation is not complete until conditions of balanced growth have been established, i.e., the specific rate of change of all measures of cell mass are equal

(MacIntyre et al., 2002). Some cyanobacteria for instance may be grown at full sunlight, but only when acclimated gradually to these extreme light conditions.

For buoyant, bloom-forming phytoplankton species like *Microcystis* the time spend at or near the lake surface determines the risk of photoinhibition. Cells will float to the surface only if they become disentrained from turbulent mixing, i.e. only at low wind speeds (generally below 3 m s^{-1}). Photoinhibition of photosynthesis will occur if cells are exposed to an irradiance that is (much) higher than the level to which they were acclimated (MacIntyre et al., 2002). Light stress results from irradiance in excess of that which can be used directly in photosynthesis (Powles, 1984). Under these conditions safe dissipation of the excess of excitation energy is required to protect the photosystems from long-term damage (Niyogi, 2000). Surface bloom formation by buoyant cyanobacteria is perhaps one of the best natural examples of a process bringing about the abrupt increase in irradiance that may cause severe photoinhibition, even in nutrient replete or otherwise unstressed cells. The effect is all the more damaging since surface bloom formation is more likely after a period of deep mixing / low average irradiance. Cyanobacteria synthesise new gas vesicles at low irradiances. Once mixing subsides the resulting over-buoyant colonies can no longer loose buoyancy and they become trapped at the surface (Reynolds and Walsby, 1975). Photoinhibition sets in quickly and there is then a real risk of photoinhibition causing long lasting damage.

Photosynthesis in cyanobacteria and higher plants is carried out by two sequentially placed photosystems, PS2 and PS1, linked by a chain of redox carriers. Light energy is captured by the antennae of both photosystems and transferred to the reaction centres. The central target of photoinhibition is the D1 protein of PS2. Safety valves include nonphotochemical mechanisms for quenching of excited chlorophyll, as well as alternative electron acceptors like oxygen (Niyogi, 2000), and changes in pigment composition. With light saturation of growth rate, the proportion of photosynthetic pigments decreases and the proportion of photoprotective pigments increases; the capacity for energy dissipation is thereby greatly enhanced (MacIntyre et al., 2002). In eukaryotic algae excess irradiance induces the conversion of the xanthophyll violaxanthin (a carotenoid), ultimately to zeaxanthin (Demmig-Adams and Adams, 1996). This reaction is reversed under low irradiance. The conversion to zeaxanthin is accompanied by a lower pH within the lumen of the photosynthetic membranes and an increase in non-photochemical quenching of fluorescence, indicative of the harmless dissipation of excess energy as heat. Low pH is required not only for the conversion of violaxanthin to zeaxanthin but also for the xanthophyll-cycle dependent energy dissipation itself. Cyanobacteria lack a xanthophyll cycle; they can produce zeaxanthin but they do so much more slowly. Other carotenoids typically found in cyanobacteria are myxoxanthophyll, echinenone and beta-carotene. Like zeaxanthin, these carotenoids are mainly involved in photoprotection: in cyanobacteria the carotene is present in the reaction centres and zeaxanthin is located in the cytoplasmic membranes (MacIntyre et al., 2002).

Ibelings et al. (1994) directly compared acclimation of *Microcystis* and the (non-buoyant) green alga *Scenedesmus protuberans* to high and fluctuating irradiances. The light regimes mimicked those received in lakes with various speeds and depths of mixing. *Microcystis* was more sensitive to photoinhibition than its green algal competitor *Scenedesmus*. At first sight this may seem surprising. Because of its buoyant nature and its migration behaviour, colonies of *Microcystis* will have a greater chance of being exposed to high irradiance at or near the lake surface. If *Microcystis* has evolved mechanisms to encounter high irradiances, then why the greater sensitivity? It has been argued that photoinhibition is actually part of the normal physiology of cells that are exposed to high irradiance. Photoinhibition that occurs without delay would protect cells from more damaging effects; it provides a mechanism for the long-term protection of photosystem 2 (Oquist et al., 1992). If protection is too slow or incomplete, cells that are caught in a surface bloom for longer periods risk photooxydative death (Zohary and Pais-Madeira, 1990; Abeliovich and Shilo, 1972).

Excess excitation energy is dissipated harmlessly as heat, thereby protecting the photosystem. The fact that photoinhibition occurred more readily in *Microcystis* may in fact be an adaptation to life as a buoyant species. Ibelings et al. (1994) argued that the constitutive presence of zeaxanthin was instrumental in the prompt quenching. Likewise Demmig-Adams et al. (1990) noted that cyanobacterial lichens containing zeaxanthin readily increased non-photochemical quenching of fluorescence. In contrast, Campbell et al. (1998) put forward the suggestion that the redistribution of excitation energy between PS2 and PS1 – so called state transitions - are the more important regulatory mechanism in cyanobacteria exposed to high irradiance.

Photoinhibition that is readily provoked may protect PS2. Nevertheless, Ibelings et al. (1994) found that *Microcystis* apparently depressed its rate of photosynthesis at times when the green alga (*Scenedesmus*) maintained uninhibited rates of photosynthesis. Campbell et al. (1998) argued that non-photochemical quenching of fluorescence in cyanobacteria does not necessarily cause a lower overall photochemical efficiency, since energy may be redirected towards PS1. It appears, however, that *Microcystis* is not as well adapted to fluctuating light as its eukaryotic competitors and is unable to benefit optimally from the saturating irradiance levels that are temporarily available when mixing takes cells to the upper layers of the watercolumn. Others have also found that mixing not only prevents surface bloom formation but also arrests growth of the bloom forming species (e.g. Reynolds et al., 1983). This re-emphasises the dependence of *Microcystis* on its buoyancy in lakes with a partially stable water column. Buoyancy enables the colonies to maintain themselves in a shallow near-surface mixed layer where light fluctuations are reduced and where irradiance levels are constantly (relatively) high, so that the continuous presence of zeaxanthin would be beneficial to the cells.

2.3 TOXINS

The hepatotoxic microcystins (MC) were first characterised in *Microcystis* (Botes et al., 1984) and named after this genus, but they were later also found in other cyanobacteria. *Microcystis* produces not only microcystins but also a variety of other cyclic or linear bioactive oligopeptides (Namikoshi and Rinehart, 1996). Some are known peptides (Erhard et al., 1999; Clemens et al., 1995), but others remain unidentified. The oligopeptide composition of different species was investigated in single colonies isolated from a natural bloom and analysed directly with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) by Fastner et al. (2001). Strains of *M. wesenbergii, M. ichthyoblabe* and also *M. novacekii* seldomly produce microcystins (Watanabe et al., 1988; Neilan et al., 1997; Otsuka et al., 2000).

Detailed information on microcystins and other aspects of toxicity in cyanobacteria are reviewed in other chapters of this book (see Chapters 1-3).

2.4 RESISTANCE TO GRAZING

2.4.1 Grazing by zooplankton

Is *Microcystis* too large to be eaten by zooplankton? Recently, Ghadouani et al. (2003) concluded that zooplankton communities are negatively affected by cyanobacterial blooms and therefore the potential to reduce such blooms through herbivorous grazing is only limited. Colonial cyanobacteria are deemed unsuitable food for zooplankton for three reasons: i) mechanical interference; ii) inadequate chemical composition that reduces growth and reproduction of zooplankton; iii) the production of cyanobacterial toxins. There are insufficient field-based, experimental studies to allow firm conclusions to be drawn (Ghadouani et al., 2003). The uptake of filamentous and colonial cyanobacteria by filter-feeding zooplankton is still a matter of discussion (Gulati et al., 2001). Evidence has been presented for positive, negative and no relationships between cyanobacterial abundance and zooplankton density (Paterson et al., 2002); the negative effects of cyanobacteria on zooplankton growth have been discussed by Paerl et al. (2001). The fact that microcystin is found in zooplankton (e.g. Thostrup and Christoffersen, 1999; Ferrao et al., 2002) during *Microcystis* blooms clearly indicates that cyanobacteria are taken up from the seston: without ingestion and assimilation of toxic cells, microcystins would not be found in *Daphnia* and other zooplankters. Grazing by zooplankton must therefore be a loss factor for *Microcystis*. Lampert (1987) concluded that large *Microcystis* colonies cannot be handled by zooplankton, but neither do these colonies seriously interfere with the filtration. Smaller colonies, however, (analogous to filamentous cyanobacteria) may severely reduce filtration rates through mechanical interference. The correlation between the clearance rate of *Daphnia galeata* and total phytoplankton biomass in Bautzen Reservoir was negative only if biomass of *Microcystis aeruginosa* was excluded. This suggested that *Microcystis aeruginosa* was the main grazing-resistant phytoplankton species in the reservoir (Boing et al., 1998).

Cyanobacterial toxins have been shown to have adverse effects on filtration, growth, reproduction and survival of zooplankton (e.g. Thostrup and Christoffersen, 1999; Rohrlack et al., 1999a, 1999b). *Daphnia* populations present during a bloom of toxic *Microcystis* may decrease in numbers and in individual body growth. Through these effects, blooms of *Microcystis* may affect the overall functioning of a lake ecosystem, since *Daphnia* is a key organism in the foodweb. The microcystin-producer *Microcystis* PCC 7806 was found to be poisonous to *Daphnia galeata*, whereas the non-producing mutant did not have lethal effects. Both variants of PCC7806, however, were able to reduce the *Daphnia* ingestion rate. Rohrlack et al. (1999c) conclude from this that while microystins may poison *Daphnia* (cf. Jungmann and Benndorf, 1994), these toxins are not responsible for inhibiting the ingestion process. It is important to distinguish in these experiments the three separate ways that *Microcystis* may affect zooplankton growth: through colony size interfering with filtration, through unidentified substances inhibiting feeding, and through direct toxic effects of microcystins. The possibility of correlations between the separate factors should be investigated, e.g., the occurrence of microcystin genes might be correlated with colony size: the *Microcystis* colonies of largest size (>100 μm) can have the highest proportion of microcystin-producing genotypes, the lowest proportion cells that lack microcystin, and the highest microcystin cell quotas (Kurmayer et al., 2003).

Larger cladocerans are often negatively correlated with blooms of toxic cyanobacteria, and hence these blooms may indirectly promote the smaller cladocerans, rotifers and copepods. Filter feeding and a capacity to ingest relatively large phytoplankton particles may increase the exposure of large *Daphnia* species to blooms of toxic *Microcystis,* which in addition to potential toxic effects provide little nutritional value. Yet it is not the colonial form per se that makes *Daphnia* more vulnerable than smaller species of zooplankton, which tend to leave large colonial *Microcystis* untouched. Fulton and Paerl (1987) found that unicellular strains of *Microcystis* inhibited the clearance rate of *Daphnia* on other algae more than colonial *Microcystis*. Rohrlack et al. (1999b), on the other hand, demonstrated the role of the mucilaginous envelope of colonial *Microcystis* strains in the inhibition of ingestion by daphnids. Results by DeMott (1999) further complicate the picture. Toxic strains of *Microcystis* inhibited feeding by *Daphnia* species, but *D. magna*, in which the inhibition was strongest after one hour incubation, subsequently recovered after 24 h incubation. These results seem consistent with a foraging strategy that needs to balance the benefits of a reduced microcystin intake with the disadvantage of reduced energy intake in a non-selective filter feeder. Epp (1996) does not agree with the view that *Daphnia* is non-selective. In his experiments *Daphnia pulicaria* was able selectively to avoid (filamentous) cyanobacteria while successfully feeding on other species. The sensitivity of zooplankton to cyanobacterial toxins varies widely. Selection of zooplankton strains with lower susceptibility to cyanobacterial toxins may increase in populations over several decades of eutrophication (Hairston et al., 1999). Hence, over time, *Microcystis* would have to rely more and more on morphological, rather than chemical, deterrents of zooplankton grazing. Rohrlack et al. (2001), however, conclude that differences in survival among *Daphnia* clones were due to variations

in microcystin intake rather than due to differences in susceptibility to the toxins. It is not only microcystins that harm zooplankton: cyanobacteria contain many other bioactive peptides that might be toxic in some way. One example is the peptide microviridin J, which has been found in *Microcystis* and appeared to cause a lethal molting disruption in *Daphnia* spp., upon ingestion of living cyanobacterial cells (Rohrlack et al., 2003).

Overall, we can paraphrase Lampert (1987) in writing that there is no general answer to the question whether zooplankton is affected by cyanobacteria (or vice versa). Although in most cases cyanobacteria have an adverse effect on growth and reproduction of zooplankton, there are no universal patterns. The effects seem to depend on the strains of zooplankton and cyanobacteria present. Nevertheless, it seems that grazing by zooplankton affects *Microcystis* less than it affects most other phytoplankton species (since *Microcystis* is protected by size and/or toxin production) although grazing losses for *Microcystis* by herbivorous zooplankton cannot be neglected.

2.4.2 Grazing by zebra mussels

Filter-feeders, like the zebra mussel *Dreissena polymorpha*, may have a similar or even larger effect on *Microcystis* populations than zooplankton. In Lake IJsselmeer (The Netherlands), at places where their densities are high enough, zebra mussels keep the water clear of cyanobacteria. As a consequence, cyanobacteria and *Dreissena* occupy different areas of the lake (see Vos et al., 2003; Pires and van Donk, 2002; Ibelings et al., 2003). Grazing experiments with seston from the IJsselmeer showed that *Dreissena* was capable of removing particles in a size range that included colony-forming cyanobacteria – different size classes were cleared at equal rates (Pires et al., unpublished manuscript) – and cyanobacteria were not discriminated against. The dominant *Daphnia* species in the IJsselmeer (*D. galeata*), on the other hand, showed an optimum for clearing particles with a diameter between 1 and 20 μm, which includes only some of the smaller *Microcystis* colonies. Interestingly, the amount of microcystin in the mussels in the IJsselmeer is an order of magnitude lower than that in the zooplankton (Ibelings et al., 2004).

Vanderploeg et al. (2001) state that *Dreissena* has reversed progress made by nutrient control measures. Whereas in Lake IJsselmeer *Dreissena* is believed to moderate the negative effects of eutrophication (and may even stimulate a shift from *Microcystis* to green algae), in Lake Erie and Lake Huron (USA), zebra mussels are believed to promote succession in the opposite direction, resulting in *Microcystis* blooms. Even if *Dreissena* was not instrumental in the return of *Microcystis* to Lake Erie, at least it can be said that zebra mussels were incapable of preventing the blooms of *Microcystis* (Vanderploeg et al., 2001). Of course, during periods of water column stability mussels refilter water from a benthic boundary layer (Ackerman et al., 2001), whilst floating cyanobacteria like *Microcystis* concentrate in a near-surface mixed layer, escaping grazing by the mussels.

Vanderploeg et al. (2001) put forward the idea that selective filtration by the mussels has promoted development of *Microcystis* in Lake Erie and Lake Huron. *Dreissena* is capable of sorting particles on the pallial organs (Baker et al., 1998).

Viable *Microcystis* was rejected via the pseudofaeces of the mussel, i.e. the (toxic) colonies did not enter the digestive tract (Vanderploeg et al., 2001). A similar result was obtained for a unicellular strain isolated from Lake Erie.

Size alone seems to be an unlikely criterion for rejection since Vanderploeg et al. (2001) found that colonies up to a diameter of 153 μm did not present a great problem. Ten Winkel and Davids (1982) observed that cyanobacteria even as large as 750 μm could be ingested. Pires and van Donk (2002) showed that the presence of toxic *Microcystis* cells inhibited clearance rates of the green alga *Chlamydomonas*. Non-toxic *Microcystis* cells had no such effect. Toxic *Microcystis* enhanced the production of pseudofaeces, containing viable cells. Perhaps contrary to what may be expected, the pseudofaeces mainly contained *Chlamydomonas* cells, whilst toxic *Microcystis* cells were assimilated. *Chlamydomonas* has a thick cell wall, which makes it hard to digest. Baker et al. (1998) also found that *Microcystis* was ingested preferentially over green algae and diatoms.

In most experiments in which toxic strains are assimilated, the mussels did not seem to suffer from exposure to microcystin, with the exception of a reduction in clearance rate (Pires et al., 2003). Yet Vanderploeg et al. (2001) suggested that a reduction in filtration activity of natural *Dreissena* populations (54% of the time instead of more than 90%) may be related to chronic exposure to toxic *Microcystis*. The larvae of *Dreissena* seem highly sensitive to microcystin, even during short-term exposure (Pires et al., 2003). Survival of larvae fed on toxic *Microcystis* was lower than for larvae feeding on non-toxic *Microcystis* (but higher than that of starved larvae).

2.5 THE LIFE CYCLE OF *MICROCYSTIS*

2.5.1 Summer and autumn

Microcystis may minimise its population losses in two ways: gas vesicles prevent sedimentation losses and colony formation may reduce grazing losses. And yet throughout the growing season cells are lost, by photooxidation in scums, by stranding on lee shores or in reed beds, and by flushing from lakes. Nevertheless in many temperate lakes large populations persist throughout the summer, only to disappear in autumn. Small numbers of colonies may survive the winter and form the inoculum for the next season's growth but there is also evidence that colonies overwinter on the sediments (Reynolds et al., 1981; Takamura et al., 1984; Oliver et al., 1985; Thomas and Walsby, 1986; Tsujimura et al., 2000). There are several explanations for what causes *Microcystis* colonies to sink out of suspension in the autumn.

The loss of buoyancy in autumn has been related to events accompanying autumnal overturn, such as an increase in the ballast of storage products in cyanobacteria (Reynolds and Rogers, 1976; Reynolds et al., 1981). Experiments with a laboratory strain of *Microcystis* showed that whilst cells kept at 20 °C regained buoyancy at night by metabolising the dense stores of carbohydrate accumulated during the day, those kept at 8 °C failed to metabolise the carbohydrate and remained negatively buoyant (Thomas and Walsby, 1986). Such

changes in carbohydrate levels suggest differences in relative rates of respiration and photosynthesis at lower temperatures. From a study in laboratory cultures at lower temperatures, Visser et al. (1995) concluded that the accumulation of carbohydrate at reduced temperature was the result of a lowered rate of protein synthesis during the light period. Although the photosynthetic rate itself decreased at reduced temperature, the decreased incorporation of carbon into protein resulted in more of the fixed CO_2 being stored as carbohydrate, thus increasing the ballast of the cells.

Another explanation for the autumnal sedimentation is co-precipitation with suspended particles. In Blelham Tarn, English Lake District, *Microcystis* colonies were confined to the aerobic epilimnion throughout the summer and were absent from the hypolimnion, which became anoxic from late May to early October. At holomixis in mid-October, reduced iron compounds, which were freely soluble in the anaerobic water of the hypolimnion, became oxidised when mixed with the aerated water from the epilimnion, and formed a yellow precipitate, which adhered to the *Microcystis* colonies causing them to become denser than water and to sink (Oliver et al., 1985). Recent findings (Verspagen et al., 2004) in the rather shallow Lake Volkerak, The Netherlands, showed a similar co-precipitation of buoyant *Microcystis* colonies with clay particles that caused sedimentation of the colonies. In this case, sedimentation took place at all times of the year.

2.5.2 Winter and spring

Some of the *Microcystis* colonies from the summer blooms overwinter on the lake sediment, not in special akinetes but as vegetative cells (Brunberg and Böstrom, 1992; Fallon and Brock, 1981; Reynolds et al., 1981; Takamura et al., 1984). In spring, these benthic colonies may reinvade the water column (Preston et al., 1980). Various conditions responsible for the recruitment have been suggested. Low oxygen concentrations, resulting from the onset of stratification, may enhance the recruitment of cyanobacteria (Càceres and Reynolds, 1984; Reynolds et al., 1981; Trimbee and Harris, 1984; Trimbee and Prepas, 1988). Oliver et al. (1985) suggested that some colonies regain buoyancy under anaerobic conditions, when the ferric iron compounds that weigh them down are reduced to soluble ferrous compounds. Càceres and Reynolds (1984) concluded from their experiments that an increase in water temperature may be critical for the renewed activity of *Microcystis,* but anoxic conditions and light may also play a role. Penetration of relatively high irradiance levels to the sediment seems necessary for this process, since gas vesicle formation in the dark occurs only after earlier accumulation of energy reserves (Deacon and Walsby, 1990). Reynolds and Bellinger (1992) showed on the basis of an 18-year data set that a population of *Microcystis* in the water column could be established more quickly when the water was clear after the onset of stratification, allowing light to penetrate to the lake bottom.

An alternative is that recruitment may not be triggered by specific conditions, but may be a more or less continuous process during the winter and spring as result of the ongoing decrease of the carbohydrate content in the colonies. In autumn, colonies that sink down may initially have a high carbohydrate content but will metabolise the carbohydrate in the very low irradiance at or near the sediment. At a

certain time in winter or spring, dependent on the initial carbohydrate concentration, oxygen conditions and temperature, buoyancy will be regained if the gas vesicles have remained intact. When the temperature is high enough to sustain regular protein synthesis, *Microcystis* colonies will be able to stay in the epilimnion, grow and re-establish a planktonic population.

Buoyant colonies buried in the sediment, however, may find it hard to free themselves from the lake bottom: numerous buoyant colonies can sometimes be found in the sediment of lakes. In Lake Volkerak, recruitment of *Microcystis* from the sediment was probably not the result of an active buoyancy change in the benthic *Microcystis* population, but rather resulted from resuspension after wind-induced mixing and/or bioturbation (Verspagen et al., 2004). This happens to a larger extent in shallower areas of the lake, which consequently will contribute more of the *Microcystis* recruited (Brunberg and Blomqvist, 2003).

3 Competition

3.1 *MICROCYSTIS* VERSUS ALGAE

The characteristics of cyanobacteria that give them their competitive strength over algae are summarized and discussed in Chapter 3 (Kardinaal and Visser) and Chapter 7 (Huisman and Hulot) of this book. In this paragraph we will try to sort out under which conditions *Microcystis* has the greatest competitive strength.

Cyanobacteria are known to be strong competitors for light. However, competition experiments in light-limited continuous cultures revealed that *Microcystis* is not a particularly strong competitor for light (Huisman et al., 1999). It loses the competition when grown in mixed cultures with the green alga *Chlorella,* and also when grown in mixed cultures with the cyanobacterium *Aphanizomenon*.

Microcystis has been considered to be a K-strategist (Reynolds, 1984) because it has a low maximum growth rate but succeeds when the losses by grazing and sedimentation are low. K-strategists, however, often win the competition by their high affinity for some resource. This does not seem to be the case for *Microcystis*. Rather, its relatively low affinity for light is compensated by its buoyancy, which enables it to increase its daily light dose. In a stable water column, buoyancy thereby provides *Microcystis* with a direct competitive advantage.

3.2 *MICROCYSTIS* VERSUS *PLANKTOTHRIX*

In the temporate climate zones *Microcystis* and *Planktothrix* are arguably among the most abundant harmful cyanobacteria in freshwater systems. *Planktothrix* can be found in two distinctly different niches: (1) stratifying on the thermocline (*P. rubescens*) and (2) homogenously suspended in the epilimnion (mainly *P. agardhii*). We discuss here the principal niche differences between the epilimnetic species of *Planktothrix* and *Microcystis*. Although *Microcystis* and *Planktothrix* are

usually found in different lakes, they sometimes coexist or alternate in the same waterbodies, e.g. in Lake IJsselmeer, *Microcystis* sp. and *Planktothrix agardhii* dominate in different years.

By floating up, *Microcystis* colonies obtain a higher daily light dose (provided the water column is sufficiently stable). Moreover, if they subsequently accumulate sufficient carbohydrate ballast, *Microcystis* will migrate rapidly down the water column and avoid photooxidation early in the day. *Planktothrix agardhii* on the other hand, being filamentous, can only regulate its vertical migration in the stable metalimnion (Section 2.1.3). Once entrained in the epilimnion the light it receives is determined by circulation through the surface layers. It is more sensitive to light than *Microcystis* and suffers photoinhibition and photodamage at lower irradiances (Eloff et al., 1976; Van Liere and Mur, 1980; Paerl et al., 1983). If the light is steeply attenuated, however, *Planktothrix* will avoid prolonged exposure to high irradiance near the surface. The steepest light gradients are commonly found in shallow waters with dense populations of phytoplankton.

Planktothrix populations occur most commonly in waters less than 4 m deep. In more northern climate zones with their lower maximum irradiance, *Planktothrix* is also found in deeper lakes. Very shallow lakes usually do not have the right conditions for *Microcystis*, which prefers somewhat deeper, diurnally or seasonally stratifying lakes where it can profit most from its buoyancy. In lakes with a depth of 4-6 m, like Lake IJsselmeer, the two niches overlap and interesting patterns of competition can be found. Differences in the affinity for light between the competitors become more important. *Planktothrix* has a higher affinity for light than *Microcystis* and if the phytoplankton biomass is sufficiently concentrated, it creates the 'shade' conditions in which *Planktothrix agardhii* flourishes (Scheffer et al., 1997); *Microcystis* then loses the competition. At lower biomass concentrations, however, the growth of *Planktothrix* is adversely affected by photoinhibition and under such conditions *Microcystis* may win the competition.

4 Control of *Microcystis* in lakes and reservoirs

We summarise here some of the methods developed to control cyanobacterial blooms. Many options are available to the lake manager: "bottom up" control refers to limiting the availability of nutrients or light; "top down" refers to foodweb control, encouraging predation by animals. The latter option, a form of biomanipulation, is often used as an adjunct to phosphorus reduction and may help to shift eutrophic lakes from the turbid to the clear state (Perrow et al., 1997; Meijer et al., 1999). Biomanipulation has had some success with *Microcystis* (e.g., Anadotter et al., 1999), although small blooms of *Microcystis* may persist in the clear state (Korner, 2001) or once the more-or-less permanent blooms of *Planktothrix agardhii* have disappeared (Meijer et al., 1999). Ghadouani et al. (2003) have questioned the usefulness of herbivory to control phytoplankton in lakes dominated by *Microcystis.*

Lake flushing has been successfully used to reduce the amount of cyanobacteria, and especially filamentous species, in lakes. Davis et al. (2003) compared the observed changes in the *Planktothrix* population in Blelham Tarn (English Lake District) with the changes calculated from growth rate and found good agreement if allowance was made for the proportion lost from the surface mixed layer by flushing, which was calculated from the rainfall and catchment area around the lake. Walsby et al. (1989) suggested that one explanation for the absence of faster-floating colonial cyanobacteria in Lake Rotongaio (New Zealand) was that a proportion of the cyanobacteria in the surface layer was lost in the outflow into Lake Taupo each day and that the losses would have been greater for colonies concentrated in the surface film than for the small *Anabaena minutissima* filaments that dominated the phytoplankton. Because of their relatively low growth rates *Microcystis* blooms may often be correlated with reduced flushing rates (e.g. Jacoby et al., 2000). In the remainder of this section on control of *Microcystis* we focus on those measures that are specific for control of bloom-forming cyanobacteria like *Microcystis* by targeting their special adaptations.

4.1 REMOVAL BY PRESSURE DEVICES

4.1.1 Loss and recovery of gas vesicles in darkness

Without gas vesicles, colonial cyanobacteria like *Microcystis* not only lack an advantage over other phytoplankton, they are at a considerable disadvantage. Their large colony size, which confers the necessary high floating velocity when gas vesicles provide buoyancy, confers a high sinking velocity when the gas vesicles are collapsed. The colonies are then rapidly lost to the hypolimnion or the sediment. This potentially offers a method of controlling these organisms. We consider briefly the consequences of sedimentation, the pressures that must be applied, and some methods of destroying gas vesicles.

An organism that sediments to a depth exceeding the compensation depth will not easily re-enter the euphotic zone, unless it is resuspended by strong turbulent mixing, or regains its buoyancy by making new gas vesicles. Kromkamp et al. (1989) found that *Microcystis* cells required light to support *de novo* gas vesicle synthesis (e.g., when phosphate inhibition was relieved); Deacon and Walsby (1990) found that after all existing gas vesicles had been collapsed by pressure, there was some gas vesicle production in the dark but that this occurred only if the cells had been pre-incubated at high irradiance and thereby increased their energy reserves. Even then, insufficient new gas vesicles were made to render the cells buoyant. The general conclusion, therefore, is that if, after gas vesicle collapse, cells sink into the aphotic zone they will not recover buoyancy and will not easily rejoin the population in the epilimnion.

In engineering a device for collapsing gas vesicles, the cost will rise with the pressure that must be applied and it is therefore necessary to determine the minimum pressure required. The mean critical pressure of gas vesicles in *Microcystis* varies from 0.6 to 0.9 Mpa, but in each cell the critical collapse pressure (p_c) of individual gas vesicles varies and that of the strongest may be as

much as 0.2 MPa above the mean value. The maximum p_c may therefore be in the range of 0.8 to 1.1 MPa. From this may be subtracted the cell turgor pressure, which usually exceeds 0.2 MPa. To collapse all of the gas vesicles in turgid cells will therefore require application of 0.6 to 0.9 MPa, depending on the strain.

To minimise the pressure costs, it might be argued that it is not necessary to collapse all of the gas vesicles: usually, not more than 50% need to be collapsed to make cells sink, requiring application of the median critical pressure, which is close to the mean p_c (Walsby and Bleything, 1988). The danger, however, is that some cells will be left close to neutral buoyancy and with the production of few more gas vesicles will become positively buoyant again.

The recommendation, therefore, is to design for the maximum required, 0.9 MPa for *Microcystis* spp.

4.1.2 Devices for collapsing gas vesicles

A number of different devices have been trialled for the lake-scale collapse of gas vesicles in cyanobacteria. There are considerations of efficacy and cost in the construction and operation of the devices, which are made in more detail by Walsby (1992).

Ultrasonic transducer. D.A. Hill and R.F. Packman (Lowestoft Water Company, U.K., 1957: see Walsby, 1992) described an attempt to use gas vesicle collapse in the removal of floating cyanobacteria. The cyanobacteria passing through a pipe were subjected to ultrasonic radiation from a transmitter in the pipe wall. Gas vesicle collapse and buoyancy loss occurred but was incomplete in the device used. There are problems in treating large volumes of water because the intensity of the radiation decreases as the square of distance. In small waterbodies this method has been used in combination with mixing and flushing to combat *Microcystis* blooms (Nakano et al., 2001).

Explosives. The detonation of a firework over a water bath containing floating *Microcystis* colonies was shown to generate sufficient pressure to collapse the gas vesicles and cause the colonies to sink (Walsby, 1968). The method was scaled up by Menday and Buck (1972) who detonated a charge of the explosive Cordex in a flooded quarry; it caused gas vesicle collapse and buoyancy loss of cyanobacteria up to 30 m away. They analysed the costs of treating a reservoir with a grid of explosive charges and assessed the collateral damage, e.g. to fish, some of which were killed.

Deep concentric pipes. The excess hydrostatic pressure (above the overlying atmospheric pressure) at a depth h in a water column is equal to $p = h\rho g$, where ρ is the density of water (998 kg m^{-3}) and g is gravitational acceleration (9.81 m s^{-2}). With each additional meter in depth, the pressure therefore rises by 9790 Pa. The pressure of 0.9 MPa suggested for treating *Microcystis* is hence obtained at a depth of 88 m. Clarke and Walsby (1988) described a deep concentric pipe through which water could be circulated to expose it to hydrostatic pressure sufficient to collapse gas vesicles in cyanobacteria. Only a small amount of energy is required to drive the water through the pipe. A concentric pipe 86-m deep sunk at the Lound Reservoir near Lowestoft removed all of the gas vesicles from *Microcystis* colonies entering the treatment works and prevented cyanobacteria floating above the

primary filtration system. Pressurised cyanobacteria could be removed by sedimentation in shallow ponds as a pretreatment to reduce the cyanobacterial loading before entering the treatment plant. Colonial cyanobacteria could also be removed from a lake by cycling the water through such a deep pipe.

An unintended deep-pipe treatment: the Lake Kinneret pipe. The water from Lake Kinneret (the Sea of Galilee), which is 210 m below mean sea level, is lifted through a height of 256 m as it enters the distribution system of the Israeli National Water Carrier. In 1994, a bloom of the toxic gas-vacuolate cyanobacterium *Aphanizomenon ovalisporum* appeared in the lake. As it was pumped into the system it was subjected to a pressure of 2.5 MPa, greatly exceeding the critical pressure of the *Aphanizomenon* gas vesicles, which were collapsed. This caused a loss of turbidity in the water at the top of the pipe, giving a spurious indication that the water quality had improved. In the time it took for water to flow from the lake to the storage reservoirs the *Aphanizomenon* was subjected twice more to pressures exceeding 0.8 MPa as it passed through siphons carrying the water across deep gorges. The *Aphanizomenon* entering the reservoir was not buoyant and what few cyanobacteria remained at the treatment works were easily removed (Porat et al., 1999, 2000).

4.2 ARTIFICIAL MIXING

4.2.1 Effects of artificial mixing on Microcystis *and non-buoyant algae*

Buoyancy is crucial for *Microcystis* but the advantages of buoyancy are largely removed when colonies are entrained in intensely circulating water. Artificial mixing of lakes and reservoirs aims to reduce *Microcystis*. Decreased *Microcystis* biomass due to artificial mixing was found by Toetz (1981), Visser et al. (1996a) and Lindenschmidt (1999); cyanobacterial dominance shifted to negatively buoyant green algae and diatoms. Diatoms and green algae may in fact profit from artificial mixing, as artificial mixing reduces their sedimentation losses (Visser et al., 1996c) and may lower the pH thus shifting the inorganic carbon complex to CO_2.

In a recent study, turbulence was recorded in Lake Nieuwe Meer, The Netherlands, using a Self-Contained Autonomous Micro-Profiler (SCAMP) in a situation with and without artificial mixing (Huisman et al., 2004; see also Chapter 7). The data were compared with a competition model that included turbulent mixing of the water column, buoyancy of *Microcystis,* as well as sedimentation losses of diatoms and green algae. The model predicts that changes in turbulent diffusivities may shift the competitive balance between buoyant and non-buoyant phytoplankton. For an average water-column depth of 18 m, as in Lake Nieuwe Meer, a critical turbulent diffusivity of about 3.4 $cm^2\ s^{-1}$ was predicted. If vertical turbulent diffusivities remain below this critical value, *Microcystis* profits from its buoyancy and is predicted to become dominant. Conversely, if vertical turbulent diffusivities exceed this critical value, surface blooms of *Microcystis* would be prevented, and diatoms and green algae are predicted to become dominant. The critical vertical diffusivities predicted by the model matched well with the diffusivities achieved by artificial mixing of Lake Nieuwe Meer. This novel

quantitative approach allows prediction of the mixing intensities required for the prevention of surface blooms of buoyant cyanobacteria.

4.2.2 Unsuccessful artificial mixing

Artificial mixing is not always successful in reducing the cyanobacterial biomass (e.g. Knoppert et al., 1970; Lackey, 1973; Osgood and Stiegler, 1990). Pastorok et al. (1980) summarised the results of a large number of destratification experiments: out of twenty-four cases examined, the abundance of cyanobacteria decreased in twelve, increased in eight, and showed no change in four cases. The following explanations may be offered for the failure of artificial mixing: i) mixing relieved nutrient limitation; (ii) insufficient account was taken of lake bathymetry; iii) mixing was insufficient; iv) changes in the mixing regime did not produce the required downshift in pH. These reasons will be further discussed below.

Artificial mixing is most successful in reducing the biomass of light-limited cyanobacterial populations, because deep and permanent mixing will increase light limitation. When cyanobacteria are not light-limited but nutrient-limited prior to mixing, the cyanobacterial biomass per surface area of lake may even increase as a result of artificial mixing, due to a higher nutrient availability when water from the nutrient-rich hypolimnion gets mixed into the epilimnion.

A study in a reservoir where *Microcystis* remained dominant despite artificial mixing (Visser et al., 1996b) showed that growth of *Microcystis* occurred mostly in a large, relatively shallow (6-m depth) area of the reservoir. No aerators were present to mix the water in this shallow area. *Microcystis* colonies demonstrated a higher buoyancy loss during the day in the shallow area, indicating that they received a higher light dose than those at the deep site. Apparently, the large shallow, unmixed area provided a source of *Microcystis* that interfered with mixing of the cyanobacteria in the deeper parts of the reservoir.

Another reason for the failure to decrease the amount of *Microcystis* is that the mixing velocity of the water is insufficient to prevent the (larger) colonies from escaping entrainment as occurs when the installed equipment is too weak for the lake area. The work in Lake Nieuwe Meer revealed that there is a critical threshold value for the turbulent diffusion coefficient that must be exceeded to shift the competitive dominance from buoyant cyanobacteria to sinking phytoplankton (Huisman et al., 2004) The distribution of the aeration tubes over the lake is important as well: in Lake Nieuwe Meer, it was found that the mixing efficiency diminished rapidly with distance from the aeration tubes.

Forsberg and Shapiro (1980) showed that in most cases with rapid mixing in enclosures, a shift from cyanobacteria to eukaryotic algae occurred. The shift from a system dominated by cyanobacteria to a system dominated by green algae may be augmented if mixing lowers the pH and increases the relative availability of free CO_2 (Shapiro, 1997). Deppe et al. (1999) showed that a strategy that combined phosphorus reduction with the transport of hypolimnetic water rich in free CO_2 to the epilimnion completely suppressed *Microcystis*.

4.2.3 Mixing devices

There are various devices for artificial mixing of lakes: usually, air is injected close to the lake bottom by a perforated pipe, or by a special diffuser producing very small bubbles. Mechanical destratification devices make use of centrifugal pumps or propellers. Mechanical mixing is less efficient than aeration (Symons et al., 1967, 1970). The depth of the air inlet is important: the greater the depth the more efficient the aeration and mixing (Cooke et al., 1993). Lorenzen and Fast (1977) concluded that an air flow rate of 9.2 m^3 min^{-1} km^{-2} lake area should be sufficient to accomplish adequate mixing in most lakes.

4.3. REDUCTION OF NUTRIENT LOADING

In many lake restoration programmes, decreasing the phosphorus (P) loading in a lake has resulted in a lower biomass of all phytoplankton, including *Microcystis*. There may, however, be hysteresis effects such that reduced P-loading does not result in reduced phytoplankton biomass, for instance because of internal loading of P stored in the sediment or the presence of a large benthic *Microcystis* population. A nice example is Lake Trummen (USA): only a modest reduction in *Microcystis* blooms occurred after reducing the external P-load, but the blooms were greatly diminished after a second step, dredging the sediment (Cronberg et al., 1975).

In lakes where eutrophication has been reduced, *Microcystis* populations may lose their dominance and be partly replaced by other organisms like diatoms (*Asterionella*, *Fragillaria*), chrysophytes (*Mallomonas*, *Dinobryon*, *Synura*) and dinoflagellates (*Ceratium hirundinella*) (see for examples Sas, 1989). With further P-reduction *Microcystis* blooms may disappear altogether, or remain at a reduced biomass after lake restoration.

In several lakes, however, *Microcystis* blooms are a natural phenomenon and they occurred long before the peak in anthropogenic eutrophication in the second half of the 20th century. Although nutrient reduction measures may eventually reduce the problem of noxious blooms of *Microcystis*, it is perhaps wishful thinking that they will disappear altogether. Surface blooms have a tendency to appear during periods of warm, stable weather, i.e., at those moments when lakes are used most intensively by the public.

5 Outlook: effects of climate change on *Microcystis* blooms

There is evidence that the increase in atmospheric CO_2 has caused global warming by approximately 0.6 °C over the last century and that this trend is likely to continue (Houghton et al., 2001). Consequences of global warming are changes in ice-cover, wind, solar insolation and precipitation. Water column stability, nutrient-loading and lake residence time may all be affected, with implications for phytoplankton. Higher temperatures will affect physiological processes such as photosynthesis, respiration and growth of the phytoplankton (Reynolds, 1997). In combination with i) the anticipated increase in the availability of phosphorus, ii) a

reduction in some loss processes like sedimentation, and iii) a lengthened growing season, global warming is expected to result in an increase in phytoplankton biomass (e.g. Kilham et al., 1996)

Elevated water temperature may promote *Microcystis* blooms via different mechanisms, including an enhanced growth rate. *Microcystis* seems to have an exceptionally large Q_{10} for growth: 9-10 compared to 1-3 for other phytoplankton species (Reynolds, 1997). Indeed, growth rate of *Microcystis* increased 9-fold when temperature was increased from 10 to 20 ^{o}C, whereas growth rates of *Aphanizomenon* and *Planktothrix* increased 3-4 fold (Laboratory of Aquatic Microbiology, University of Amsterdam, unpublished results).

Higher temperatures from global warming may enhance the stability of the water column and thus suppress turbulent mixing, which would be highly advantageous for buoyant cyanobacteria like *Microcystis*. Conversely, the predicted increase in cloud cover and wind speed would weaken water-column stability and this would reduce the competitive strength of cyanobacteria. Howard and Easthope (2002) produced a model of cyanobacterial growth that incorporated climate change, and concluded that cyanobacterial production will (slightly) diminish in the next 90 years, mainly as a consequence of light limitation under an increased cloud cover; more intense short blooms were predicted during periods of higher insolation and high water temperature, although scum formation should decrease in a windier future (Ibelings et al., 2003). In reality, however, there are so many uncertainties involved in the prediction of climate scenarios that it is hard to foretell how water-column stability in different areas of the world will be affected by global warming.

6 Conclusions

We have discussed several adaptations that support the widespread distribution of *Microcystis* species and the attendant problems in lakes of different types. Arguably, the most important characteristics of *Microcystis* are its buoyancy and large colony size. Without buoyancy the large colonies would sink and would thus be unable to form surface blooms. Without large size the organism would not float fast enough to regain the euphotic zone after a mixing event. Moreover, without large size, grazing losses would be greater. Smaller algae must sustain larger grazing losses whilst these losses seem to be much reduced for colony-forming cyanobacteria like *Microcystis*. There are still many questions on the functional role of the toxins produced by *Microcystis*, but it seems these might deter potential predators. Other adaptations of *Microcystis* (and other cyanobacteria) to life in eutrophic lakes with a dense biomass of phytoplankton include its ability to use inorganic carbon efficiently and to grow at high pH. However, compared to other phytoplankton species, *Microcystis* species have a low specific growth rate and a relatively low affinity for phosphorus, which make them less successful in waters with a short residence time and in oligotrophic waters. All these features help to explain the potential dominance of *Microcystis* over other phytoplankton species in a wide range of eutrophic lakes, and they indicate measures that can be taken by water management to combat these harmful cyanobacteria.

7 Acknowledgements

We like to thank Geoff Codd, Jef Huisman and an anonymous reviewer for critical reading of the manuscript and their valuable suggestions. Part of this work was supported by a grant from the UK Natural Environment Research Council to AEW. PMV was supported by a MEERVOUD grant of the Earth and Life Sciences Foundation (ALW), which is subsidized by the Netherlands Organization for Scientific Research (NWO).

8 References

Abeliovich, A. and Shilo, M. (1972) Photooxidative death in blue-green algae, *Journal of Bacteriology* **111**, 682-689.

Annadotter, H., Cronberg, G., Aagren, R., Lundstedt, B., Nilsson, P.A. and Strobeck, S. (1999) Multiple techniques for lake restoration, *Hydrobiologia* **396**, 77-85.

Anagnostidis, K. and Komárek, J. (1988) Modern approach to the classification system of cyanophytes. 3 – Oscillatoriales, *Archiv für Hydrobiologie (Algological Studies)* **80**, 327-472.

Ackerman, J.D., Loewen, M.R. and Hamblin, P.F. (2001) Benthic-pelagic coupling over a zebra mussel reef in western Lake Erie, *Limnology and Oceanography* **46**, 892-904.

Agustí, S. and Phlips, E.J. (1992) Light absorption by cyanobacteria: implications of the colonial growth form, *Limnology and Oceanography* **37**, 434-441.

Baker, S.M., Levinton, J.S., Kurdziel, J.P. and Shumway, S.E. (1998) Selective feeding and biodeposition by zebra mussels and their relation to changes in phytoplankton composition and seston load, *Journal of Shellfish Research* **17**, 1207-1213.

Beard, S.J., Handley, B.A., Hayes, P.K. and Walsby, A.E. (1999) The diversity of gas vesicle genes in *Planktothrix rubescens* from Lake Zürich, *Microbiology* **145**, 2757-2768.

Beard, S.J., Davis, P.A., Iglesias-Rodríguez, D., Skulberg, O.M. and Walsby, A.E. (2000) Gas vesicle genes in *Planktothrix* spp. from Nordic lakes: strains with weak gas vesicles possess a longer variant of *gvpC*, *Microbiology* **146**, 2009-2018.

Boing, W.J., Wagner, A., Voigt, H., Deppe, T. and Benndorf, J. (1998) Phytoplankton responses to grazing by *Daphnia galeata* in the biomanipulated Bautzen reservoir, *Hydrobiologia* **389**, 101-114.

Bourelly, P. (1970) Les algues d'eau douce **3**, N. Boubée and Cie, Paris (in French)

Bormans, M., Sherman, B.S. and Webster, I.T. (1999) Is buoyancy regulation in cyanobacteria an adaptation to exploit separation of light and nutrients?, *Marine and Freshwater Research* **50**, 897-906.

Botes, D.P., Tuiman, A.A., Wessels, P.L, Viljoen, C.C., Kruger, H., Williams, D.H., Santikarn, S., Smith, R.J. and Hammond, S.J. (1984) The structure of cyanoginosin-LA, a cyclic peptide from the cyanobacterium *Microcystis aeruginosa, Journal of the Chemical Society - Perkin Transactions* **I**, 2311-2319.

Brunberg, A.K. and Blomqvist, P. (2003) Recruitment of *Microcystis* (Cyanophyceae) from lake sediments: the importance of littoral inocula, *Journal of Phycology* **39**, 58-63.

Brunberg, A.K. and Böstrom, B. (1992) Coupling between benthic biomass of *Microcystis* and phosphorus release from the sediments of a highly eutrophic lake, *Hydrobiologia* **235/236**, 375-385.

Cáceres, O. and Reynolds, C.S. (1984) Some effects of artificially-enhanced anoxia on the growth of *Microcystis aeruginosa* Kutz. emend. Elenkin, with special reference to the initiation of its annual growth cycle in lakes, *Archiv für Hydrobiologie* **99**, 379-397.

Campbell, D., Hurry, V., Clarke, A.K., Gustafsson, P. and Oquist, G. (1998) Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation, *Microbiology and Molecular Biology Reviews* **62**, 667-671.

Chen, Y.W., Qin, B.Q., Teubner, K. and Dokulil, M.T. (2003) Long-term dynamics of phytoplankton assemblages: *Microcystis*- domination in Lake Taihu, a large shallow lake in China, *Journal of Plankton Research* **25**, 445-453.

Clarke, K.B. and Walsby, A.E. (1988) Improvements in the treatment of water to remove gas vacuolate cyanobacteria, U.K. Patent Application 2196330A.

Clemens, J., Oberer, L., Quiquerez, C., Konig, W.A. and Weckesser, J. (1995) Cyanopeptolin-S, a sulfate-containing depsipeptide from a water bloom of *Microcystis* sp., *FEMS Microbiology Letters* **129**, 129-133.

Cooke, G.D., Welch, E.B., Peterson, S.A. and Newroth, P.R. (1993) Artificial circulation, in G.D. Cooke, E.B. Welch, S.A. Peterson and P.R. Newroth (eds.), *Restoration and Management of Lakes and Reservoirs,* Lewis Publishers**,** pp. 419-449.

Cronberg, G., Gelin, C. and Larsson, K. (1975) Lake Trummen restoration project. II. Bacteria, phytoplankton and phytoplankton productivity, *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **19**, 1088-1096.

Davis, P.A., Dent, M., Parker, J., Reynolds, C.S. and Walsby, A.E. (2003) The annual cycle of growth rate and biomass change in *Planktothrix* spp. in Blelham Tarn, English Lake District, *Freshwater Biology* **48**, 852-867.

Deacon, C. and Walsby, A.E. (1990) Gas vesicle formation in the dark, and in light of different irradiances, by the cyanobacterium *Microcystis* sp., *British Phycological Journal* **25**, 133-139.

Demmig-Adams, B. and Adams, W.W. (1996) The role of the xanthophyll cycle carotenoids in the protection of photosynthesis, *Trends in Plant Science* **1**, 21-26.

Demmig-Adams, B., Maguas, C., Adams, W.W., Meyer, A., Kilian, E. and Lange O.L. (1990) Effect of high light on the efficiency of photochemical energy-conversion in a variety of lichen species with green and blue-green phycobionts, *Planta* **180**, 400-409.

DeMott, W.R. (1999) Foraging strategies and growth inhibition in five daphnids feeding on mixtures of a toxic cyanobacterium and a green alga, *Freshwater Biology* **42**, 263-274.

Deppe, T., Ockenfeld, K., Meybohm, A., Opitz, M. and Benndorf, J. (1999) Reduction of *Microcystis* blooms in a hypertrophic reservoir by a combined ecotechnological strategy, *Hydrobiologia* **409**, 31-38.

Dinsdale, M.T. and Walsby, A.E. (1972) The interrelations of cell turgor pressure, gas-vacuolation, and buoyancy in a blue-green alga, *Journal of Experimental Botany* **23**, 561-570.

Elser, J.J. (1999) The pathway to noxious cyanobacteria blooms in lakes: the food web as the final turn, *Freshwater Biology* **42**, 537-543.

Eloff, J.N., Steinitz, Y. and Shilo, M. (1976) Photooxydation of cyanobacteria in natural conditions, *Applied and Environmental Microbiology* **31**, 119-126.

Epp, G.T. (1996) Grazing on filamentous cyanobacteria by *Daphnia pulicaria*, *Limnology and Oceanography* **41**, 560-567.

Erhard, M., von Döhren, H. and Jungblut, P.R. (1999) Rapid identification of the new anabaenopeptin G from *Planktothrix agardhii* HUB 011 using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, *Rapid Communications in Mass Spectrometry* **13**, 337-343.

Fallon, R.D. and Brock, T.D. (1981) Overwintering of *Microcystis* in Lake Mendota, *Freshwater Biology* **11**, 217-226.

Falkowski, P.G. and LaRoche, J. (1991) Acclimation to spectral irradiance in algae, *Journal of Phycology* **27**, 8-14.

Fastner, J., Erhard, M. and von Döhren, H. (2001) Determination of oligopeptide diversity within a natural population of *Microcystis* spp. (Cyanobacteria) by typing single colonies by matrix-assisted laser desorption ionization-time of flight mass spectrometry, *Applied and Environmental Microbiology* **67**, 5069-5076.

Ferrão, A.D., Kozlowsky-Suzuki, B. and Azevedo, S.M.F.O. (2002) Accumulation of microcystins by a tropical zooplankton community, *Aquatic Toxicology* **59**, 201-208.

Fogg, G.E. and Walsby, A.E. (1971) Buoyancy regulation and the growth of planktonic blue-green algae, *Mitteilungen der Internationale Vereinigung für Theoretisch und Angewandte Limnologie* **19**, 182-188.

Forsberg, B.R. and Shapiro, J. (1980) Predicting the algal response to destratification, in *Restoration of Lakes and Inland Waters, EPA 440/5-81-010, United States Environmental Protection Agency, Washington, D.C.*, pp. 134-145.

Fulton, R.S. and Paerl, H.W. (1987) Toxic and inhibitory effects of the blue-green alga *Microcystis aeruginosa* on herbivorous zooplankton, *Journal of Plankton Research* **9**, 837-855.

Ganf, G.G. (1974) Diurnal mixing and the vertical distribution of phytoplankton in a shallow equatorial lake (Lake George, Uganda), *Journal of Ecology* **62**, 611-629.

Ganf, G.G., Oliver, R.L. and Walsby, A.E. (1989) Optical properties of gas-vacuolate cells of *Microcystis* in relation to light attenuation in a turbid, stratified reservoir (Mount Bold Reservoir, South Australia), *Australian Journal of Marine and Freshwater Research* **40**, 595-611.

Ghadouani, A., Pinel-Alloul, B. and Prepas, E.E. (2003) Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities, *Freshwater Biology* **48**, 363-381.

Geitler, L. (1932) *Cyanophyceae*, in Rabenhorst's *Kryptogamen-Flora* Vol. **14**, Akademische Verlagsgesellschaft, Leipzig.

George, D.G. and Edwards, R.W. (1976) The effect of wind on the distribution of chlorophyll *a* and crustacean plankton in a shallow eutrophic reservoir, *Journal of Applied Ecology* **13**, 607-690.

Gulati, R.D., Bronkhorst, M. and Van Donk, E. (2001) Feeding in *Daphnia galeata* on *Oscillatoria limnetica* and on detritus derived from it, *Journal of Plankton Research* **23**, 705-718.

Hairston, N.G., Lampert, W., Cáceres, C.E., Holtmeier, C.L., Weider, L.J., Gaedke U., Fischer, J.M., Fox, J.A. and Post, D.M. (1999) Lake ecosystems: rapid evolution revealed by dormant eggs, *Nature* **401**, 446-446.

Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., Van der Linden, P.J. and Xiaosu, D (eds). (2001) *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the IPCC*, Cambridge University Press, Cambridge, U.K., 944 pp.

Howard, A. (2001) Modeling movement patterns of the cyanobacterium *Microcystis*, *Ecological Applications* **11**, 304-310.

Howard, A. and Easthope, M.P. (2002) Application of a model to predict cyanobacterial growth patterns in response to climatic change at Farmoor Reservoir, Oxfordshire, UK, *Science of the Total Environment* **282**, 459-469.

Huisman, J., Jonker, R.R., Zonneveld, C. and Weissing, F.J. (1999) Competition for light between phytoplankton species: experimental tests of mechanistic theory, *Ecology* **80**, 211-222.

Huisman, J., Sharples, J., Stroom, J.M., Visser, P.M., Kardinaal, W.E.A., Verspagen, J.M.H. and Sommeijer, B. (2004) Changes in turbulent mixing shift competition for light between phytoplankton species, *Ecology* **85,** 2960-2970.

Humphries, S.E. and Lyne, V.D. (1988) Cyanophyte blooms: the role of buoyancy, *Limnology and Oceanography* **33**, 79-91.

Ibelings, B.W. (1996) Changes in photosynthesis in response to combined photon irradiance and temperature stress in cyanobacterial surface waterblooms, *Journal of Phycology* **32**, 549-557.

Ibelings, B.W. and Maberly, S.C. (1998) Photoinhibition and the availability of inorganic carbon restrict photosynthesis by surface blooms of cyanobacteria, *Limnology and Oceanography* **43**, 408-419.

Ibelings, B.W., Mur, L.R. and Walsby, A.E. (1991a) Diurnal changes in buoyancy and vertical distribution in populations of *Microcystis* in two shallow lakes, *Journal of Plankton Research* **13**, 419-436.

Ibelings, B.W., Mur, L.R., Kinsman, R. and Walsby, A.E. (1991b) *Microcystis* changes its buoyancy in response to the average irradiance in the surface mixed layer, *Archiv für Hydrobiologie* **120**, 385-401.

Ibelings, B.W., Kroon, B.M.A. and Mur, L.R. (1994) Acclimation of photosystem II in a cyanobacterium and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes, *New Phytologist* **128**, 407-424.

Ibelings, B.W., Vonk, M., Los, H.F.J., van der Molen, D.T. and Mooij, W.M. (2003) Fuzzy modeling of cyanobacterial surface waterblooms: validation with NOAA-AVHRR satellite images*, Ecological Applications* **13**, 1456–1472.

Ibelings, B.W., Bruning, K., de Jonge, J., Wolfstein, K., Pires, L.M.D., Postma, J. and Burger, T. (2004) Distribution of microcystins in the foodweb of a large shallow lake: no evidence for bioaccumulation or biomagnification, *Microbial Ecology*, in press.

Jacoby, J.M., Collier, D.C., Welch, E.B., Hardy, F.J. and Crayton, M. (2000) Environmental factors associated with a toxic bloom of *Microcystis aeruginosa*, *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 231-240.

Jungmann, D. and Benndorf, J. (1994) Toxicity to *Daphnia* of a compound extracted from laboratory and natural *Microcystis* spp., and the role of microcystins, *Freshwater Biology* **32**, 13-20.

Kaplan, A. and Reinhold, L. (1999) CO_2 concentrating mechanisms in photosynthetic microorganisms, *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 539-570.

Kemka, N., Njine, T., Togouet, S.H.Z., Niyitegeka, D., Monkiedje, A. and Menbohan, S.F. (2003) Quantitative importance of cyanobacteria populations in a hypertrophic shallow lake in the

subequatorial African region (Yaounde Municipal Lake, Cameroon), *Archiv für Hydrobiologie* **156**, 495-510.

Kilham, S.S., Theriot, E.C. and Fritz, S.C. (1996) Linking planktonic diatoms and climate change in the large lakes of the Yellowstone ecosystem using resource theory, *Limnology and Oceanography* **41**, 1052-1062.

Kinsman, R., Ibelings, B.W. and Walsby, A.E. (1991) Gas vesicle collapse by turgor pressure and its role in buoyancy regulation by *Anabaena flos-aquae*, *Journal of General Microbiology* **137**, 1171-1178.

Klemer, A.R. (1978) Nitrogen limitation of growth and gas vacuolation in *Oscillatoria rubescens, Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **20**, 2293-2297.

Klemer, A.R., Feuillade, J. and Feuillade, M. (1982) Cyanobacterial blooms: carbon and nitrogen limitation have opposite effects on the buoyancy of *Oscillatoria*, *Science* **215**, 1629-1631.

Knoppert, P.L., Rook, J.J., Hofker, T. and Oskam, G. (1970) Destratification experiments in Rotterdam, *Journal of American Water Works Association* **62**, 448-454.

Komárek, J. and Anagnostidis, K. (1986) Modern approach to the classification system of cyanophytes. 2 – Chroococcales, *Archiv für Hydrobiologie (Algological Studies)* **43**, 157-226.

Komárek, J. and Hindák, F. (1988) Taxonomic review of natural populations of the cyanophytes from *Gomphosphaeria*-complex, *Archiv für Hydrobiologie (Algological Studies)* **80**, 203-225.

Komárek, J. and Anagnostides, K. (1999) *Cyanoprokaryota 1. Teil Chroococcales,* Gustav Fischer Verlag Jena, Jena, Germany.

Konopka, A., Kromkamp, J. and Mur, L.R. (1987a) Buoyancy regulation in phosphate-limited cultures of *Microcystis aeruginosa*, *FEMS Microbiology Ecology* **45**, 135-142.

Konopka, A., Kromkamp, J. and Mur, L.R. (1987b) Regulation of gas vesicle content and buoyancy in light- or phosphate-limited cultures of *Aphanizomenon flos-aquae* (Cyanophyta), *Journal of Phycology* **23**, 70-78.

Korner, S. (2001) Development of submerged macrophytes in shallow Lake Muggelsee (Berlin, Germany) before and after its switch to the phytoplankton-dominated state, *Archiv für Hydrobiologie* **152**, 395-409.

Kromkamp, J. and Walsby, A.E. (1990) A computer model of buoyancy and vertical migration in cyanobacteria, *Journal of Plankton Research* **12**, 161-183.

Kromkamp, J., Van den Heuvel, A. and Mur, L.R. (1989) Formation of gas vesicles in phosphorus-limited cultures of *Microcystis aeruginosa*, *Journal of General Microbiology* **135**, 1933-1939.

Kurmayer, R., Christiansen, G. and Chorus, I. (2003) The abundance of microcystin-producing genotypes correlates positively with colony size in *Microcystis* sp. and determines its microcystin net production in Lake Wannsee, *Applied and Environmental Microbiology* **69**, 787-795.

Lackey, R.T. (1973) Artificial reservoir destratification effects on phytoplankton, *Journal of the Water Pollution Control Federation* **45**, 668-673.

Lampert, W. (1987) Laboratory studies on zooplankton-cyanobacteria interactions, *New Zealand Journal of Marine and Freshwater Research* **21**, 483-491.

Lindenschmidt, K.E. (1999) Controlling the growth of *Microcystis* using surged artificial aeration, *International Review for Hydrobiology* **84**, 243-254.

Lorenzen, M.W. and Fast, R. (1977) A guide to aeration/circulation techniques for lake management, *Research Series EPA-600/3-77-004*, *United States Environmental Protection Agency, Washington D.C.*

MacIntyre, H.L., Kana, T.M., Anning, T. and Geider, R.J. (2002) Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria, *Journal of Phycology* **38**, 17-38.

Meijer, M.L., de Boois, I., Scheffer, M., Portielje, R. and Hosper, H. (1999) Biomanipulation in shallow lakes in The Netherlands: an evaluation of 18 case studies, *Hydrobiologia* **409**, 13-30.

Menday, D.C. and Buck A.A. (1972) A shock-wave technique to collapse vacuoles of blue-green algae, *Water Research* **6**, 279-284.

Mitrovic, S.M., Bowling, L.C. and Buckney, R.T. (2001) Vertical disentrainment of *Anabaena circinalis* in the turbid, freshwater Darling River, Australia: quantifying potential benefits from buoyancy, *Journal of Plankton Research* **23**, 47-55.

Murphy, T.P., Irvine, K., Guo, J., Davies, J., Murkin, H., Charlton, M. and Watson, S.B. (2003) New microcystin concerns in the lower Great Lakes, *Water Quality Research Journal of Canada* **38**, 127-140.

Nakano, S., Hayakawa, K., Frenette, J.J., Nakajima, T., Jiao, C.M., Tsujimura, S. and Kumagai, M. (2001) Cyanobacterial blooms in a shallow lake: a large-scale enclosure assay to test the importance of diurnal stratification, *Archiv für Hydrobiologie* **150**, 491-509.

Namikoshi, M. and Rinehart, K.L. (1996) Bioactive compounds produced by cyanobacteria, *Journal of Industrial Microbiology and Biotechnology* **17**, 373-384.

Neilan, B.A., Jacobs, D., DelDot, T., Blackall, L.L., Hawkins, P.R., Cox, P.T. and Goodman, A.E. (1997) rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*, *International Journal of Systematic Bacteriology* **47**, 693-697.

Niyogi, K.K. (2000) Safety valves for photosynthesis, *Current Opinions in Plant Biology* **3**, 455-460.

Oliver, R.L. (1994) Floating and sinking in gas-vacuolate cyanobacteria, *Journal of Phycology* **30**, 161-173.

Oliver, R.L. and Ganf, G.G. (2000) Freshwater blooms, in B.A. Whitton and M. Potts (eds.), *The Ecology of Cyanobacteria*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 149-194.

Oliver, R.L. and Walsby, A.E. (1984) Direct evidence for the role of light-mediated gas vesicle collapse in the buoyancy regulation of *Anabaena flos-aquae* (cyanobacteria), *Limnology and Oceanography* **29**, 879-886.

Oliver, R.L., Thomas, R.H., Reynolds, C.S. and Walsby, A.E. (1985) The sedimentation of buoyant *Microcystis* colonies caused by precipitation with an iron-containing colloid, *Proceedings of the Royal Society of London* B **223**, 511-528.

Oquist, G., Chow, W.S. and Anderson, J.M. (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term protection of photosystem II, *Planta* **186**, 450-460.

Osgood, R.A. and Stiegler, J.E. (1990) The effects of artificial circulation on a hypereutrophic lake, *Water Research Bulletin* **26**, 209-217.

Otsuka, S., Suda, S., Li, R.H., Matsumoto, S. and Watanabe, M.M. (2000) Morphological variability of colonies of *Microcystis* morphospecies in culture, *Journal of General and Applied Microbiology* **46**, 39-50.

Paerl, H.W. and Ustach J.F. (1982) Blue-green algal scums: an explanation for their occurrence during freshwater blooms, *Limnology and Oceanography* **27**, 212-217.

Paerl, H.W., Tucker, J. and Bland, P.T. (1983) Carotenoid enhancement and its role in maintaining blue-algal (*Microcystis aeruginosa*) surface blooms, *Limnology and Oceanography* **28**, 847-857.

Paerl, H.W., Fulton, R.S., Mosiander, P. and Dyble, J. (2001) Harmful freshwater algal blooms, with an emphasis on cyanobacteria, *The Scientific World* **1**, 76-113.

Pastorok, R.A., Ginn, T.C. and Lorenzen, M.W. (1980) Review of aeration/circulation for lake management, in *Restoration of Lakes and Inland Waters, EPA 440/5-81-010, United States Environmental Protection Agency, Washington D.C.*, pp. 124-133.

Paterson, M.J., Findlay, D.L., Salki, A.G., Hendzel, L.L. and Hesslein, R.H. (2002) The effects of *Daphnia* on nutrient stoichiometry and filamentous cyanobacteria: a mesocosm experiment in a eutrophic lake, *Freshwater Biology* **47**, 1217-1233.

Perrow, M.R., Meijer, M.L., Dawidowicz, P. and Coops, H. (1997) Biomanipulation in the shallow lakes: state of the art, *Hydrobiologia* **342**, 355-365.

Pires, L.M.D. and Van Donk, E. (2002) Comparing grazing by *Dreissena polymorpha* on phytoplankton in the presence of toxic and non-toxic cyanobacteria, *Freshwater Biology* **47**, 1855-1865.

Pires, L.M.D., Kusserow, R. and Van Donk, E. (2003) Influence of toxic and non-toxic phytoplankton on feeding and survival of *Dreissena polymorpha* (Pallas) larvae, *Hydrobiologia* **491**, 193-200.

Porat, R., Teltsch, B., Mosse, R., Dubinsky, Z. and Walsby, A.E. (1999) Turbidity changes caused by collapse of cyanobacterial gas vesicles in water pumped from Lake Kinneret into the Israeli National Water Carrier, *Water Research* **33**, 1634-1644.

Porat, R., Teltsch, B., Dubinsky, Z. and Walsby, A.E. (2000) Effects of light and pressure on gas vesicle formation and buoyancy in *Aphanizomenon ovalisporum* Forti (Cyanobacteria) from Lake Kinneret, Israel, *Archiv für Hydrobiologie, Special Issues, Advances in Limnology* **55**, 333-348.

Porat, R., Teltsch, B., Perelman, A. and Dubinsky, Z. (2001) Diel buoyancy changes by the cyanobacterium *Aphanizomenon ovalisporum* from a shallow reservoir, *Journal of Plankton Research* **23**, 753-763.

Powles, S.B. (1984) Photoinhibition of photosynthesis induced by visible light, *Annual Review of Plant Physiology* **35**, 15-44.

Preston, T., Stewart, W.D.P. and Reynolds, C.S. (1980) Bloom-forming cyanobacterium *Microcystis aeruginosa* overwinters on the sediment, *Nature* **288**, 365-367.

Rabouille, S., Thébault, J.M. and Salençon, M.J. (2003) Simulation of carbon reserve dynamics in *Microcystis* and its influence on vertical migration with Yoyo model, *Comptes Rendus Biologies* **326**, 349-361.

Reynolds, C.S. (1976) Succession and vertical distribution of phytoplankton in response to thermal stratification in a lowland mere, with special reference to nutrient availability, *Journal of Ecology* **64**, 529-551.

Reynolds, C.S. (1984) *The Ecology of Freshwater Phytoplankton,* Cambridge University Press, Cambridge, United Kingdom.

Reynolds, C.S. (1987) Cyanobacterial water-blooms, *Advances in Botanical Research* **13**, 67-143.

Reynolds, C.S. (1991) Toxic blue-green algae: the " problem" in a perspective, *Freshwater Forum* **1**, 29-39.

Reynolds, C.S. (1997) *Vegetation Processes in the Pelagic: a Model for Ecosystem Theory*, Ecology Institute, Oldendorf, Germany.

Reynolds, C.S. and Walsby, A.E. (1975) Water-blooms, *Biological Reviews* **50**, 437-481.

Reynolds, C.S. and Rogers, D.A. (1976) Seasonal variations in the vertical distribution and buoyancy of *Microcystis aeruginosa* Kutz. emend. Elenkin in Rostherne Mere, England, *Hydrobiologia* **48**, 17-23.

Reynolds, C.S., Jaworski, G.H.M., Cmeich, H.A. and Leedale, G.F. (1981) On the annual cycle of the blue-green alga *Microcystis aeruginosa* Kutz. emend. Elenkin, *Philosophical Transactions of the Royal Society B* **293**, 419-477.

Reynolds, C.S., Wiseman, S.W., Godfrey, B.M. and Butterwick, C. (1983) Some effects of artificial mixing on the dynamics of phytoplankton populations in large limnetic enclosures, *Journal of Plankton Research* **5**, 203-232.

Reynolds, C.S. and Bellinger, E.G. (1992) Patterns of abundance and dominance of the phytoplankton of Rostherne Mere, England: evidence from an 18-year data set, *Aquatic Sciences* **54**, 10-36.

Rippka, R., Deruelles, J.B., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria, *Journal of General Microbiology* **111**, 1-61.

Robarts, R.D. and Zohary, T. (1984) *Microcystis aeruginosa* and underwater light attenuation in a hypertrophic lake (Hartbeespoort Dam, South Africa), *Journal of Ecology* **72**, 1001-1017.

Rohrlack, T., Henning, M. and Kohl, J.G. (1999a) Does the toxic effect of *Microcystis aeruginosa* on *Daphnia galeata* depend on microcystin ingestion rate? *Archiv für Hydrobiologie* **146**, 385-395.

Rohrlack, T., Henning, M. and Kohl, J.G. (1999b) Mechanisms of the inhibitory effect of the cyanobacterium *Microcystis aeruginosa* on *Daphnia galeata'*s ingestion rate, *Journal of Plankton Research* **21**, 1489-1500.

Rohrlack, T., Dittmann, E., Henning, M., Börner, T. and Kohl, J.G. (1999c) Role of microcystins in poisoning and food ingestion inhibition of *Daphnia galeata* caused by the cyanobacterium *Microcystis aeruginosa*, *Applied and Environmental Microbiology* **65**, 737-739.

Rohrlack, T., Dittmann, E., Börner, T. and Christoffersen, K. (2001) Effects of cell-bound microcystins on survival and feeding of *Daphnia* spp., *Applied and Environmental Microbiology* **67**, 3523-3529.

Rohrlack, T., Christoffersen, K., Hansen, P.E., Zhang, W., Czarnecki, O., Henning, M., Fastner, J., Erhard, M., Neilan, B.A. and Kaebernick, M. (2003) Isolation, characterization, and quantitative analysis of microviridin J, a new *Microcystis* metabolite toxic to *Daphnia*, *Journal of Chemical Ecology* **29**, 1757-1770.

Sas, H., Ed. (1989) *Lake Restoration by Reduction of Nutrient Loading: Expectations, Experience, Extrapolations*, Academia Verlag Richarz, St. Augustin.

Scheffer, M., Rinaldi, S., Gragnani, A., Mur, L.R. and van Nes, E.H. (1997) On the dominance of filamentous cyanobacteria in shallow, turbid lakes, *Ecology* **78**, 272-282.

Shapiro, J. (1997) The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes, *Freshwater Biology* **37**, 307-323.

Smith, V.H. (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton, *Science* **221**, 669-671.

Symons, J.M., Irwin, W.H., Robinson, E.L. and Robeck, G.G. (1967) Impoundment destratification for raw water quality control using either mechanical or diffused-air-pumping, *Journal of the American Water Works Association* **59**, 1268-1291.

Symons, J.M., Carswell, J.K. and Robeck, G.G. (1970) Mixing of water supply reservoirs for quality control, *Journal of the American Water Works Association* **62**, 322-334.

Takamura, N., Yasuno, M. and Sugahara, K. (1984) Overwintering of *Microcystis aeruginosa* Kutz. in a shallow lake, *Journal of Plankton Research* **6**, 1019-1029.

Talling, J.F. (1976) The depletion of carbon dioxide from lake water by phytoplankton, *Journal of Ecology* **64**, 79-121.

Ten Winkel, E.H. and Davids, C. (1982) Food selection by *Dreissena polymorpha* Pallas (Mollusca: Bivalva), *Freshwater Biology* **12**, 553-558.

Thomas, R.H. and Walsby, A.E. (1985) Buoyancy regulation in a strain of *Microcystis*, *Journal of General Microbiology* **131**, 799-809.

Thomas, R.H. and Walsby, A.E. (1986) The effect of temperature on recovery of buoyancy by *Microcystis*, *Journal of General Microbiology* **132**, 1665-1672.

Thostrup, L. and Christoffersen, K. (1999) Accumulation of microcystin in *Daphnia magna* feeding on toxic *Microcystis*, *Archiv für Hydrobiologie* **145**, 447-467.

Toetz, D.W. (1981) Effects of whole lake mixing on water quality and phytoplankton, *Water Research* **15**, 1205-1210.

Trimbee, A.M. and Harris, G.P. (1984) Phytoplankton population dynamics of a small reservoir: effect of intermittent mixing on phytoplankton succession and the growth of blue-green algae, *Journal of Plankton Research* **6**, 699-713.

Trimbee, A.M. and Prepas, E.E. (1988) The effect of oxygen depletion on the timing and magnitude of blue-green algal blooms, *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* **23**, 220-226.

Tsujimura, S., Tsukada, H., Nakahara, H., Nakajima, T. and Nishino, M. (2000) Seasonal variations of *Microcystis* populations in sediments of Lake Biwa, Japan, *Hydrobiologia* **434**, 183-192.

Utkilen, H.C., Oliver, R.L. and Walsby, A.E. (1985) Buoyancy regulation in a red *Oscillatoria* unable to collapse gas vacuoles by turgor pressure, *Archiv für Hydrobiologie* **102**, 3l9-329.

Vanderploeg, H.A., Liebig, J.R., Carmichael, W.W., Agy, M.A., Johengen, T.H., Fahnenstiel, G.L. and Nalepa, T.F. (2001) Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie, *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1208-1221.

Van Liere, L. and Mur, L.R. (1980) Occurrence of *Oscillatoria agardhii* and some related species, a survey, in J. Barica and L.R. Mur (eds.), *Hypertrophic Ecosystems*, Junk Publishers, The Hague, The Netherlands, pp. 67-77.

Van Rijn, J. and Shilo, M. (1985) Carbohydrate fluctuations, and vertical migration of scum-forming cyanobacteria in fishponds, *Limnology and Oceanography* **30**, 1219-1228.

Verspagen, J.M.H., Snelder, E.O.F.M., Visser, P.M., Huisman, J., Mur, L.R. and Ibelings, B.W. (2004) Recruitment of benthic *Microcystis* (Cyanophyceae) to the water column: internal buoyancy changes or resuspension? *Journal of Phycology* **40,** 260-270.

Visser, P.M., Ibelings, B.W. and Mur, L.R. (1995) Autumnal sedimentation of *Microcystis* spp. as result of an increase in carbohydrate ballast at reduced temperature, *Journal of Plankton Research* **17**, 919-933.

Visser, P.M., Ibelings, B.W., Van der Veer, B., Koedood, J. and Mur, L.R. (1996a) Artificial mixing prevents nuisance blooms of the cyanobacterium *Microcystis* in Lake Nieuwe Meer, the Netherlands, *Freshwater Biology* **36,** 435-450.

Visser, P.M., Ketelaars, H.A.M., Van Breemen, L.W.C.A. and Mur, L.R. (1996b) Diurnal buoyancy changes of *Microcystis* in an artificially mixed storage reservoir, *Hydrobiologia* **331**, 131-141.

Visser, P.M., Massaut, L., Huisman, J. and Mur, L.R. (1996c) Sedimentation losses of *Scenedesmus* in relation to mixing depth, *Archiv für Hydrobiologie* **136**, 289-308.

Visser, P.M., Passarge, J. and Mur, L.R. (1997) Modelling vertical migration of the cyanobacterium *Microcystis*, *Hydrobiologia* **349**, 99-109.

Vos, R.J., Hakvoort, J.H.M., Jordans, R.W.J. and Ibelings B.W. (2003) Multiplatform optical monitoring of eutrophication in temporally and spatially variable lakes, *Science of the Total Environment* **312**, 221-243.

Wallace, B.B. and Hamilton, D.P. (1999) The effect of variations in irradiance on buoyancy regulation in *Microcystis aeruginosa*, *Limnology and Oceanography* **44**, 273-281.
Walsby, A.E. (1968) An alga's buoyancy bag, *New Scientist* **40**, 436-437.
Walsby, A.E. (1969) The permeability of blue-green algal gas-vacuole membranes to gas, *Proceedings of the Royal Society of London* B **173**, 235-255.
Walsby, A.E. (1970) The nuisance algae: curiosities in the biology of planktonic blue-green algae, *Water Treatment and Examination* **19**, 359-373.
Walsby, A.E. (1992) The control of gas-vacuolate cyanobacteria, in D.W. Sutcliffe and J. Gwynfryn Jones (eds.), *Eutrophication: Research and Application to Water Supply*, Freshwater Biological Association, Ambleside, United Kingdom, pp. 150-162.
Walsby, A.E. (1994) Gas vesicles, *Microbiological Reviews* **58**, 94-144.
Walsby, A.E. (1997) Numerical integration of phytoplankton photosynthesis through depth and time in a water column, *New Phytologist* **136**, 189-209.
Walsby, A.E. and Booker, M.J. (1980) Changes in buoyancy of a planktonic blue-green alga in response to light intensity, *British Phycological Journal* **15**, 311-319.
Walsby, A.E., Utkilen, H.C. and Johnsen, I.J. (1983) Buoyancy changes of a red coloured *Oscillatoria agardhii* in Lake Gjersjøen, Norway, *Archiv für Hydrobiologie* **97**, 18-38.
Walsby, A.E. and McAllister, G.K. (1987) Buoyancy regulation by *Microcystis* in Lake Okaro, *New Zealand Journal of Marine and Freshwater Research* **21**, 521-524.
Walsby, A.E. and Bleything, A. (1988) The dimensions of cyanobacterial gas vesicles in relation to their efficiency in providing buoyancy and withstanding pressure, *Journal of General Microbiology* **134**, 2635-2645.
Walsby, A.E., Reynolds, C.S., Oliver, R.L. and Kromkamp, J. (1989) The role of gas vacuoles and carbohydrate content in the buoyancy and vertical distribution of *Anabaena minutissima* in Lake Rotongaio, New Zealand, *Archiv für Hydrobiologie/ Ergebnisse der Limnologie* **32**, 1-25.
Walsby, A.E., Hayes, P.K., Boje, R. and Stal, L.J. (1997) The selective advantage of buoyancy provided by gas vesicles for planktonic cyanobacteria in the Baltic Sea, *New Phytologist* **136**, 407-417.
Walsby, A.E., Ng, G., Dunn, C. and Davis, P.A. (2004) Comparison of the depth where *Planktothrix rubescens* stratifies and the depth where the daily insolation supports its neutral buoyancy, *New Phytologist* **162**, 133-145.
Watanabe, M.F., Oishi, S., Harada, K.I., Matsuura, K., Kawai, H. and Suzuki, M. (1988) Toxins contained in *Microcystis* species of cyanobacteria (blue- green algae), *Toxicon* **26**, 1017-1025.
Whitelam, G.C. and Codd, G.A. (1983) Photoinhibition of photosynthesis in the cyanobacterium *Microcystis aeruginosa*, *Planta* **157**, 561-566.
Xie, L.Q., Xie, P. and Tang, H.J. (2003) Enhancement of dissolved phosphorus release from sediment to lake water by *Microcystis* blooms - an enclosure experiment in a hyper-eutrophic, subtropical Chinese lake, *Environmental Pollution* **122**, 391-399.
Zohary, T. and Robarts, R.D. (1989) Diurnal mixed layers and the long-term dominance of *Microcystis aeruginosa*, *Journal of Plankton Research* **11**, 25-40.
Zohary, T. and Pais-Madeira, A.M. (1990) Structural, physical and chemical characteristics of *Microcystis aeruginosa* from a hypertrophic lake, *Freshwater Biology* **23**, 339-352.
Zohary, T. and Robarts, R.D. (1990) Hyperscums and the population dynamics of *Microcystis aeruginosa*, *Journal of Plankton Research* **12**, 423-432.